OPTOGENETICS

Beyond the brain: Optogenetic control in the spinal cord and peripheral nervous system

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Optogenetics offers promise for dissecting the complex neural circuits of the spinal cord and peripheral nervous system and has therapeutic potential for addressing unmet clinical needs. Much progress has been made to enable optogenetic control in normal and disease states, both in proof-of-concept and mechanistic studies in rodent models. In this Review, we discuss challenges in using optogenetics to study the mammalian spinal cord and peripheral nervous system, synthesize common features that unite the work done thus far, and describe a route forward for the successful application of optogenetics to translational research beyond the brain.

INTRODUCTION

At the interface between the internal and external worlds, the spinal cord and peripheral nervous system are fundamental to the behavior of any vertebrate animal. These systems are implicated in a range of disease states, and new approaches to modulating neural activity in these systems have generated much interest (1). One such approach to neuromodulation, optogenetics, was initially applied in rodent models for the control of neural circuits in the brain, but recently has shown promise in modulating activity in the spinal cord and peripheral nervous system (fig. S1) (2–55). Optogenetic methods involve expressing light-activated microbial opsin proteins in desired neural or nonneural cell populations (56, 57). Expression of these opsins is followed by light delivery to the cell population and opsin activation. Depending on the opsin used, a wide variety of light-induced changes in cell activity are possible. Most frequently, the opsins used are light-activated ion channels, which then enable temporally precise control over action potential generation in neural circuits. Optogenetics has revolutionized neuroscience research by enabling causal exploration of how activity in neural circuits produces behavior.

Spinal cord and peripheral neural circuits are complex and heterogeneous and include tissues of vastly different sizes, shapes, stiffness, opacity, and cellular and molecular structure, thereby imposing unique challenges for optogenetic modulation (Fig. 1). The spinal cord and peripheral nervous system flex with the body during movement, and this flexibility can impede delivery of light in freely moving animals. Immune responses induced by injection of viral vectors or by expression of light-activated opsin proteins have the potential to reduce opsin expression below effective levels (Fig. 1), an issue that is prevalent in the peripheral nervous system because of increased exposure to immune surveillance (6, 58). Muscle and connective tissue surrounding peripheral neurons may block light or contribute electrical noise during experiments, complicating optogenetic implementation or clouding interpretation of results (Fig. 1).

Researchers have overcome many of these challenges in rodents by developing new opsin transduction procedures and light delivery strategies (Fig. 2) to optogenetically control the spinal cord and peripheral nervous system (Fig. 3), although much work will need to be done to extend these strategies to primates. Here, we describe guiding principles that have governed progress in optogenetics in the spinal cord and peripheral nervous system over the last decade, distill the key scientific and therapeutic results achieved thus far (Fig. 4 and Table 1), and discuss the translational potential of optogenetics beyond the brain (59, 60).

WHY OPTOGENETICS?

The spinal cord and periphery are rich with excitable cells that are targets for therapeutic modulation. These include motor neurons, sensory neurons, interneurons, visceral afferents and efferents, various types of muscle, and a variety of other nonneural targets (Fig. 3). Modulating activity in these spinal and peripheral targets has traditionally been accomplished through electrical stimulation, which has been used clinically in conditions ranging from spinal cord injury to epilepsy. However, even under conditions for which electrical neuromodulation is mature, such as spinal cord stimulation for the treatment of chronic pain (61–63), this approach to neuromodulation fails in many patients for reasons that are incompletely understood. This remains true even after a half-century (64) of clinical and basic science research and derives in part from the nonselective nature of electrical stimulation (58).

Similarly, although electrical stimulation of the vagus nerve is now used to treat some forms of epilepsy, off-target effects including throat pain, cough, and shortness of breath limit the degree to which this approach can be used (65). For motor control, electrical stimulators have been deployed for decades to stimulate paralyzed muscles in patients with spinal cord injury and stroke (66). However, such stimulators induce muscle fatigue when stimulating paralyzed muscles due to the disorderly recruitment of motor units (67), and they cannot prevent muscle contractions in patients with spasticity. Optogenetics, which can be implemented with cell type specificity (4, 17, 21), has been used to inhibit cells (6, 19) and has the potential to solve some of these problems, both by directly treating the underlying conditions without off-target effects and by elucidating the mechanisms underlying successful neuromodulation (68, 69).

OPSIN EXPRESSION

Sufficiency and specificity of opsin expression are the fundamental considerations vital to the success of optogenetic neuromodulation. Achieving sufficient expression and trafficking of opsin proteins without

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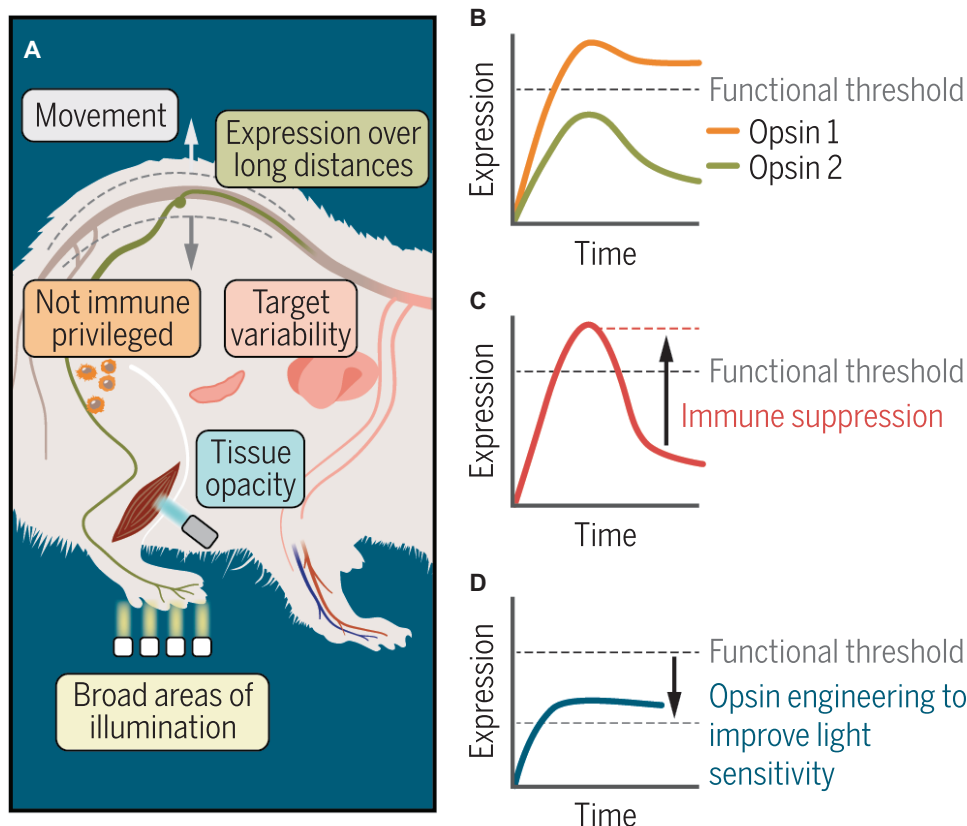*Corresponding author. Email: delp@stanford.edu

Fig. 1. Challenges of optogenetically targeting cells outside of the brain. (**A**) Wide variations in expression of opsin proteins, tissue structure, and the mechanical environment of the peripheral nervous system may make it difficult to develop modular and adaptable light and opsin expression systems. Excitable cells in the peripheral nervous system may be sparsely scattered throughout a large volume of tissue, necessitating broad areas of illumination. Opsin expression over long distances may be required because of the length of axons in the spinal cord and peripheral nervous system. Outside of the spinal cord and brain, the immune system may impair opsin expression. Various tissues, such as muscle, may be opaque to light. Relative movement of targeted cells during locomotion may complicate light delivery; because of large variations in targeted cell size and structure, custom light delivery strategies may be required. (**B**) There may be variations in opsin expression even when using the same viral construct and promoter. (**C**) Opsin expression in the peripheral nervous system may be improved over time by modulating the immune response. (**D**) Engineering opsins to have greater sensitivity to light may lower the expression threshold for functional optogenetic experiments.

ral subtypes, either in combination with Cre-dependent viral vector injection or by crossing Cre-driver lines with lines carrying Cre-dependent genes encoding opsins. In the peripheral nervous system, this strategy has limitations. Whereas many Cre-driver lines are available and have been characterized for expression in brain regions, they are frequently not fully characterized in the spinal cord and peripheral nervous system or are not developed with the requirements of these systems in mind. As a result, differences in opsin expression patterns between the brain and spinal cord can preclude direct use of existing genetic lines without de novo characterization in the region being targeted. Improvements in our knowledge of the genetic diversity of the spinal cord and periphery (*76*) will enable parallel efforts to develop new driver lines, allowing for more granular control of the systems being studied. An important limitation of transgenic approaches to optogenetic control is that they are not currently considered translatable into human patients (*77*).

Viral transduction

Strategies that use gene therapy viral vectors to deliver DNA are more tractable solutions for inducing opsin expression in human patients (*78, 79*). Many clinical trials that use viral vectors to deliver other types of therapeutic genes are under way (*80*). In addition, viral vectors provide opportunities for precise spatial targeting of opsin expression by restricting delivery to defined areas of the body. Examples of such an approach include injecting viral vectors intraspinally to target ascending circuits projecting from a defined spinal cord segment (*81*), intratu-

sacrificing specificity is challenging, especially in experiments that attempt optogenetic inhibition or involve low-yield transduction strategies, such as retrograde transport (*4*). Here, we discuss three approaches for achieving opsin expression: transgenesis, viral transduction, and cellular transplantation. Other important considerations for choosing an approach include the time course of expression and the consistency of expression among animals. Although these considerations are shared by researchers focusing on the brain, in our experience, extra care must be taken with the peripheral nervous system, particularly of nonhuman primates, because any difficulties with opsin expression in rodents will be amplified in these larger animals (*70*).

Transgenesis

Selectively breeding rodents to specifically express opsins from birth is an attractive option. A plethora of Cre-driver mouse (*71–73*) and rat lines (*74, 75*) allow for targeting of opsins to genetically specified neu-

morally to target metastatic glioma (*5*), intraneurally to target afferents from a particular dermatome (*6*), or intramuscularly to target the motor neurons of a specified muscle (*4*). Similar to transgenesis, genetic specificity can be accomplished through the use of gene promoters (*27*), although viral packaging capacity limits which promoters can be used (*82*). Cell type restriction does not have to be accomplished through promoters alone. Researchers can take advantage of the intrinsic tropism that individual viruses exhibit to restrict opsin expression. In addition to tropism differences across virus classes, individual viral vectors can be engineered with different envelope proteins, such that each serotype may display varying tropism and different retrograde or anterograde transport propensities (*7, 40, 83–87*). As is the case with transgenic lines, it cannot be assumed that viral expression patterns will be consistent between the brain and periphery or that serotype tropism will be conserved across species. For example, traditional versions of the rabies virus are well characterized and travel exclusively in a retrograde direction in
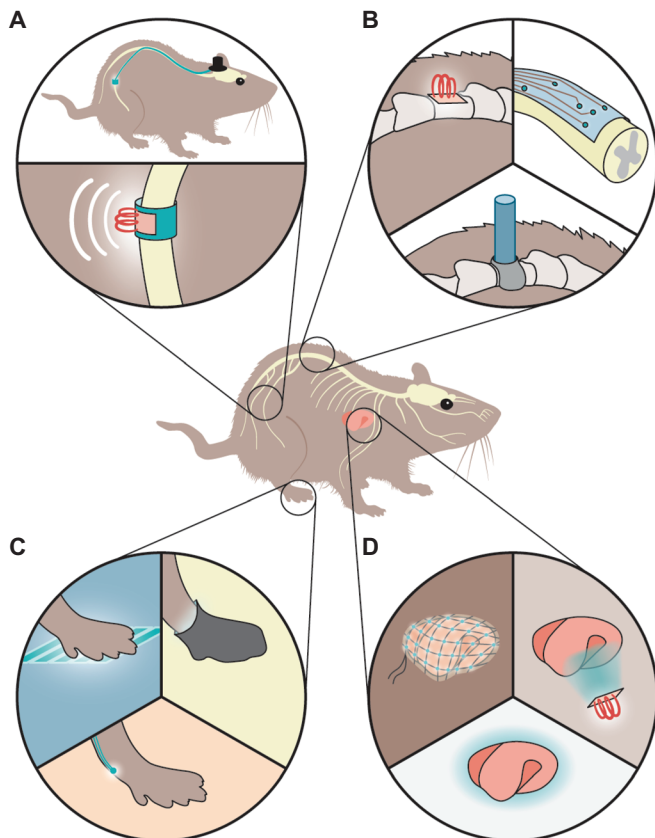
Fig. 2. Light delivery strategies in the spinal cord and peripheral nervous system. (**A**) For peripheral nerves, such as the sciatic nerve, pudendal nerve, or vagus nerve, fiber-optic coupled nerve cuffs can be placed under the skin from an attachment site on the skull (top) (*4*). Small wirelessly powered devices can directly illuminate the nerve (bottom) (*48*). (**B**) For the spinal cord, wirelessly powered devices can be implanted dorsal to the spinal column (top left) (*48*), or epidural flexible light-emitting diode (LED) arrays can be placed within the spinal column itself (top right) (*49*). In the future, optical fibers may be directly cemented dorsal to the intervertebral space (bottom). (**C**) Transdermal illumination is a frequently used approach for light delivery to sensory nerve endings (top left) (*6, 7, 9, 11, 12, 14*). Alternatives include LEDs implanted subcutaneously (bottom) (*48*). In the future, light may be delivered using light-emitting fabrics (top right). (**D**) For internal organs, wirelessly powered devices could be implanted next to the organs (top right), flexible light-emitting meshes could be developed that would wrap around opsin-expressing organs (top left), or intrinsic light-emission systems such as luciferin/luciferase expressed within the organ could be used to activate opsins (bottom) (*106*).

the brain. However, a recent study has reported that the same virus travels in an anterograde direction in primary sensory afferents (*88*).

In using viruses to transduce peripheral targets of rodents, we and others have observed the best results (*4, 6*) when using viral doses that are significantly higher [~10^{10} to 10^{11} viral genomes (*4, 6, 7*)] than those typically used in the brain [~10^{9} viral genomes (*89*)]. These required doses are likely to vary considerably depending on the viral system used, the neural target, and the host species. We have also noted modest variability in opsin expression between batches of virus. The performance of optogenetic viral constructs in primates, at least in the brain, may be best predicted by initial performance in rats (*70*); however, this has yet to be validated in the spinal cord or peripheral nervous system.

A caveat of higher viral doses is the risk of provoking a greater immune response. In a recent phase 1/2 trial of the efficacy of adeno-associated virus type 2 (AAV2)–mediated gene therapy in the degenerative retinal disease Leber's congenital amaurosis, dose-dependent immune and inflammatory responses were reported, and only transient improvements in retinal sensitivity after vector administration peaking at 6 to 12 months after treatment were observed (*90*). Similar transient effects in some end points have also been observed in alipogene tiparvovec, the AAV1 gene therapy approved by the European Medicines Agency for patients with lipoprotein lipase deficiency (*91*). As in these gene therapy studies, careful titration of the viral dose delivered will be critical to achieving a stable transduction level that is sufficient to drive the required changes in neural activity while not overburdening the transduced cells with excessive transgene expression.

Cellular transplantation

Grafts of opsin-expressing cells may be an attractive opsin delivery strategy for situations in which endogenous cellular dysfunction is at the root of pathology. This approach also offers an opportunity for greater specificity of expression beyond what can be achieved through genetic targeting strategies because opsin-expressing cells can be sorted on the basis of nongenetic characteristics before engraftment. Immunogenic consequences could also be reduced through the use of autologous cell transplants or autologous induced pluripotent stem cells. Pioneering efforts to use cell grafts for optogenetic control have demonstrated feasibility for modulation of muscle (*8*) and glucose homeostasis (*9*) in the mouse.

Persistence of expression

Regardless of how cells are modified to express opsins, in order to conduct chronic experiments or demonstrate the potential for therapeutic translation, expression must be long-lasting. Although researchers have observed AAV2 transduction with channelrhodopsin-2 for as long as 64 weeks in rat retinal ganglion cells (*92*), there is increasing evidence that virally induced expression of transgenes outside of the central nervous system can decrease within a few weeks (*6, 93, 94*). This limits the temporal scope of optogenetic experiments that are possible and raises concerns for therapeutic development. Characterization of the persistence of opsin expression and the immune response, both to the viral construct used and to the opsin expressed, should be a part of optogenetic experiments in the periphery, particularly if the study may have clinical feasibility or requires stable optogenetic neuromodulation over long time periods (*95*). As with other forms of gene therapy, successful clinical application of optogenetics may require engineering of the viral construct or opsin protein (*96*) to reduce transgene burden, increase opsin photosensitivity, reduce the immune response, and facilitate persistent expression of opsins after viral delivery (Fig. 1B). Other gene therapy applications will likely inspire immune modification strategies including inducing tolerance to specific antigens, transient immunosuppression, and suppression of humoral immunity (*97, 98*) to enable long-lasting virally induced opsin expression (Fig. 1C).

LIGHT DELIVERY

Light delivery has proven to be a vexing challenge for optogenetic applications in the spinal cord and periphery. In the brain, researchers have converged on a standard approach for light delivery in which a
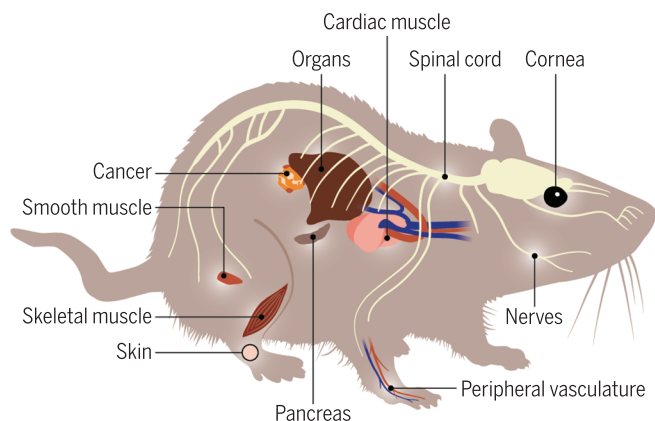
Fig. 3. Optogenetic targets outside of the brain. Given recent technical innovations, spinal cord sensory and motor circuits are ripe for optogenetic interrogation. Early demonstrations of genetically specified control of sympathetic nerves should encourage new explorations of parasympathetic and sympathetic nervous system function. Optogenetic studies of sensory terminals in the skin and cornea will improve our understanding of pain and other types of sensation. In addition to neuronal targets, other peripheral excitable cells may be appropriate targets for optogenetic modulation. These include endothelial cells for vasoconstriction, pancreatic β cells for glucose homeostasis, glioma tumor cells for elucidating therapeutic potential against cancer, skeletal muscle cells for direct motor control, smooth muscle cells in erectile tissue and organ vasculature for direct effector control, and cardiomyocytes for pacing of the heart.

fiber-optic tip of a cannula is inserted into the brain, and then the cannula is cemented to the skull. Such an approach has proven to be modular and can be readily adapted to target virtually any brain structure (99–101). However, it is unlikely that such a globally applicable system can be developed for the spinal cord and periphery, which exhibit great variance in anatomy and structure that depends on the ganglion, plexus, nerve, organ, or portion of the spinal cord being illuminated.

Not surprisingly, a broad range of light delivery strategies have been used in the spinal cord and periphery (Fig. 2). These range from fiber-optic nerve cuff implants (4) to epidural fiber-optic implants (55) to complex surgical interventions that fuse spinal vertebrae and implant glass windows over the spinal cord (10). Recent advances in wireless power transfer have enabled miniature, fully internal implants that can target light to spinal and peripheral structures in mice without the need for tethered optical fibers (48, 49). In the periphery, it has also been possible to achieve optogenetic control through noninvasive means such as transdermal illumination (Fig. 2). Transdermal illumination has proved surprisingly powerful in enabling control of peripheral sensory afferents (6, 7, 11–14, 47) and smooth muscle (15), allowing for activation and inhibition of these structures in freely moving untethered mice and rats. Despite the diversity of strategies that have been used, it is possible to propose principles that guide the development of light delivery systems for the spinal cord and periphery and to propose designs for future applications.

Superficial stimulation to achieve deep control
A critical difference between optogenetic control of the brain and of the spinal cord and periphery lies in the type of stimulation that is possible. Optical fibers are often extended deep into brain tissue to illuminate cortical and subcortical structures, with minimal reported changes in animal behavior occurring as a result of the tissue death caused by the optical fiber track. This may derive from redundancy in central nervous system circuits, although it may also be due to the lack of sensitivity of behavioral measures. Unfortunately, it appears unlikely that these sorts of penetrating light delivery systems can be used in the spinal cord and periphery. For example, the likely path taken by a penetrating optical fiber in the spinal cord will require the severing of white matter tracts, which carry high information density and have minimal redundancy and where local damage can have global consequences.

As a result, light delivery systems in the spinal cord and periphery are typically superficial, wrapping around a nerve, or are placed immediately dorsal to the spinal cord. Improvements in opsin design to render opsins more photosensitive (102) and also sensitive to far-red colors of light (103, 104) will be critical to enable optogenetic control of deep structures without the use of penetrating devices, particularly in larger organisms (Fig. 2D). Internal light-generating systems (105–107), for example, luciferase/luciferin, can achieve opsin activation for optogenetic control without the need for exogenous devices and may be well suited for neuromodulation of deep structures over long time scales.

Constraints imposed by relative motion
Neural structures outside the brain exhibit significant relative motion during movement of the organism. This limits the amount of mechanical coupling that is possible between the implanted light source and the underlying neural structure. For example, the sciatic nerve, a common target for treating peripheral pain, undergoes major displacement during normal animal movement. Silica fiber-optic nerve cuff implants, which typically have limited flexibility, can constrain the natural movement of the nerve and so exert marked force on the nerve. In our experience, careful modulation of the degree of "give" in an optical fiber–coupled nerve cuff is essential to allow for in vivo light delivery without inducing nerve injury (4). The spinal cord also undergoes meaningful displacement relative to the vertebral column during animal movement, which can result in shear-induced damage in penetrating light delivery systems.

Similar constraints exist for delivery of light to other deep neural structures, such as the enteric nervous system or vagus nerve. The miniaturization and added flexibility of a new generation of optogenetic devices (16, 108, 109) as well as the development of wireless optogenetic systems for the brain (109–112) and beyond (48, 49) should alleviate concerns about implant-induced tissue damage (Fig. 2).

The risks and rewards of transdermal illumination
Transdermal illumination has proven to be a powerful tool for controlling primary afferent neurons (6, 7, 9, 11, 12, 14). However, as a light delivery method, it is not without its limitations. A major challenge for extensive use of transdermal illumination in somatosensory and pain research is to develop tools to enable this modality to be used in concert with traditional pain and sensation assays, which frequently require concurrent access to the paw (for example, von Frey assays) or are conducted in a dimly lit environment (for example, place preference assays). Because transdermally delivered light is visible to the animal, care must be taken with adequate habituation (and controls) to ensure that the animal is not merely responding to the light or developing an unwanted association between illumination and pain-related stimuli,

which can occur through repeated testing. Transdermally evoked optogenetic responses have now been observed in both rats (7, 11) and mice (6, 9, 13–15, 27), but there are no published reports that this approach has been extended to nonhuman primates. There are reports that nociceptors in nonhuman primates exist at depths (200 μm) (113) that are sufficiently superficial to enable transdermal illumination (penetration depth of 500 μm), even after accounting for scattering and absorption (114).

Challenges of illumination location and timing

Many neurons in the spinal cord and peripheral nervous system project over large distances or are specialized to form sensory receptors. Optogenetic control (and in particular inhibition) of fibers of passage or projections has been challenging (115, 116). A further complication is that the same light stimulus can produce different effects on neurons depending on the site and duration of illumination (117). Recent experiments have demonstrated that the same illumination can produce different action potential patterns depending on whether it is delivered at a distal sensory ending or at a more proximal location (18). This may result in variations in the observed behavioral responses resulting from activation or inhibition of the same neural population.

Another fundamental consideration is that optogenetic stimulation, like electrical stimulation, results in an intrinsically artificial pattern of activation that does not necessarily mimic complex physiological stimulus–evoked neural activity (118). Although this is a commonly discussed criticism of optogenetics in general (119), it may be particularly relevant to the study of sensory processing, where a typical experiment involves studying the behavioral (6, 7, 11–14, 18, 48) or downstream electrophysiological (22) effects of activating or inhibiting a specific subpopulation of sensory neurons. "Single-channel" optogenetic stimulation of a given neural population does not recapitulate the combinatorial nature of physiological somatosensory activation. This is especially important in the study of pain where it is known that crosstalk in the spinal cord, as a result of simultaneously applied yet distinct stimuli, affects pain processing. Systematic exploration of how optogenetic stimulation parameters affect sensory perception will be necessary to enable the creation of optogenetic sensory neuroprosthetics (120, 121).

The temporal constraints on optogenetic control are also worth stressing. High-frequency optogenetic stimulation of channelrhodopsin-2 (122), particularly in cold temperatures (20), can result in a depolarization block at light frequencies as low as 10 Hz. Heating induced by constant illumination, which is frequently used for optogenetic inhibition, is also a concern. The recent development of inhibitory channelrhodopsins with slower off-kinetics may alleviate this concern (115, 123) by enabling persistent inhibition of neural circuits in vivo through intermittent illumination.

OPTOGENETIC CONTROL OF SOMATOSENSATION AND PAIN

Difficulties in light delivery and opsin expression initially delayed the in vivo use of optogenetics in the somatosensory system (3, 124). However, researchers were still able to use optogenetics to perform technically complex in vitro experiments (22). Examples include in vitro slice electrophysiology experiments that explored the role of dorsal horn parvalbumin interneurons in GABAergic inhibition (29), and the con-

sequences of glycinergic interneuron ablation through recording changes in the characteristics of optogenetically evoked inhibitory synaptic transmission (21). In addition, some in vitro studies involved optical stimulation of central terminals of primary afferents (22, 28, 30) while recording from spinal neurons. These experiments have increased our knowledge regarding the connectivity and function of peripheral and spinal sensory neurons under both normal and pathological conditions.

A key advance toward in vivo control of somatosensation has been the realization that, in cases where opsin expression was sufficient, transdermal illumination could be used to modulate activity in primary sensory neurons. Initial experiments used transgenic rodent lines with high opsin expression to examine the results of activating putative proprioceptive mechanoreceptors (11) and nociceptors expressing $Na_V1.8$ channels (13). Using an alternative strategy, our group demonstrated that injection of viral constructs could specifically transduce nociceptors with stimulatory and inhibitory opsins, thus enabling bidirectional control over pain perception in mice (6). In subsequent work, many groups have adopted the transdermal illumination approach, using it to answer questions about many fiber types including the function of Aδ fibers (7), somatosensory representation of whisker follicle activation (31), the effect of inhibiting $Na_V1.8$-expressing nociceptors (54), and the effects of inactivating TRPV1 channels on pain sensation (27). In the future, transdermal illumination could also allow the manipulation of sensory end organs for behavioral studies in living animals; to date, optogenetic manipulation of these cells has been restricted to ex vivo preparations (26, 47).

Whereas the transdermal illumination approach has improved our understanding of primary afferent sensory neurons, control of somatosensory circuits within the dorsal horn of the spinal cord in vivo has been more difficult. Recent improvements in methods to deliver light to the spinal cord dorsal horn (48, 49) could enable a new genre of experiments in which researchers can determine the behavioral consequences of activation or inhibition of defined classes of dorsal horn neurons without the confounds introduced by genetic ablation or pharmacological intervention.

A question that will continue to affect interpretation of all somatosensory optogenetic excitation and inhibition experiments is whether a lack of optogenetically derived effects on behavior can be ascribed to a failure of opsin-mediated control (a false-negative result) or reflects the physiological role played by the neural population under investigation (a true-negative result). Disentangling the two can be challenging, particularly because the efficacy of optogenetic stimulation or inhibition can be driven by a number of factors, including the type of fibers transduced, the color and thickness of the skin (in the case of transdermal illumination), the percentage of relevant neurons expressing opsins, the level of opsin expression within each neuron, the wavelength and power of the light source, and, most importantly, the assay by which animal behavior is being evaluated. For example, researchers have found that optogenetic inhibition of primary afferent neurons expressing VGlut3 (vesicular glutamate transporter 3) affected behavioral responses only in a small subset of cases tested involving a variety of assays in many chronic pain rodent models (14). These results highlight the importance of using a varied repertoire of behavioral tests to assess the effects of an optogenetic intervention.

As with other experiments assessing subjective phenomena such as pain, translation of results in rodents to humans is challenging. Such translation may be aided by using optogenetics to modulate behavior

Fig. 4. Overview of studies using optogenetics beyond the brain. The schematic indicates studies using optogenetics to control mammalian excitable cells by delivering light to intact tissue outside of the brain. Each square represents one or more studies indicated by the reference numbers in parentheses at the top. The key explains the features of each study through the background color of the square. Also indicated in each square is the approximate location of light delivery, the approximate color of light used, and the name of the opsin or chemical employed. The top three rows represent studies completed in ex vivo samples of intact mammalian tissue, such as excised vascular smooth muscle (40). The second three rows represent studies completed in anesthetized mammals. The bottom three rows represent studies completed in awake mammals. If more than one type of experiment was used in a study, the study square is placed in the lowest row applicable. The first three columns represent studies in which transgenesis was used to confer light sensitivity. The second three columns represent studies that used more translationally relevant strategies such as viral transduction, cell transplant, and chemical photoswitches. The bottom three rows and right three columns represent studies that are the most translationally relevant including the demonstration of the optogenetic inhibition of pain (6), the optogenetic stimulation of motor neurons (4), and the optogenetic control of glucose homeostasis (9). Arch, archaerhodopsin; bPAC, a photoactivated adenylate cyclase derived from *Beggiatoa*; ChETA, engineered channelrhodopsin-2 mutants with fast-time kinetics; ChR2, channelrhodopsin-2; EROS, an erectile optogenetic stimulator; NpHR, halorhodopsin; QAQ, quaternary ammonium–azobenzene–quaternary ammonium.

in nonreflexive tests of pain (*125*). Recent advances in wireless opto-genetic techniques (*48, 49, 109, 126*) as well as integration of simultaneous tether-free delivery of pharmacological compounds (*126*) for control of neural structures will help make possible complex behavioral experiments in naturalistic rodent environments.

OPTOGENETIC CONTROL OF MOTOR CIRCUITS

A complex network of spinal interneurons and motor neurons play critical roles in regulating animal movement. To improve our ability to restore function after spinal cord injury or motor neuron disease as well as to enhance our understanding of how movement arises, it will be necessary to examine the role played by individual neural populations in guiding motor behavior. Some researchers have exploited the temporal and genetic specificity provided by optogenetics to begin this process (*8, 10, 19, 20, 23, 32–37, 81*).

The following studies use a mouse ex vivo spinal cord slice model to assess electrical activity in motor neuron axons or cell bodies as a proxy for locomotion. Researchers have used optogenetics to demonstrate the sufficiency and necessity of neurons expressing VGlut2 to initiate central pattern generator activity that resembles hindlimb locomotion (*32, 33*). In a related study, these investigators found that spinal cord excitatory interneurons expressing Shox2 were responsible for modulating locomotor frequency (*34*). Another group found surprising differences in the way that forelimb and hindlimb neural circuitry was reorganized during development (*35*). These ex vivo investigations highlight the utility of simplifying complex motor tasks to dissect precise mechanisms.

Some motor circuits, however, are only functional in intact, non-anesthetized animals and are better studied in this context. For example, researchers found that optogenetic activation of V2a neurons recruited cerebellar feedback and disrupted a reach motor task in awake mice (*81*). Because V2a neurons are found throughout the brain, the researchers were able to use well-characterized optogenetic tools for brain illumination to deliver light to the awake mouse. Optogenetically interrogating spinal motor circuits in awake mice has only recently become possible. The development of a new fiber-optic system to directly deliver light to the dorsal spinal cord enabled the investigation of the role of VGAT+ inhibitory interneurons in locomotion and led to the demonstration of a rostral bias in the organization of inhibitory signaling in mouse hindlimb circuits (*10*).

In an early example of the power of optogenetic approaches for studying the spinal cord, Alilain *et al.* (*36*) demonstrated how optogenetic stimulation may be able to therapeutically restore function to damaged spinal circuits. Here, researchers hemisected the spinal cords of rats, paralyzing half of the diaphragm. They then virally transduced spinal neurons ipsilateral and distal to the lesion so that they expressed channelrhodopsin-2. Optogenetic stimulation of the spinal neurons during surgery under anesthesia led to independent breathing by the paralyzed diaphragm for at least 24 hours after the cessation of optogenetic stimulation. Such prolonged effects may be indicative of neural plasticity and demonstrate how permanent opsin expression and implanted light sources may not be required for some types of optogenetic therapies.

Therapeutic modulation of lower motor neurons outside of the spinal cord is also possible. Compared to electrical stimulation, optogenetic stimulation of axons recruits motor units in an orderly physiological way, resulting in less muscle fatigue (*37*). In addition to transgenic (*19, 20, 37*) and viral strategies (*4*) to induce opsin expression in lower motor neurons, implantation of channelrhodopsin-2–expressing stem cell–derived motor neurons into the sciatic nerve space led to functional innervation of denervated muscle fibers, allowing for optogenetic stimulation of those cells to restore muscle activity (*30*).

NONNEURAL TARGETS

Researchers have begun to use optogenetics to study nonneural cells (Fig. 3). Direct optogenetic control of muscle cells has received the most attention. Like neurons, these cells endogenously exhibit temporally precise activation patterns. Direct optogenetic control of muscle cells could be useful in situations where optogenetic control of the efferent nerve is less practical. In vitro optogenetic activation of rodent skeletal (*127–129*) and cardiac muscle (*130*) has improved tissue engineering strategies and has provided insights into cellular function. Recent studies of optogenetic activation of skeletal muscle in vivo to control laryngeal muscle and reduce denervation atrophy are exciting demonstrations of the spatial specificity of optogenetics (*40, 51*). Additionally, researchers have used optogenetics to control erectile smooth muscle in awake rats (*15*) and in ex vivo preparations of the peripheral vasculature in mice (*41*).

Control of cardiac muscle in vivo has also recently been demonstrated. Initial studies used optogenetics to pace the heart in a transgenic mouse line expressing channelrhodopsin-2 in cardiomyocytes (*38*). Researchers have also induced expression of opsin in mouse cardiomyocytes through systemic viral gene delivery (*39*), enabling optogenetic pacing of mouse heart. Other studies have exploited the spatial power that optogenetics provides to demonstrate multisite cardiac pacing and cardiac resynchronization therapy in vivo (*46*) and to pinpoint regions of the heart susceptible to the formation of extrasystoles (*53*). Other applications of optogenetics in cardiomyocytes have been reviewed elsewhere (*131, 132*).

Preliminary reports have also described how optogenetics may be used to study cancer (*5, 133*), fertilization (*42*), insulin secretion (*9, 43, 44*), endothelial regulation of vasoconstriction (*50*), and immune function (*134*), opening the door to a diverse host of targets for manipulation (Fig. 3).

THERAPEUTIC PROMISE

The pace of discovery in the spinal cord and periphery enabled by optogenetic techniques is accelerating (fig. S1), perhaps, in part, because some of the key technical challenges have been addressed (Fig. 4). Many therapeutically relevant neural and nonneural structures are likely to first be optogenetically controlled in preclinical models, and some, eventually, will be transferred to the clinic (Fig. 3). Such targets include the enteric nervous system, which remains understudied; endocrine regulation, which is particularly suited to optogenetic intervention because of its temporal variation; the sympathetic control of various organs and adipose tissue, an area in which preliminary work has begun (*45, 52*); and the optogenetic dissection of neuroinflammation, through the control of the vagus nerve (*135*).

Optogenetic control of the motor system is beginning to enable real discovery and has therapeutic potential. In the spinal cord, opsin-expressing stem cells and optically induced neural plasticity (*36*) are

Table 1. Selected studies of translational optogenetics.

Target	Key translational findings and innovations
Diabetes	Melanopsin-mediated transcription to optically control glucose tolerance in a mouse model of type 2 diabetes (9)
	Optogenetic control of insulin release through transplantation of a mouse β cell line transfected with ChR2 (44)
Obesity	Direct neural control of lipolysis and fat mass reduction using optogenetic activation of sympathetic nerve fibers (52)
Cancer	ChETA-mediated membrane depolarization leading to selective apoptosis of transduced glioma cells and increased survival (5)
Skeletal muscle	Optogenetic control of contraction in mammalian skeletal laryngeal muscle through muscle transduction (40)
	Attenuated atrophy of denervated skeletal muscle through optogenetic stimulation of muscle cells (51)
Cardiovascular dysfunction	ChR2 used for optogenetic control of heart muscle in vivo (38)
	Systemic gene delivery of ChR2 enabled optogenetic pacing of mouse hearts (39)
	Multisite optogenetic control of cardiac resynchronization (46)
Motor disorders	Rescue of patterned breathing after spinal cord hemisection (36)
	Optogenetic control of peripheral motor neurons reduced muscle fatigue (37)
	Optogenetic inhibition of peripheral motor neurons (19)
	Restoration of muscle function in a denervated mouse model through embryonic stem cell–derived optically sensitive motor neuron engraftment (8)
Pain	Optogenetic stimulation of pain-related behavior using a $Na_v1.8$-ChR2 transgenic mouse (13)
	Viral vector delivery to express opsins in nociceptors enabled optogenetic stimulation and suppression of pain (6)
	Increased mechanical thresholds in rats with optogenetic inhibition of fast-conducting high-threshold mechanoreceptors (7)
	A TRPV1 promoter driving expression of ArchT in nociceptors enabled optogenetic inhibition of pain (27)
New devices	Optogenetic modulation of the spinal cord in freely moving mice (10)
	Implantable light delivery system with targeted viral transduction of peripheral motor neurons controlled muscle activity in walking rats (4)
	Fully internal wireless device for optogenetic control of the spinal cord and periphery (48)
	Implantable, flexible wireless optogenetic system for control of spinal and peripheral neural circuits (49)

likely early candidates for therapeutic modulation by optogenetics. In the peripheral nervous system, because muscle-specific optogenetic control has now been demonstrated, many translationally relevant experiments are possible. For example, the combinatorial capabilities that spectrally separated opsins (102) may potentially be exploited to control agonist-antagonist muscle pairs in a temporally coordinated closed-loop manner to animate limbs in animal models of paralysis or denervation. A major advance in the field that may now be possible is temporally precise inhibition of lower motor neurons with the goal of treating disorders such as bladder dysfunction or spasticity. Such a feat has eluded electrical stimulation in the clinic, although extended neuromuscular stimulation has been used to improve overall spasticity with modest results (136). Direct optogenetic inhibition of lower motor neurons has been demonstrated in transgenic mice (19), but virally mediated inhibition of motor neurons has not yet been accomplished.

In the somatosensory system, the future of optogenetic control also looks bright. Well-established techniques now exist for bidirectional transdermal control of primary afferent nerves in rodent models. If these methods can be extended to larger animals, this may allow for noninvasive light delivery to achieve optogenetic suppression of neural activity, potentially enabling implant-free optogenetic control of chronic pain disorders. The expansion of optogenetics to nonanesthetized rodents for the control of spinal neurons (48) (or afferent nerves that cannot be accessed by transdermal light delivery) represents an important avenue for future work. Analogous to clinically deployed electrical spinal cord stimulators, systems for optogenetic control of spinal circuits could be used to treat a range of pain disorders, with fewer off-target effects.

In this Review, we have focused on approaches that use light to control neural activity. From a translational perspective, the development of magnetic (137), acoustic (138), or thermal stimuli for neuromodulation through the use of genetically encoded effectors may have particular advantages, potentially allowing for control of deep neural structures without the use of light-emitting implants while retaining temporal specificity. Similarly, in applications where temporal specificity is not important, such as long-term inhibition of neural structures, optogenetic approaches may be most effective when combined with internal light-generating systems (105, 106). The complementary DREADD (designer receptors exclusively activated by designer drugs) technology that uses chemical ligand–activated synthetic G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptors may also be an alternative that avoids the challenges of light delivery (139–141).

The 10-year anniversary of the use of channelrhodopsin-2 in mammalian neurons has prompted much discussion about the establishment of optogenetics as a powerful scientific tool in neuroscience and its future translational potential (56, 142, 143). Over the next decade, we anticipate clinical trials of therapies that harness the specificity and temporal precision of optogenetics. A human clinical trial has recently begun to examine whether channelrhodopsin-2 can be used to restore light sensitivity in the degenerative eye disease retinitis pigmentosa (144, 145). Whether optogenetics is embraced as a clinical therapy will depend on the success of these early trials.

A common critical step is the importance of the continued development of nonhuman primate models to demonstrate safety and efficacy of opsin targeting, expression, and light delivery. Although optogenetics to modulate neurons in the brains of nonhuman primates has been demonstrated by a few groups (*70, 146–152*), such studies have not yet been extended beyond the brain. Some of these groups have described opsin expression in nonhuman primates as being substantially more difficult to achieve than in rodents (*70*). It is likely, therefore, that optogenetic control of the nonhuman primate peripheral nervous system and spinal cord will also prove challenging. However, with the increasing availability of transgenic marmoset models (*153, 154*), and research efforts toward modifying AAVs for human use (*155*), it is likely that these difficulties will be overcome. Although not strictly necessary as a precursor to a clinical trial, the development of nonhuman primates as a preclinical model for optogenetic therapies will be instrumental in the demonstration of safety and efficacy for many of the therapeutic interventions we discuss here. The critical challenges we have identified—sustained opsin expression, a controllable immune response, and efficient light delivery—are all tractable and, with sustained effort, can be overcome.

We have summarized both the current applications and the future promise of optogenetics to modulate the activity of excitable cells beyond the brain. These techniques are likely to allow genetically specified, temporally precise control of many combinations of diverse cell types within the spinal cord and peripheral nervous system. Along with their potential for direct therapeutic application, these techniques are also likely to improve development of new ways to treat human disease as they continue to enable a deeper understanding of human physiology and pathology.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/8/337/337rv5/DC1
Fig. S1. Increase in the number of publications since the initial development of optogenetics.

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Abstracts

One-sentence summary: This Review discusses the use of optogenetics in the spinal cord and peripheral nervous system, distills the key results achieved thus far, and discusses the translational potential.

Editor's Summary:

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