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Optical stimulation for restoration of motor function following spinal cord injury

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Abstract

Spinal cord injury (SCI) can be defined as a loss of communication between the brain and the body due to disrupted pathways within the spinal cord. While many promising molecular strategies have emerged to reduce secondary injury and promote axonal regrowth, there is still no effective cure and recovery of function remains limited. Functional electrical stimulation (FES) represents a strategy developed to restore motor function without the need for regenerating severed spinal pathways. Despite its technological success, however, FES has not been widely integrated into the lives of spinal cord injury survivors. In this review, we briefly discuss the limitations of existing FES technologies. Additionally, we discuss how optogenetics, a rapidly evolving technique used primarily to investigate select neuronal populations within the brain, may eventually be used to replace FES as a form of therapy for functional restoration following SCI.

Keywords

Optogenetics; spinal cord injury; functional electrical stimulation; sensorimotor; FES; SCI

Spinal cord injury

Despite efforts to elucidate the pathophysiology of spinal cord injury (SCI) in the last few decades, the search for a cure continues [1-3]. Currently, the gold standard of care is to provide intense physical rehabilitation following the acute injury phase, in an attempt to maximize

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any spontaneous recovery of respiratory, hand, arm, leg, bowel, bladder and sexual function [4]. While this paradigm increases the possibility of some degree of recovery, particularly in patients with incomplete injuries, most patients do not experience a full recovery and have only limited gains with current rehabilitation therapy [4-7].

The poor chance of recovery following SCI has inspired a significant amount of research aimed at restoring lost function in SCI survivors. From a biological standpoint, these efforts have primarily focused on molecular manipulations to lessen the degree of secondary injury that occurs via ischemia and excitotoxicity [5,8-14], replacement of lost neurons and glia via stem cell transplantation [15,16], and remyelination or axonal regeneration by either reducing glial scar formation [17] or by inserting biomaterial substrates [18] that promote neural regrowth [19-23]. Unfortunately, these approaches have been met with limited success due to the complexity involved with degrading glial scarring while regenerating neural tissue and directing appropriate neural connections required to restore severed spinal pathways [24].

An alternative to molecular manipulations is to activate remaining neuromuscular components, which, despite the loss of descending input, can still be activated via external stimuli. Historically, the most common form of stimuli has been electricity. Namely, functional electrical stimulation (FES) has been successfully used to restore breathing [25,26], lower [27-29] and upper extremity function [30,31], and bladder and bowel control [32-35].

Presently, FES systems can restore lost function, however, they have a narrow scope of application and generally only restore one previously lost function at a time. For example, phrenic pacing has allowed for individuals with high cervical injuries and intact phrenic nerves to successfully wean from mechanical ventilation, leading to increased survival rates and improved quality of life [36,37]. Additionally, Parastep ®, a commercially available device that relies on surface stimulation of the quadriceps, gluteal muscles, and peroneal nerves, permits individuals with lower SCI to ambulate for distances over a quarter of a mile [38]. Furthermore, Vocare ® utilizes anterior sacral root stimulation to restore micturition [39,40].

Despite the proven effectiveness of the systems described above, technological shortcomings and practical limitations such as inadequate activation control strategies [41], electrical current spillover [42-44], and muscle fatigue [45] have led to a limited integration of FES systems into the daily lives of SCI survivors [41]. Optogenetics, a novel stimulation modality that uses light to either excite or inhibit genetically modified neurons, has the potential to overcome some of the limitations facing current FES strategies [1,46,47].

Optogenetics

Optogenetics is a rapidly evolving technique originally developed to study neural activity in select neuronal populations [48]. The genetic material of specific cell populations is modified via viral vectors to express a trans-membrane protein reactive to light (opsins). These trans-membrane proteins undergo a conformational change when light of a specific wavelength (390–700 nm) is directly applied to the cells, resulting in selective ionic current flow across the cell membrane. In turn, positively-charged (cations) or negatively-charged (anions) ionic movement will lead to cell depolarization or hyperpolarization, respectively. Therefore, specific viral vectors can be chosen and modified to transduce specific neuronal populations

allowing for selective modulation with light. Excitatory responses can be achieved by activating Channelrhodopsin-2 (ChR-2) cation channels (responsive to 470 nm wavelength blue light), which allow entry of positively-charged sodium and calcium ions into the cell (Figure 1A) [50,51]. In contrast, inhibitory responses can be evoked by activating Halorhodopsin (HR), a trans membrane ion-pump, using 580 nm yellow light, which facilitates the movement of negatively-charged chloride ions (Figure 1B) [5,47].

The use of optogenetics has previously focused on characterization of neuronal mechanisms of excitation and inhibition within the brain [5,8,10,12-14,52]. However, increased interest in translational applications of optogenetics technology has resulted in the pursuit of novel clinical avenues for restoration of vision, seizure control, and treatment of cardiac arrhythmias [19,53,54]. Light offers clear advantages for modulating neuronal behavior. Specifically, optical stimulation can provide real-time, selective control of cellular activity [51]. Additionally, efforts to expand the toolbox for controlling neurons via light have led to an increased variety of ChR-2s that are altered to respond to various light wavelengths with enhanced ion channel kinetics and selectivity [55]. More recent efforts have led to the first light-gated chloride channel, engineered from the ChR-2 trans membrane family of proteins, which is designed to decrease the latency between light activation and cell inhibiton that is observed with HR ion pumps [56,57]. Furthermore, optical control of muscle function has been achieved by controlling murine stem cells, previously engineered to express ChR-2, followed by implantation distal to a nerve ligation in an attempt to establish a possible regenerative medicine therapeutic intervention [58]. Moreover, the combination of genes that express ChR-2 with genes that express the light-generating protein luciferase demonstrated that it is possible to activate neurons by exogenous application of the luciferase substrate, leading to cell luminescence and light-driven auto-activation *in vitro* [59]. Finally, computational modeling evidence has shown that optogenetic activation of axons follows a physiologic, small-to-large diameter axon recruitment order [60], which could prove invaluable for restoring motor function following SCI.

Restoration of motor function following SCI via optical stimulation

Applications of optogenetic technology for restoring function following SCI are already underway in small animal models. In fact, optogenetics has recently been used to dissect select spinal cord circuitry responsible for evoking both rhythmic, and stimulation-triggered limb movements [46,47,61]. Specifically, Towne and colleagues demonstrated the ability of using optical stimulation to selectively activate hind limb muscles in a rodent model of SCI using retrograde transduction of motor neurons with ChR-2 via intramuscular inoculation with an adeno-associated virus (AAV) [50]. Similarly, Alilain and colleagues showed that it is possible to restore motor activity in the diaphragm muscle of rodents that sustained a cervical SCI using optical stimulation of the spinal cord at cervical vertebral levels 3-6 [62]. Additionally, Hagglund and colleagues showed rhythmic activation of selective muscles necessary for locomotion using optical stimulation in a transgenic mouse line expressing ChR-2 channels in spinal interneurons [47].

The continued development of optogenetic technology promises to overcome several limitations of electrical stimulation techniques for restoring motor function following SCI.

First, optical stimulation allows selective muscle activation and fine motor control due to increased specificity associated with viral transduction of select motor neurons [61], as well as the theoretical possibility of direct transduction and control of skeletal itself [63]. Second, optogenetics may restore function in a more physiologic manner particularly for functions that involve complex patterns of excitation and inhibition of different neuronal populations. An example is micturition, which requires activation of parasympathetic neural circuitry to initiate bladder emptying and simultaneous inhibition of sphincter contraction. While electrical stimulation can be used to achieve bladder emptying, [53,64,65] it occurs in a suboptimal tetanic fashion. Third, muscle fatigue associated with existing electrical stimulation technologies [66-68] can be delayed by activating slow twitch, fatigue-resistant fibers before any fast fatigable fibers are activated (Figure 2) [61,69,70]. Despite the significant advantages of optical stimulation over electrical stimulation, multiple limitations have to be addressed before optogenetics can be clinically used to restore function in SCI survivors.

Limitations of optogenetic applications

Numerous studies using direct administration of AAV vectors with different serotypes in small animal models have demonstrated robust transduction rates [71-74]. Additionally, gene therapy using viral vectors has been successfully translated to clinical practice, however, its use has uncovered multiple issues that need to be addressed before viral delivery of optogenetics can be used clinically in humans. First, efforts to reproduce efficient transduction in large animal models have been largely unsuccessful in the past. More recently, improvements in transduction efficiency have been reported in both the brain and spinal cord in swine [75-77] and nonhuman primate models [76,78,79]. Second, integration of foreign genomic material can also result in numerous adverse events including expression of proto-oncogenes [80], silencing of tumor-suppressor genes [81,82] which could lead to neoplastic transformation or protein mutations, leading to undesired changes in downstream cellular functions [81,82]. Third, peripherally-administered vectors can initiate immune responses leading to inhibition of vector function, decreased expression duration, and cytotoxic effects [83-85]. Current strategies to lessen immune responses include altering the capsid of the viral vector, modifying the vector delivery route, or applying techniques to inhibitor or modulate immune system activity [86,87]. Alternatively, non-viral techniques could be used along with biomaterial and molecular strategies to systemically deliver genetic material into target locations [20,88,89].

Further work is also needed to identify optimal vectors (viral or nonviral) and specific administration routes for targeting specific neuronal populations. For example, efficient and selective transduction of alpha motor neurons within the ventral spinal cord will likely require intraparenchymal or intrathecal vector injection into the spinal gray matter. Alternatively, this could be achieved by retrograde transport from intraneural or intramuscular injection sites.

Finally, multiple barriers must be overcome before chronically implantable optical systems can be developed. Some of these barriers include 1) minimizing glial responses to the implanted light guides, similar to the glial scarring observed with other chronic neural interface systems such as deep brain stimulation and intracortical recording systems; 2)

optimizing light delivery paradigms to enhance temporal and spatial activation of target neurons while improving light penetration through tissue surrounding the light source [90]; and 3) reducing heating effects on tissue surrounding the light source.

Future directions

Small animal studies suggest that optogenetics offers multiple advantages over electrical stimulation techniques. However, multiple steps need to be taken before optogenetics can be clinically used to restore function in SCI survivors. First, it is necessary to devise appropriate strategies for safe transgene delivery to target cell types *in vivo*. These strategies will require controlled transduction (via appropriate vectors and serotypes) and expression (via appropriate gene regulation promoters). Second, it is paramount to extend the expression lifetime to allow for single (or minimally repeated) administration of viral vectors and promoters. Third, stimulation systems need to be developed that optimize light delivery paradigms in a tissue specific manner while reducing glial responses to light delivery devices. Finally, stimulation will need to be controlled in a natural manner by the user while also allowing for real-time adjustment to account for perturbations within the user's environment [41,91].

Conclusions

While there is still no cure for SCI, advances in stimulation and neural interfacing technology show promise for restoring neurologic function. Optogenetics offers to improve upon existing FES technology by better following physiologic muscle activation, increasing selectivity, and providing simultaneous control of excitatory and inhibitory responses. In turn, advances in optogenetics technology could provide an avenue for optimal restoration of function following SCI, thereby improving the quality of life for those living with paralysis.

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Abbreviations

AAV	adeno-associated virus
ChR-2	channelrhodopsin
FES	functional electrical stimulation
HR	halorhodopsin
SCI	spinal cord injury

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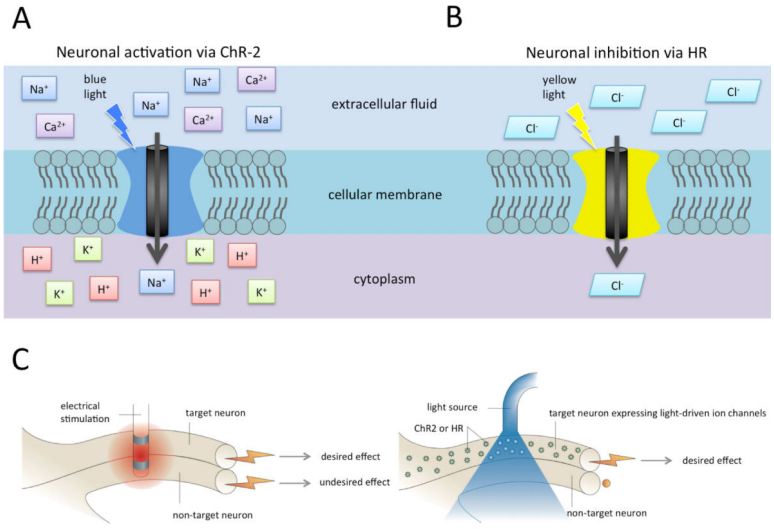

Figure 1. Mechanisms of neuromodulation via optogenetics

A) Application of blue light (470 nm wavelength) leads to a conformational change of the trans membrane ion channel protein, channelrhodopsin, allowing a flow of positively charged ions into the cytoplasm, ultimately leading to neuron depolarization.

B) Yellow light application (580 nm wavelength) changes the conformation of the trans membrane ion pump protein, halorhodopsin, allowing negatively charged ions to move into the cytoplasm, leading to neuron hyperpolarization.

C) Schematic comparing the non-specific activation characteristic of electrical activation, leading to both desired and undesired effects, and optical activation of only targeted neurons, leading to only desired effects.

Ca^{2+} = calcium ion; ChR-2 = channelrhodopsin; Cl^- = chloride ion; H^+ = hydrogen ion; HR = halorhodopsin; K^+ = potassium ion; Na^+ = sodium ion

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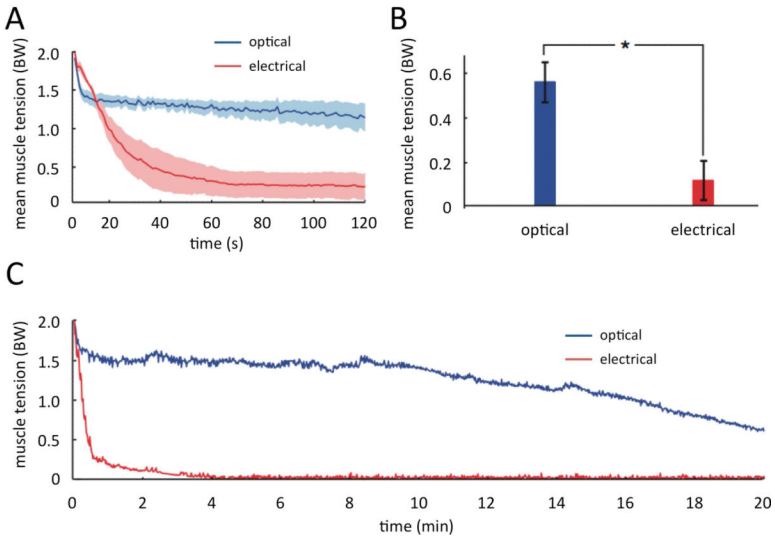

Figure 2. Fatigue resistance comparison between optical and electrical stimulation

A) Average tetanic muscle tension during 2 min stimulation with 250 ms trains of stimulation at 1 Hz using electrical and optical stimulation (n=7, shaded region is standard error of the mean, average body weight = 0.258 ± 0.01 N).

B) Average fatigue index measured as decline in tetanic muscle tension over 2 min (n=7).

C) Tetanic tension from a single mouse during optical and electrical stimulation in the hind limbs over 20 minutes.

BW = average body weight

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