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Optogenetic approaches for neural tissue regeneration: A review of basic optogenetic principles and target cells for therapy

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From the Contents

Abstract

Optogenetics has revolutionized the field of neuroscience by enabling precise control of neural activity through light-sensitive proteins known as opsins. This review article discusses the fundamental principles of optogenetics, including the activation of both excitatory and inhibitory opsins, as well as the development of optogenetic models that utilize recombinant viral vectors. A considerable portion of the article addresses the limitations of optogenetic tools and explores strategies to overcome these challenges. These strategies include the use of adeno-associated viruses, cell-specific promoters, modified opsins, and methodologies such as bioluminescent optogenetics. The application of viral recombinant vectors, particularly adeno-associated viruses, is emerging as a promising avenue for clinical use in delivering opsins to target cells. This trend indicates the potential for creating tools that offer greater flexibility and accuracy in opsin delivery. The adaptations of these viral vectors provide advantages in optogenetic studies by allowing for the restricted expression of opsins through cell-specific promoters and various viral serotypes. The article also examines different cellular targets for optogenetics, including neurons, astrocytes, microglia, and Schwann cells. Utilizing specific promoters for opsin expression in these cells is essential for achieving precise and efficient stimulation. Research has demonstrated that optogenetic stimulation of both neurons and glial cells—particularly the distinct phenotypes of microglia, astrocytes, and Schwann cells—can have therapeutic effects in neurological diseases. Glial cells are increasingly recognized as important targets for the treatment of these disorders. Furthermore, the article emphasizes the emerging field of bioluminescent optogenetics, which combines optogenetic principles with bioluminescent proteins to visualize and manipulate neural activity in real time. By integrating molecular genetics techniques with bioluminescence, researchers have developed methods to monitor neuronal activity efficiently and less invasively, enhancing our understanding of central nervous system function and the mechanisms of plasticity in neurological disorders beyond traditional neurobiological methods. Evidence has shown that optogenetic modulation can enhance motor axon regeneration, achieve complete sensory reinnervation, and accelerate the recovery of neuromuscular function. This approach also induces complex patterns of coordinated motor neuron activity and promotes neural reorganization. Optogenetic approaches hold immense potential for therapeutic interventions in the central nervous system. They enable precise control of neural circuits and may offer new treatments for neurological disorders, particularly spinal cord injuries, peripheral nerve injuries, and other neurodegenerative diseases.

Key Words: adeno-associated virus; astrocytes; bioluminescent optogenetics; channelrhodopsins; halorhodopsins; microglia; neural stem cells; neurons; oligodendrocyte; optogenetics

Introduction

Optogenetics is considered an innovative approach capable of revolutionizing various scientific disciplines, including neuroscience, behavioral research, and genetics. Researchers are exploring new directions to enhance the efficiency and accuracy of neural activity control (Rajalingham et al., 2021; Rost et al., 2022; Spreen et al., 2024) by applying a range of concepts that expand the boundaries of this methodology. Development of new, faster, or more sensitive opsins is a key factor in improving the spatial and temporal resolution of optogenetics. Researchers are creating chimeric or genetically modified opsins with enhanced properties, such as a broader spectral range, greater conductivity, and faster deactivation speeds. These advancements allow for the study of neuronal activity at shorter or longer time intervals and in response to different wavelengths of light (Kralik et al., 2022; Tichy et al., 2022; Geng et al., 2023).

As more specific promoters and genetic constructs are developed, optogenetic methods can be improved for the targeted expression of opsins in various cell types, subcellular compartments, and neuronal circuits (Figueiredo et al., 2011; Yizhar et al., 2018). Selectivity is crucial for understanding the roles of different neurons in complex circuits and identifying ways for their mutual modulation. Optogenetic tools are not limited to the activation and inhibition of neurons. Researchers are developing optogenetic methods to manipulate various cellular processes, including intracellular signaling pathways (Zhang et al., 2015; Kwon et al., 2020; Huang et al., 2022), synaptic plasticity (Xie et al., 2012; Rost et al., 2022; Surdin et al., 2023), and gene expression (Milias-Argeitis et al., 2016; Arancibia et al., 2023; Qian et al., 2023).

The knowledge gained from the aforementioned studies can lead to the development of new treatment methods, such as optogenetic deep brain stimulation for Parkinson's disease, targeted illumination for suppressing epileptic activity, restoration of motor function following spinal cord injury (SCI) through the reactivation of spinal circuits below the site of injury, and enhancement of neuroplasticity (Ikefuama et al., 2022; Ledri et al., 2023; Mondello et al., 2023).

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Since optogenetics continues to be considered a promising tool in the field of neuroscience, constant efforts are being made to expand its applications, improve the technology, and harness its potential to unravel the mysteries of the brain and revolutionize our understanding of the nervous system. Accordingly, this review aims to provide a comprehensive overview of optogenetics in neurobiology, outlining the underlying principles, the models used in fundamental research, and the biomedical fields where this revolutionary technology finds application. It emphasizes cell-specific promoters in modulating the activity of specific cells in the nervous system and explores new technologies, such as bioluminescent optogenetics, for the treatment of SCI and other neurological diseases.

Search Strategy

An electronic search of the Google Scholar database, which includes developments of light-sensitive opsins from 1980 to 2024, was conducted using the following terms: "Optogenetics" (MeSH terms) AND "Channelrhodopsins" (MeSH terms) OR "Halorhodopsins" (MeSH terms) OR "Archaerhodopsins" (MeSH terms). Inclusion criteria consisted of peer-reviewed review and experimental articles published in English, while exclusion criteria included non-reviewed articles, reviews, commentaries, or editorials with limited data. Additionally, an electronic search of the Google Scholar database was conducted to identify the most used delivery vectors for light-sensitive opsins and to analyze advancements in this field of optogenetics.

Publications up to and including 2024 were included, and the following keywords were used: "Optogenetics" (MeSH terms) AND "Adeno-associated virus" AND "Peripheral Nerve Disease" (MeSH terms) OR "Spinal Cord Injuries" (MeSH terms). Next, the Google Scholar database was searched for the latest developments in optogenetics related to bioluminescent optogenetics in the context of therapy for Peripheral Nerve Disease (MeSH terms) OR Spinal Cord Injuries (MeSH terms). Finally, publications on the use of cell-specific promoters for various nervous system cell types in both optogenetics applications and neurobiological practice were analyzed using the following keywords: "Optogenetics" (MeSH terms) AND "Microglia" (MeSH terms) AND "Neurons" (MeSH terms) OR "Neural Stem Cells" (MeSH terms) OR "Oligodendrocytes" (MeSH terms) OR "Schwann cells" (MeSH terms), as well as "cell promoters" AND "Microglia" (MeSH terms) OR "Neurons" (MeSH terms) OR "Neural Stem Cells" (MeSH terms) OR "Oligodendrocytes" (MeSH terms) OR "Schwann cells" (MeSH terms).

Data were extracted by summarizing the key findings from each selected article concerning the following aspects: 1) Basic principles of optogenetics. 2) Specific optogenetic techniques used. 3) Various target cells for therapy and their distinct roles in neuronal regeneration. 4) The results, efficacy, and limitations of optogenetic interventions in animal models, along with potential applications in clinical practice.

Evolution of Excitatory Opsins to Modulate Neuronal Activity

The primary goal of optogenetics is to enhance the precision and control of cellular activity. This technology is based on proteins known as opsins, which are a diverse group of light-sensitive proteins found throughout the evolutionary spectrum. There are two types of opsins: (1) excitatory opsins, which include cation channel rhodopsins, and (2) inhibitory opsins, comprising chloride pump halorhodopsins, proton pump archaerhodopsins, and chloride channel rhodopsins. By manipulating the influx or efflux of specific ions, these two types of opsins can modulate the membrane potential in different ways, thereby controlling the excitability of neurons.

Excitatory opsins, known as channelrhodopsins (ChRs), are cation channel opsins that, upon light stimulation, induce the influx of positively charged ions such as Na^+, K^+, and Ca^{2+} (**Figure 1**). This influx results in the depolarization of the cell membrane and subsequent cell activation. Two of the most used ChRs in optogenetics are ChR1 and ChR2, which are derived from the green algae *Chlamydomonas reinhardtii* (Berndt et al., 2009; Lin et al., 2009, 2011; Deisseroth et al., 2017).

Some of the earliest experiments investigating ChRs were conducted by Nagel et al. (2002). In their groundbreaking study, the researchers identified ChR1 as a light-regulated proton channel in green algae. They discovered that ChR1 exhibited light-dependent conductance, paving the way for future optogenetic applications. Several studies focused on the use of opsins in fundamental research were pivotal in demonstrating the potential of optogenetic control using channelrhodopsins in neurons (Boyden et al., 2005; Li et al., 2005). Boyden et al. (2005) utilized ChR2 to demonstrate a method for noninvasive, genetically targeted, and temporally precise control of neuronal activity. By illuminating ChR2-positive neurons with blue light, they induced rapid depolarizing currents, achieving a maximal rise rate of (160 ± 111) pA/ms within (2.3 ± 1.1) ms after the onset of the light pulse. Furthermore, prolonged light exposure resulted in the rapid recovery of peak ChR2 photocurrents in neurons and the generation of a single spike with reliable timing, followed by irregular spikes. Thus, the technology developed for controlling neuronal firing and both excitatory and inhibitory synaptic transmission, as described by Boyden et al. (2005), represents a widely applicable tool in neurobiology. Li et al. (2005) conducted a similar study, demonstrating the potential of using ChR2 for noninvasive activation and inhibition of neurons and neuronal networks. They showed that ChR2 functions somato-dendritically to depolarize neurons and elicit action potential firing. Together with Boyden's research, this study contributed significantly to establishing the utility of channelrhodopsins in optogenetics.

Scientists have developed numerous variants of ChR2 with modified kinetic and spectral properties, enhancing membrane transport activity and expression in mammalian cells, while providing a more stable spectral response. These advancements contribute to improved optogenetic control. Specifically, red-shifted ChRs, which respond to longer wavelengths of light, have been created to enable deeper tissue penetration and reduce phototoxicity, both of which are crucial for *in vivo* applications. Red light is less scattered by tissues and less absorbed by blood compared to the blue and green wavelengths required for other variants. Red-shifted excitatory opsin variants, such as Chronos and Chrimson (Klapoetke et al., 2014), oChIEF (Hass et al., 2016), ChRmine (Chen et al., 2021), and ReaChR (Lin et al., 2013), are commonly used in biomedical research. Additionally, the Na^+-translocating channelrhodopsin ChETA-ChR2 with E123T mutations (Gunaydin et al., 2010) and the Ca^{2+}-translocating channelrhodopsin CatCh (Kleinlogel et al., 2011; Majumder et al., 2018) have effectively replaced standard cation channel opsins (Geng et al., 2023).

The separate class of high-sensitivity step-function opsins (SFOs) represents reengineered ChRs resulting from modifications at position C128. These opsins exhibit bistable light responses, converting short light pulses into stable membrane potential steps (Berndt et al., 2009). SFOs are genetically engineered proteins that, in response to light stimulation, maintain a prolonged open state, allowing for cell activation over an extended period. Recently developed new step-function opsins with ultra-high light sensitivity (SOUL), based on SFO (ChR2 C128S/D156A) and incorporating T159C mutations, have demonstrated extremely high light sensitivity. These opsins can undergo photoactivation even under conditions of significantly attenuated light power (Gong et al., 2020; Lewis et al., 2020; Sun et al., 2022).

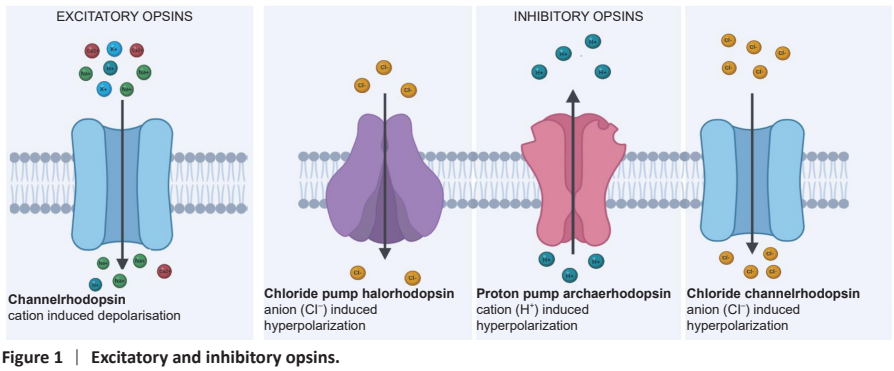

Figure 1 | **Excitatory and inhibitory opsins.**
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Genetically modified opsins, such as SFOs and SOULs, are actively used in biomedicine and offer several advantages. First, they require reduced light exposure in the form of short light pulses to maintain opsin activation (or inhibition) for a longer duration compared to traditional opsins like ChR2. This reduced light exposure minimizes phototoxicity and potential cell damage, making SFOs and SOULs more suitable for *in vivo* applications (Mattis et al., 2011; Zhang et al., 2022; Zhou et al., 2023). Second, SFOs can induce stable depolarization or hyperpolarization of neurons, while SOULs exhibit an ultra-long open-state lifetime, allowing for the study of processes that require prolonged cell activation or inhibition. Just a few seconds of low-level light stimulation are sufficient to maintain neuronal activity for 10–30 minutes. This property is particularly valuable for studying and manipulating neural activity over extended periods, which is crucial for understanding chronic or adaptive processes in the nervous system (Gong et al., 2020; Lewis et al., 2020; Zhou et al., 2023). Third, the reduced light intensity required for the activation of step-function opsins decreases the likelihood of photobleaching (Gong et al., 2020), which occurs due to prolonged light exposure on opsins (Pashaie et al., 2012; Dempsey et al., 2016). This enhancement increases the reliability and duration of optogenetic manipulations. Fourth, the increased energy efficiency and minimally invasive nature of optogenetic stimulation achieved through the short light pulses required for SFOs and SOULs lead to reduced energy costs in light delivery, making these opsins more energy-efficient compared to other optogenetic tools. Successful photo-stimulation of cells expressing ChR2 requires at least 5 mW/mm^2 (Zhang et al., 2006), while cells with SOUL and SFO only require approximately 5 $\mu W/mm^2$ (Gong et al., 2020). Additionally, SOUL can activate deep brain layers in animals through transcranial illumination, significantly reducing the risks associated with invasive interventions (Zhang et al., 2022; Geng et al., 2023; Zhou et al., 2023).

Despite the advantages, SFOs and SOULs have certain limitations, including reduced overall photocurrent magnitude and slower channel kinetics. This issue can be addressed by co-expressing a fast channelrhodopsin with SFO, which results in a more stable photocurrent that can overcome spike failures (Bansal et al., 2022). Nevertheless, SFO and SOUL opsins have significantly expanded the optogenetic toolkit, opening new opportunities for studying and manipulating neural processes with increased precision over extended periods.

Evolution of Inhibitory Opsins to Modulate Neuronal Activity

Halorhodopsins (NpHR) and archaerhodopsins (Arch) are two major types of inhibitory opsins used in optogenetics. Upon light activation, these proteins facilitate the passage of negatively charged ions, such as Cl^- and H^+, respectively, resulting in hyperpolarization and inhibition of cellular activity.

NpHR, derived from the archaea *Natronomonas pharaonis*, is a chloride pump activated by yellow or green light through transient chloride ions (Cl^-) translocation from the extracellular environment into the cell, leading to hyperpolarization of the cell membrane and subsequent suppression of cellular activity (**Figure 2**).

Genetic modifications of NpHR and its variants have been conducted to optimize them for optogenetic experiments, resulting in improvements in properties such as efficiency, kinetics, and expression in mammalian cells. Numerous variants of NpHR have been developed, including enhanced NpHR (eNpHR), eNpHR2.0, and eNpHR3.0 (Gradinaru et al., 2010), which improve ion transport across the membrane and increase efficiency. eNpHR, an early variant of NpHR, exhibits higher levels of membrane transport and expression, demonstrating superior membrane localization in mammalian cells, making it more effective for neuronal inhibition. eNpHR2.0 and eNpHR3.0 have significantly increased photocurrents, with up to ~20 times greater efficiency compared to NpHR at an intensity of 3.5 mW/mm^2 of yellow light, which is orders of magnitude lower than what is required by proton pumps. eNpHR3.0 photocurrents are also step-like, resistant to inactivation, and highly stable over multiple light pulses and long timescales (Gradinaru et al., 2010). However, the limited penetration depth due to the maximum activation wavelength (~580 nm) reduces the efficiency of these opsins. One important feature of NpHR is its spectral compatibility with ChR2: these two opsins have largely separable action spectra and operate with the same requirements for light power density, enabling bidirectional control of neuronal activity. For activating neurons located deep within the brain, alternative NpHR variants, such as NpHR Jaws, with a red-shifted activation wavelength of approximately 632 nm are actively used. This red shift facilitates deeper tissue penetration due to the longer activation wavelength, allowing for the manipulation of neuronal circuits in previously inaccessible regions.

Arch proteins obtained from the archaeon *Halorubrum sodomense* are light-activated proton pumps. Like NpHR, the light-induced activation of Arch leads to hyperpolarization of the cell membrane, allowing for nearly 100% silencing of neurons across significant brain volumes, along with spontaneous recovery from light-dependent inactivation (Chow et al., 2010). Upon absorption of green light (~520–560 nm), Arch undergoes a photocycle in which protons are transferred from the intracellular environment to the extracellular space. This process involves several discrete intermediate steps (**Figure 3**), like the activation of microbial rhodopsins (**Figure 2**). However, the proton transfer out of the cell generates a proton motive force that can be utilized for adenosine triphosphate synthesis via ATP synthase, providing the cell with energy. It is possible to use this protein as a potential-dependent indicator. When Arch is illuminated with orange or red light, it can produce fluorescence with a maximum emission wavelength of 710 nm. This fluorescence is sensitive to membrane voltage; under excitation at 640 nm, fluorescence increased twofold from –150 to +150 mV. However, using Arch as a voltage indicator is associated with several challenges. On one hand, the fluorescence is very dim, requiring intense laser illumination to be detectable; on the other hand, the illumination of Arch slightly perturbs the membrane potential (Maclaurin et al., 2013). This observation highlights the need for modifications to Arch and the development of functionally optimized neuronal activity inhibitors.

Among the known variants of archaerhodopsin, archaerhodopsin-T (ArchT or Arch Tetramer) stands out for its higher photosensitivity, photocurrents, and expression levels (All et al., 2019). Additionally, Arch (D95E/T99C) - Arch (DETC) and Arch (DETC+A225M) exhibit greater red shifts in wavelength compared to wild-type Arch, along with approximately a five-fold increase in fluorescence (McIsaac et al., 2014). All the investigated Arch mutants developed in the McIsaac laboratory (McIsaac et al., 2014), including Arch-5 and Arch-7, demonstrated improved fluorescence in living cells and a substantially enhanced quantum yield, with Arch-7 displaying the highest quantum yield, extinction coefficient, and, therefore, absolute brightness among all tested Arch variants.

Chloride-conducting ChloCs are potentially interesting opsins for researchers (**Figure 1**). ChloCs are a class of light-sensitive ion channels created artificially through the mutagenesis of cation-conducting ChRs. The main difference is that ChloCs are designed to conduct anions, predominantly Cl^-, instead of cations such as Na^+, K^+, and Ca^{2+}, which are characteristic of ChRs. The replacement of E90 in the central domain of ChRs with positively charged residues leads to the formation of ChloCs with minimal cationic conductivity. Molecular dynamics simulations have shown the formation of a high-affinity Cl^- binding site near the gate (Wietek et al., 2014). Another approach for converting ChRs involves the structurally oriented transformation of the chimeric ChR C1C2 into a nine-fold mutated variant, iC1C2, which is more specific for transporting Cl^- ions, providing physiological inhibition without the need for membrane potential elevation (Berndt et al., 2014). Further alterations of iC1C2 resulted in the development of SwiChR, which exhibited a 25-fold increase in light sensitivity compared to iC1C2 and a 200-fold increase compared to the inhibitor NpHR (Berndt et al., 2014). Further study of optogenetic tools has led to the discovery of natural anion-conducting ChRs from the cryptophyte alga Guillardia theta (GtACR1/2) and Proteomonas sulcata (PsACR1). Anion-conducting ChRs (ACRs) are fundamentally different from cation ChRs and have been evolutionarily optimized for stringent selectivity. ACRs conduct anions while completely excluding protons and larger cations, and they hyperpolarize the membranes of cultured animal cells with much faster kinetics at light intensities below 10^{-3} mW, which is necessary for the most efficient optogenetic proteins currently available. Natural ACRs, including GtACR1, GtACR2, and PsACR1, provide optogenetic inhibition tools with unprecedented light sensitivity and temporal precision compared to Arch or ChloCs (Govorunova et al., 2015; Wietek et al., 2016).

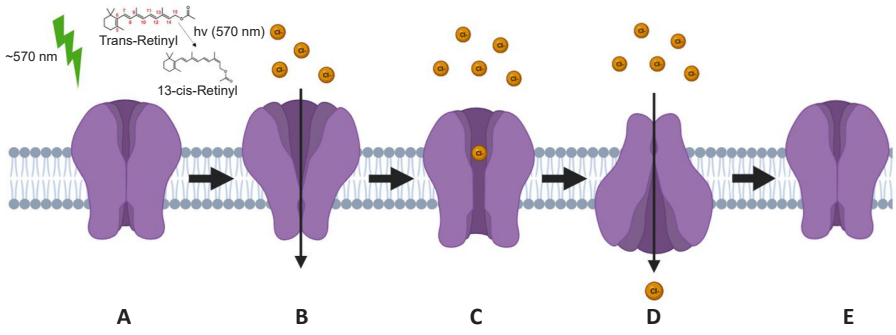

Figure 2 | **Activation of halorhodopsin involves the transient transport of chloride ions (Cl^-).**
NpHR is a chloride pump driven by yellow or green light (~570 nm) through the transient transfer of chloride ions (Cl^-) from the extracellular environment into the cell, resulting in hyperpolarization of the cell membrane and subsequent inhibition of cellular activity. (A) Initial light absorption by retinal leads to its isomerization from a fully trans- to a 13-cis configuration, causing a series of conformational changes in the protein that open the chloride-ion binding site, making it accessible from the extracellular side. (B) The chloride ion binds to halorhodopsin, and subsequent conformational changes in the protein close the binding site from the extracellular environment. (C) A second series of conformational changes opens a pathway for the chloride ion to move through the protein and eventually release it into the cell cytoplasm. (D) The release of the chloride ion into the cell allows retinal to revert to its fully trans configuration, returning the protein to its original state. (E) These sequential events lead to hyperpolarization of the cell membrane, inhibiting cellular activity by increasing the membrane potential. The inhibitory mechanism of halorhodopsins makes them valuable for use in optogenetic systems to silence or modulate neuronal excitation. Created with BioRender.com.

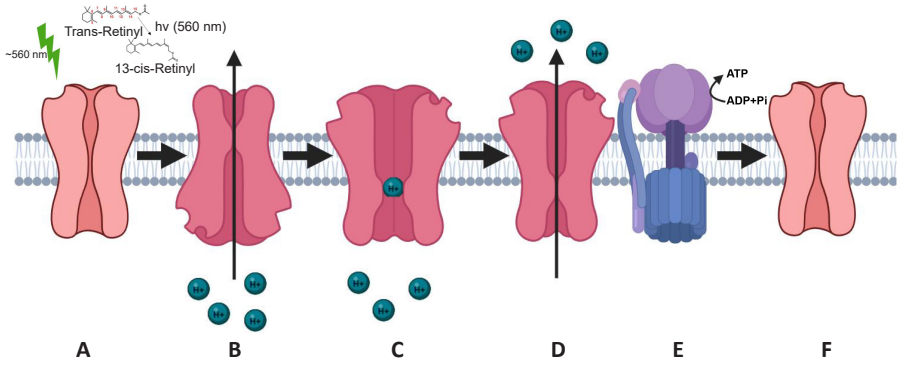

Figure 3 | **Archaerhodopsin activation with transient proton (H^+) transport.**
Upon absorption of green light (~520–560 nm), Arch protein initiates a photocycle during which a proton is translocated from the intracellular milieu to the extracellular space. This process undergoes a series of discrete intermediate stages. (A) The absorption of a photon by the retinal molecule results in its isomerization from an all-trans configuration to a 13-cis configuration. (B) The isomerization prompts conformational changes within the protein, revealing a proton-binding site accessible from the cytoplasmic side. (C) Proton binding to the protein induces further conformational changes, sequestering the bound proton from the intracellular environment. (D) Additional conformational changes open an extracellular pathway, allowing for the proton's release from the protein, culminating in its extrusion into the extracellular space. (E) By expelling protons from the cell, Arch establishes a proton gradient that can be harnessed for ATP synthesis via ATP synthase, thus providing the cell with energy. (F) Retinal reverts to its original all-trans configuration, and the protein returns to its resting state. Created with BioRender.com.

Another promising new optogenetic tool is channelrhodopsin obtained from the marine flavobacterium *Krokinobactereikastus*. The light-driven sodium pump (KR2) generates an outward sodium ion current under 530 nm light stimulation, promoting the hyperpolarization of mouse entorhinal cortex neurons. KR2 does not directly affect the neuronal chloride gradient, pumps protons with very low efficiency, and functions primarily as a sodium ion pump. This is an important advantage when using KR2 to control seizures in epilepsy, compared with other inhibitory opsins such as NpHR and Arch, which alter potassium and chloride ion concentrations and can potentially induce epileptic activity (Trofimova et al., 2023).

Thus, the development and application of NpHR and Arch have expanded the possibilities for neuronal modulation and inhibition in optogenetics. The inclusion of anionic channels, in combination with cationic ChRs, provides a more comprehensive toolkit for the precise control and tuning of neuronal activity, paving the way for new therapeutic strategies in the treatment of various neurological disorders.

Bioluminescent Optogenetics in Nerve Tissue Regeneration

A non-invasive and highly specific approach to neural stimulation is bioluminescent optogenetics (BL-OG), in which genetically expressed light-emitting luciferases are tethered to light-sensitive channelrhodopsins (luminopsins, LMO) (**Figure 4**). Bioluminescent optogenetics is a neuroscientific method that combines the principles of optogenetics and bioluminescence to manipulate the activity of specific neurons or brain circuits using light. The core concept of bioluminescent optogenetics involves using bioluminescent proteins instead of traditional light-generating sources such as lasers or LEDs to activate or inhibit neurons expressing specific opsins (Berglund et al., 2021; Jiang et al., 2023).

Bioluminescent optogenetics offers several potential advantages compared to classical optogenetics. In bioluminescent optogenetics, bioluminescent systems of natural origin, such as luciferases and their substrates, are used to generate light inside cells. Luciferases can be obtained from various organisms, including fireflies, bacteria, and other marine organisms. These enzymes typically emit visible light upon the oxidation of a specific substrate, such as luciferin, which is also delivered into the cells (**Figure 4**). After genetically modifying target neurons to express both luciferase and opsins, researchers can control their activity and manipulate cells through bioluminescent stimulation. To achieve this, a substrate for luciferase, such as luciferin, is added to the culture medium or injected into the specific brain region of interest. During the oxidation of the substrate by luciferase, photons of light are generated, which can excite opsins in the same neurons, leading to their activation or inhibition depending on the type of opsins expressed. The advantage of using bioluminescence instead of external light sources lies in the fact that light emission is localized in neurons expressing luciferase, providing better spatial and temporal control. Furthermore, bioluminescence does not require additional equipment, making it a simpler and less invasive method. Moreover, light emission in bioluminescent chemical reactions occurs without heating (Petersen et al., 2022). This modification eliminates the need for external light sources and allows for the non-invasive and autonomous activation of light-sensitive proteins. Bioluminescent optogenetics enables increased depth of penetration, reduced tissue damage, and expanded application possibilities for optogenetic technologies. However, it is important to note that bioluminescent optogenetics is a relatively new and evolving field, and further research is needed for a complete understanding of its limitations and challenges.

In experimental practice in bioluminescent optogenetics, neurons are activated by the addition of the luciferase substrate coelenterazine (CTZ) (**Figure 4**). This approach utilizes ion channels to conduct current, activating the channels through the application of a small chemical compound, thus enabling non-invasive stimulation and recruitment of all target neurons (Tung et al., 2015, 2018). *In vitro* analysis of LMO3-neural precursor cells modulated in this manner demonstrates a decrease in markers characteristic of pluripotent cells and an increase in markers characteristic of neural stem cells, indicating differentiation into functional neurons. Bioluminescent optogenetics enhances the effectiveness of LMO3-neural precursor cell transplantation in a mouse model of Parkinson's disease, leading to significant improvements in motor behavior (Zenchak et al., 2020). Additionally, bioluminescent optogenetics may serve as a potential therapeutic approach for recovery from

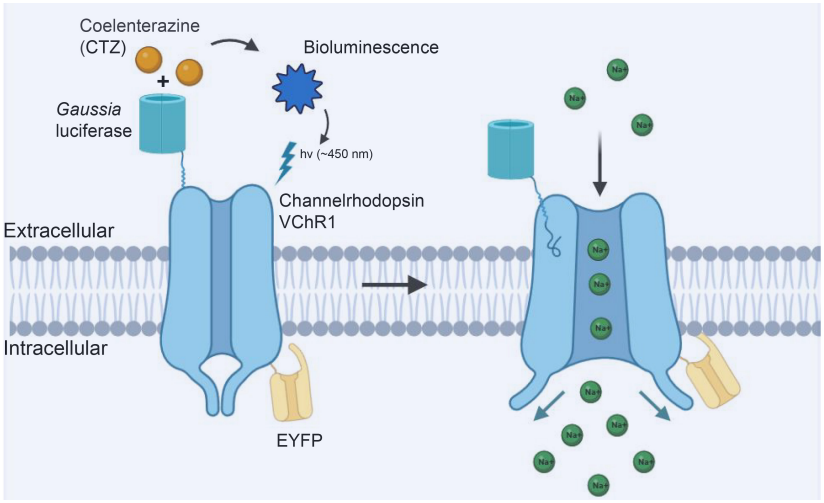

Figure 4 | **BL-OG based on the LMO3 system.**
LMO3 is a fusion protein of a mutant variant of Gaussia luciferase, sbGLuc, bound to VChR1 followed by EYFP. BL-OG uses the light emitted by luciferase upon oxidation of CTZ, a small molecule, to activate optogenetic elements. The light produced by CTZ oxidation activates the VChR1 channelrhodopsin, promoting the opening of the channel for intracellular cation transport. Created with BioRender.com. BL-OG: Bioluminescent optogenetics; CTZ: celenterazine luciferin; EYFP: enhanced fluorescent reporter protein; VChR1: volvox channelrhodopsin 1.

peripheral nerve injury. A single injection of CTZ immediately after sciatic nerve transection and repair in transgenic mice expressing excitatory luminopsin increased the number of excitable neurons, promoting the growth of regenerating motor axons and their reinnervation of the corresponding muscles (English et al., 2021). Similar results were achieved using a viral vector to induce LMO expression after peripheral nerve injury. The injection of an adeno-associated viral vector (AAV) encoding the enhanced excitatory luminopsin eLMO3 under the control of the hSyn (human Synapsin) promoter contributed to the modulation and enhancement of motor axon regeneration, complete sensory reinnervation, and accelerated recovery of neuromuscular function, even when initiated after nerve injury (Ecanow et al., 2022).

In the SCI model, it was demonstrated that the activation of neurons in the lumbar region of the spinal cord using bioluminescent-optogenetic stimulation significantly enhanced neuronal plasticity and improved the motor function of the hind limbs (Petersen et al., 2022). Injection of AAV2/5-LMO3, which consists of slow-burning Gaussia luciferase fused with rhodopsin VChR1 (**Figure 4**), under the control of the hSyn or Hb9 promoter, resulted in bioluminescence in neurons of the lumbar enlargement. Additionally, the substrate for luciferase, CTZ, was invasively injected into the lateral ventricles of the experimental animals. According to the study, 100% of the rats receiving optogenetic stimulation were able to correctly perform weight-supported stepping, achieving a Basso, Beattie, and Bresnahan (BBB) score of 10 or higher, whereas only 35% of the control group without stimulation achieved such results. Furthermore, higher levels of neuronal plasticity markers (GAP-43, MAP2, PSD-95, and NMDAR2d) were observed, indicating axonal growth, dendritic remodeling, and synaptic remodeling (Petersen et al., 2022).

Non-invasive modulation of spinal cord neuronal activity by intraperitoneal injection of CTZ substrate and modification of LMO with the opsin CheRiff, also resulted in significant improvement in motor function after SCI. However, similar peripheral delivery of substrate with a very light-sensitive opsin, which requires lower levels of substrate for optimal activation, has greater potential as a therapeutic option for patients with TCM. Peripheral delivery realistically brings the application of bioluminescent optogenetics closer to extended preclinical and clinical trials (Ikefuama et al., 2022). Activation of only a specific subpopulation of motor neurons using specific promoters during BL-OG, such as lamina IX motoneurons, seems more promising for the therapy of neurodegenerative diseases with motoneuron dysfunction. Combining this method of neuronal stimulation with trophic support for axonal regeneration through the injection of neurotrophic factors and motor training may prove to be a more effective approach. This strategy will undoubtedly complement the therapeutic arsenal of comprehensive treatments for SCI (Davletshin et al., 2022).

BL-OG is a powerful tool for studying the functioning and connectivity of specific neural circuits. By combining molecular-genetic techniques and bioluminescence, researchers can more effectively control neuronal activity and investigate their role in various physiological or pathological processes, thus expanding our understanding of the functioning of the central nervous system and mechanisms of plasticity in neurological disorders beyond the use of classical neurobiology methods.

Optogenetic Models: Recombinant Vectors and Target Cells for Opsins Delivery in Neural Tissue Regeneration

For the application of optogenetics *in vivo*, it is necessary to introduce the gene encoding the requisite opsin into target cells or tissues. Two prevalent models facilitating the expression of opsins are transgenic animals and the transduction of cells via viral vectors *in vivo*.

Transgenic animals are genetically engineered to express a specific gene, in this case, one that encodes an opsin. This enables the selective activation or inhibition of cellular populations within a living organism, depending on one or more endogenous promoters. Transgenic animals are particularly valuable for investigating the long-term effects of optogenetic manipulations in both stable and inducible forms (Rein et al., 2012; Wagdi et al., 2022; Oishi et al., 2023). Techniques for creating transgenic animals include pronuclear injection, transgenesis mediated by embryonic stem cells (encompassing systems such as Cre-Lox), and, more recently, the use of light-activated proteins within the CRISPR/Cas9 framework (Zhou et al., 2022; Bansal et al., 2023). The selected methodology involves injecting exogenous DNA into an embryo, ultimately leading to the transmission of the gene through the germline and resulting in the creation of a transgenic organism (Zeng et al., 2012).

Viral vectors in optogenetic studies offer a more efficient and targeted approach for delivering photosensitive proteins into specific cell types (Borodinova et al., 2021; Khalid et al., 2023). These vectors can provide long-term expression of opsins, enabling researchers to investigate the effects of sustained optogenetic manipulations over extended periods (Mondello et al., 2023). Different viral vectors possess unique characteristics, such as tropism, capacity, and immunogenicity, which can be advantageous under specific conditions. Researchers can select the most appropriate viral vector based on their specific requirements, making these recombinant vectors versatile tools in the design of optogenetic experiments.

Viral vectors for delivery of opsins to neural tissue

Recombinant viral vectors serve as an effective delivery platform for administering opsin-encoding genes into specific cell types. Lentiviruses, used as vectors for opsin transfer, are known for their ability to integrate into the host genome, providing long-term stability of the inserted genes while also having a large packaging capacity. This makes them particularly useful for long-term optogenetic studies (Emiliani et al., 2022; Arancibia et al., 2023). In contrast, adenoviruses typically exhibit rapid and efficient expression of inserted genes, which can be advantageous for temporary optogenetic studies, although they have a lower packaging capacity (Hagihara et al., 2020). Adeno-associated viruses are the most frequently employed viral vectors in optogenetics, especially for the modulation of neural tissue and clinical applications. They are favored for their low immunogenicity, high gene transfer efficiency, and prolonged gene expression with minimal side effects, effectively addressing the challenges associated with the limited availability of transgenic models expressing opsins. Additionally, the packaging of the viral genome can include markers such as enhanced green fluorescent protein (EGFP) or enhanced yellow fluorescent protein (EYFP), as well as monomeric red fluorescent proteins like mCherry, which facilitate

the visualization of transduced cells (Ordaz et al., 2017; Khalid et al., 2023). The injection process for viral vectors can vary depending on the target site and the depth of penetration, influencing the specificity of optically induced activity in animal models (Mondello et al., 2018).

AAVs coding for opsin genes are actively used in optogenetic applications, including exploratory research aimed at harnessing optogenetics for the treatment of neurological disorders, SCI, and neuropathic pain. However, post-delivery gene expression from AAVs may decline rapidly and exhibit variability depending on the specific promoters and AAV serotypes used (Jimenez-Gonzalez et al., 2022). For example, AAV8-ChR2 has been identified as particularly effective in inducing opsin expression in astrocytes and demonstrates the highest tangential and radial viral spread in the rat brain compared to AAV1 and AAV5 (Balachandar et al., 2022). According to Mondello et al. (2018), variations in the injection of AAV1-ChR2 in the C4–C7 spinal cord region, under photic stimulation, resulted in diverse movements in the paralyzed forelimbs, providing advantages over traditional methods such as pronuclear injection or the generation of Cre-dependent transgenic animals. Furthermore, the activation of any neuronal population below the lesion level, specifically in the T-13/L1 area caudal to the injury site, by introducing AAV2/9-LMO3 significantly enhanced motor recovery following SCI (Petersen et al., 2022). Injection of AAV-DJ-Chrimspon into the primary motor cortex, the principal origin of the corticospinal tract, may facilitate the regeneration of axons following traumatic SCI by selectively stimulating excitatory neurons (Khalid, 2023). This observed effect is consistent with other studies (Wahl et al., 2017; Yang et al., 2023) indicating that the injection of AAV2-ChR2, followed by daily patterned optical activation, increases corticospinal tract axonal outgrowth and branching. Additionally, spinal delivery of AAV2-ChR2 into the C6 segment caudal to the SCI site resulted in an increase in GAP-43^{+} fibers and enhanced vascularization of the injured spinal cord in optogenetically stimulated rats (Mondello et al., 2023). Moreover, optogenetic suppression of neurons in the anterior cingulate cortex through intracranial injection of AAV-eNpHR3.0 was found to alleviate neuropathic pain. The inhibition of overactive neurons in the anterior cingulate gyrus affected the activity of ventral posterolateral thalamic neurons, contributing to the reduction of sensory pain (Elina et al., 2021).

Less invasive variants of virus-mediated retrograde delivery of photosensitive proteins can be achieved through the intrasciatic injection of AAV6, which transports the vector to the spinal cord region, suggesting a straightforward procedure with a reduced risk of SCI. For instance, the injection of AAV6 into the peripheral nerve to express the *eNpHR3.0* gene, fused with eYFP and controlled by the hSyn promoter, enabled light-induced inhibition of acute pain perception and reversed mechanical allodynia and thermal hyperalgesia in a neuropathic pain model (Iyer et al., 2014). Additionally, optical activation of ChR2-expressing dorsal root ganglion (DRG) neurons can be achieved through retrograde injection of AAV9-ChR2 or AAV6-ChR2 into the rat's sciatic nerve. This method demonstrated the ability to modulate neuronal response properties by varying pulse duration and light stimulation intensity, effectively utilizing fast-conducting cutaneous and proprioceptive afferents *in vivo* (Kubota et al., 2019).

The recently developed approach utilizing acetylcholine-modified upconversion nanoparticles (UCNPs) in conjunction with AAV-mediated delivery of ChR2 presents a promising strategy for the treatment of peripheral nerve injuries. Acetylcholine-modified UCNPs were synthesized to specifically target acetylcholine receptors on certain neurons. When these UCNPs are excited by a laser at 960 nm, they emit upconversion fluorescence with a peak at 475 nm on the surface of the postsynaptic membrane. This upconversion fluorescence activates ChR2 on the cell membrane, leading to the depolarization of acetylcholinergic motoneurons. This depolarization facilitates axonal regeneration, promotes Schwann cell-mediated remyelination, and enhances the expression of neurotrophic factors within the central nervous system, ultimately supporting the remodeling of neuromuscular junctions (Yan et al., 2023).

The functional use of viral recombinant vectors for delivering opsins is expanding, providing a more customizable tool that enhances the specificity of opsin introduction into targeted cells. These vector modifications offer significant advantages in optogenetic research by allowing for the targeted capsid engineering of specific cell types and the use of cell type-specific promoters to limit opsin expression.

Target cells and promoters used for transgene expression in nervous tissue

The use of viral vectors enables cell-specific expression of opsins (**Table 1**), which can be achieved by incorporating cell type-specific promoters or Cre recombinase-dependent systems into the vectors (Yizhar et al., 2012). Additionally, viral serotypes that exhibit selective tropism for various cell types can be employed (Zincarelli et al., 2008). Beyond their translational significance (Bansal et al., 2023), viral delivery of light-sensitive ion channels provides enhanced control over the extent of transduction and the density of optically activated cells. The promoters described below are either currently utilized or hold potential for targeting specific cell populations, thereby expanding the toolkit available for optogenetic investigation.

Modulation of neuronal activity

The CaMKIIα (Calmodulin Kinase II alpha) promoter is used to target excitatory neurons in the forebrain, particularly in the cortex and hippocampus, as well as in the spinal cord (Mondello et al., 2018). This promoter is widely employed in optogenetics to selectively probe glutamatergic neurons. In a study by Deng et al. (2021), using a mild SCI model, motor function improvements were observed in rats following optogenetic stimulation of the M1 cortical area targeting spinal neurons above the lesion epicenter, alongside the selective activation of glutamatergic neurons via the CaMKIIα promoter.

The Sim1 transcription factor promoter can be used to selectively activate prenatal V3 neurons, providing a useful tool for investigating the activity of this cell population in SCI models. Optogenetic activation of V3 neurons through light pulses in a transgenic mouse line triggered a complex, coordinated motor neuron activity pattern, elucidating the involvement of propriospinal V3 neurons in the development of muscle spasms following SCI (Lin et al., 2019).

Some neurons contain promoters with weak transcriptional activity. In these cases, increased opsin expression can be achieved by generating transgenic mice with cell-specific expression of Cre recombinase. For example, Parvalbumin Cre targets neurons, vesicular glutamate transporter (vGluT2) Cre targets dopamine neurons, Thy1 Cre targets cells, and Visual System Homeobox 2 (Vsx2) Cre targets specific retinal neurons. Following this, a vector containing a strong upstream promoter, a stop cassette flanked by unidirectional loxP sites, and a downstream opsin gene is injected into the region of interest in the transgenic animal. Cells expressing Cre recombinase excise the stop cassette, enabling high opsin expression under the control of the ubiquitous promoter (Ordaz et al., 2017; Kathe et al., 2022). The Thy1 promoter has been shown to drive significant expression of ChR2 in pyramidal neurons, with some cortical interneurons also being targeted for stimulation (Wang et al., 2007). Optical stimulation of peripheral nerve neurons in transgenic mouse lines with the Thy1 promoter and ChR2 opsin has been shown to recruit motor units in the physiological recruitment sequence and reduce fatigue (Llewellyn et al., 2010). Additionally, optogenetic modulation of cortical circuits in Thy1-ChR2 transgenic animals induced extensive neural reorganization in both the motor cortex and spinal cord, creating an alternative motor pathway to restore impaired forelimb function through sprouting of the corticospinal tract (Wu et al., 2022).

The human synapsin promoter (hSyn) drives transcription specifically in neurons, not glial cells, making it ideal for experiments that require selective targeting of neurons. hSyn is expressed in a wide variety of neuron types across different brain regions, due to the ubiquitous expression of synapsin I protein. This includes neurons in laminae IV–VIII and X, with some expression also observed in lamina IX (Kathe et al., 2022; Petersen et al., 2022; Mondello et al., 2023).

To target more specific neuronal populations, promoters such as the Homeobox 9 (Hb9) promoter are used. Hb9 is a motor neuron-specific promoter that controls gene expression precisely within motor neurons, making it particularly valuable in studies focused on movement regulation and spinal cord function (Petersen et al., 2022). In contrast, the neuron-specific enolase (NSE) promoter is more reliable *in vitro* than *in vivo*. It does not restrict gene expression within the target cells as effectively as the synapsin promoter (Jimenez-Gonzalez et al., 2022), which can potentially reduce transduction efficiency in animal models. This results in a lower ratio of transduced neurons to the total number of transduced cells in optogenetic studies (Radhiyanti et al., 2021; Jimenez-Gonzalez et al., 2022).

Table 1 | Promoters for opsin expression in target cells. The most and least common promoters that are potentially used in optogenetic studies

Target cells	Construction/ promoters	*In vitro*/*in vivo* models	Expression in target cells/potential effects	References
Neurons	*Thy1*-Chr2-YFP transgenic mice	*In vivo* model; brain stimulation	Most cortex cells expressing ChR2 under the Thy-1 promoter are layer 5 pyramidal cells, with interneurons being an absolute minority. Illumination of ChR2-positive neurons in cortical slices produced rapid photocurrents that could elicit action potentials.	Wang et al., 2007
Neurons	*Thy1*-Chr2-YFP transgenic mice	*In vivo* model; stimulation of peripheral nerves	Recruitment of small motor units with optical stimulation, reduced fatigue of muscles in animals with optical stimulation.	Llewellyn et al., 2010
Neurons	AAV-*THp*-Cre; AAV-DIO-ChR2 (H134)-EYFP	*In vivo* model; brain stimulation	Selective and functional optogenetic labeling of dopamine neurons. Near the location where the injections were performed, ChR2 expression was seen in > 50% of dopamine neuron, as < 5% of ChR2-expressing neurons were not dopaminergic. Light stimulation drove action potentials in electrophysiologically identified dopamine neurons, but light stimulation elicited no activity in neurons that were not classified as dopamine neurons based on waveform and impulse activity.	Stauffer et al., 2016
Neurons	AAV1-*CamKIIα*-ChR2(H134R)-mCherry; AAV8-*hSyn*-Chronos-GFP	*In vivo* model; cervical spinal cord stimulation	Construction with CamKIIα promoter transduced neurons associated with both glutamatergic neurotransmissions. Construction with hSyn promoter transduced neurons that were associated with both glutamatergic and GABAergic neurotransmission, a small number of neurons associated with cholinergic neurotransmission. Optogenetic stimulation resulted in a variety of movements of the paralyzed forelimbs.	Mondello et al., 2018
Neurons	*Sim1*//ChR2 or *Sim1*// Arch3 transgenic mice	*In vivo* SCI model	V3 neurons were optogenetically activated with a light pulse, a complex coordinated pattern of motoneuron activity was evoked with reciprocal, crossed, and intersegmental activity. Overwhelming the V3 neurons with repeated optogenetic stimulation inhibited subsequent sensory evoked spasms, both *in vivo* and *in vitro*.	Lin et al., 2019
Neurons	CAG-sbGLuc-VChR1-EYFP transgenic mice.	*In vivo* Parkinson's disease model	BL-OG enhanced the efficacy of LMO3-neural precursor cell transplantation in a mouse model of Parkinson's disease, promoting a marked improvement in motor behavior	Zenchaket al., 2020
Neurons	rAAV 2/9-*CaMKIIα*-ChR2(H134R)-mCherry	*In vivo* SCI model; brain stimulation	Obvious and stable expression of the virus in the bilateral M1 region, activation of M1 glutaminergic neurons. Precise activation of M1 glutamatergic neurons could excite the descending conduction bundle, thus triggering the movement of hind limbs, increase expression of BDNF and NGF in the injured area, promote neurofilament regeneration, thus promoting the repair of the injured tissue and ultimately promoting the recovery of motor function in a rat model of SCI.	Deng et al., 2021
Neurons	*ChAT*-Cre, eLMO3 and *ChAT*-Cre/eLMO3 transgenic mice; Cre-dependent construct AAV2/9-*EF1a*-eLMO3; AAV2/9-*hSyn*-eLMO3.	*In vivo* PNI model; sciatic nerve stimulation	BL-OG increased the number of excitable neurons, promoting the growth of regenerating motor axons and their reinnervation of the corresponding muscles after transection of the sciatic nerve and its restoration.	Englishet al., 2021
Neurons	*Thy1*-Chr2 transgenic mice	*In vivo* SCI model; transcranial stimulation	Optical neurostimulation of the cortical neural circuits induced massive neural reorganization both in the motor cortex and spinal cord, mediated by an increase in BDNF expression, constructing an alternative motor pathway in restoring impaired forelimb function.	Wu et al., 2022
Neurons	Intraspinal injections AAV2/9-*hSyn*-LMO3; AAV2/9-*Hb9*-LMO3	*In vivo* SCI model; lumbar spinal cord stimulation	The highest levels of expression are primarily observed in interneuron populations located in laminae IV-VIII and X, with some expression noted more dorsally in lamina IX. The Hb9 promoter effectively restricted expression almost exclusively to motor neurons in lamina IX, though some interneurons also expressed the construct. BL-OG promotes accelerated and enhanced locomotor recovery following SCI by facilitating neuronal plasticity, which is evidenced by increased expression levels of growth and remodelling markers such as GAP-43, MAP2, PSD-95, and NMDAR2d in various regions of neurons.	Petersen et al., 2022
Neurons	AAV2/9-*hSyn*-eLMO3-EYFP	*In vivo* PNI model; stimulation of peripheral nerves	BL-OG promoted the modulation and enhancement of motor axon regeneration, complete sensory reinnervation, and accelerated recovery of neuromuscular function in the nerve injury model.	Ecanow et al., 2022
Neurons	Intraspinal injections AAV-*hSyn*-LMO3.2(CheRiff)-EGFP	*In vivo* SCI model; lumbar spinal cord stimulation	BL-OG LMO3.2 induces higher photocurrents compared to LMO3. Intraperitoneal injection of CTZ results in measurable bioluminescence emission, which is greater than that achieved through invasive delivery via lateral ventricular cannula following severe SCI. Rats that received stimulation via LMO3.2 exhibited improved locomotor function after SCI.	Ikefuama et al., 2022
Neurons	Intraspinal injection of AAV2-hSyn-ChR2-YFP	*In vivo* SCI model; cervical spinal cord stimulation	Neuron-specific optogenetic spinal stimulation significantly enhances the recovery of skilled forelimb reaching. This stimulation also increases GAP-43 and laminin labeling in the optogenetically stimulated groups, indicating that it promotes axonal growth and angiogenesis.	Mondello et al., 2023
Neuronal progenitor cells	Lenti-*Syn1*-hChR2-(E123T-T159C)-EYFP-WPRE; Lenti-*CaMKII*-hChR2-(E123T-T159C)-EYFP-WPRE; Lenti-*EF1a*-ChR2-GFP	*In vitro* model	The expression level of ChR2-eYFP was higher in cells controlled by the Syn promoter than by the CaMKII promoter. Expression driven by the universal EF1a promoter resulted in a twofold increase in ChR2-GFP expression compared to the levels regulated by the Syn and CaMKII promoters.	Lee et al., 2019
Neuronal progenitor cells	AAV9-CAG-hChR2(H134R)-mCherry	*In vitro*/*in vivo* SCImodel	Optogenetic stimulation promotes greater differentiation of NPCs into oligodendrocytes and neurons, and shifts astrocytes from a pro-inflammatory phenotype to a pro-regenerative/anti-inflammatory phenotype. Additionally, neurons derived from blue-light-stimulated ChR2-NPCs, which displayed increased branching and longer axons, showed improved axon growth.	Giraldo et al., 2020
Astrocytes	Intrathecal injection of of AVV-*GFAP*-ChR2 (H134R)-Katushka1.3; Ad-ChR2	*In vivo* neuropathic pain model; spinal cord stimulation; spared nerve injury stimulation	Photostimulation of ChR2-expressing astrocytes demonstrates the release of proalgesic mediators and electrophysiological disinhibition of spinal projection neurons, highlighting the role of astrocytes in the pathogenesis of pain.	Nam et al., 2016

Table 1 | **Continued**

Target cells	Construction/ promoters	*In vitro*/*in vivo* models	Expression in target cells/potential effects	References
Astrocytes	AAV (1,5 and 8)-*GFAP*-hChR2(H134R)-mCherry	*In vivo* model; brain stimulation	Optogenetic targeting of cortical astrocytes using serotype 8 resulted in the most efficient expression compared to the other two serotypes (1 and 5) recommended for targeting brain cells.	Balachandar et al., 2022
Astrocytes	AAV2/5-*GFAP104*-ChR2-mCherry; AAV2/5-*GFAP104*-melanopsin-mCherry	*In vivo* model; brain stimulation	Melanopsin, a G-protein-coupled photopigment, promoted a significant Ca^{2+} response in small regions of astrocytic outgrowths under blue light and induced transient changes in excitatory postsynaptic currents in hippocampal CA1 neurons, leading to long-term synaptic plasticity. In vivo activation of astrocytes expressing melanopsin via the GFAP104 promoter enhanced episodic memory in animals.	Mederos et al., 2019
Astrocytes	Intrathecal injection of AAV1-*Ef1α*-DIO-ChETA-EYFP; *GFAP*-Cre transgenic mice	*In vivo* model; spinal cord stimulation	Inhibition of pain through stimulation of Aβ-fibers and blockade of long-term depression induction in NK1R+ neurons, while maintaining non-neuronal control of pain.	Xu et al., 2021
Astrocytes	AAV2/5-*GfaABC1D*-ChR2(H134R)-mCherry; AAV2/5-*GfaABC1D*-Opto-a1AR-EYFP	*In vivo* model; brain stimulation	Opto-a1AR expressed in hippocampal astrocytes enables optogenetic modulation of longer-term synaptic plasticity and could potentially be used to normalize defects in synaptic transmission and plasticity. In contrast, no significant effect on fEPSP was observed in experiments with astrocytes expressing ChR2(H134R).	Gerasimov et al., 2021
Microglia	*CX3CR1* creER/+: R26 LSL-ReaChR/+ transgenic mice	*In vivo* model; spinal cord stimulation	Direct optogenetic stimulation of microglia leads to long-term changes in neuronal activity and IL-1β production, which enhance C-fiber-induced responses and mediate chronic pain hypersensitivity.	Yi et al., 2021
Microglia	*NG2*-tTA transgenic mice	*In vivo* model; brain stimulation	Optogenetic activation of ChR2 in mural cells decreased cerebral blood flow, while depolarization of NG2 glia reduced the proportion and density of GFP/Ki67 double-positive cells, indicating a decrease in NG2 glial proliferation. Stimulation of NG2 glia was also associated with an increase in the density of DM20/GFP double-positive cells, reflecting the differentiation of NG2 glia into premyelinating oligodendrocytes.	Oishi et al., 2023
Microglia	*Hoxb8*-IRES-Cre transgenic mice	*In vivo* model; brain stimulation	Optogenetic stimulation of Hoxb8 microglia in the dorsomedial striatum or medial prefrontal cortex induces grooming, while stimulation in the basolateral or central amygdala results in elevated anxiety. Stimulation of Hoxb8 microglia in the ventral CA1 region of the hippocampus induces both grooming and freezing behaviors. In vitro experiments directly demonstrated that optogenetic stimulation of Hoxb8 microglia in specific brain regions activates neighboring neural activity through the induction of the c-fos immediate early response.	Nagarajan et al., 2023
Oligodendrocytes	*PLP*-tTA transgenic mice	*In vivo* model; brain stimulation	Targeted optogenetic modulation of oligodendrocytes induces both short- and long-term functional plastic changes in hippocampal white matter following cell depolarization. This effect is mediated by early and late changes in the amplitude of compound action potentials, enhances axonal conduction through two distinct mechanisms, and allows precise control of synaptic activity between the axons that oligodendrocytes myelinate.	Yamazaki et al., 2014;2019
Schwann cells	pAAV-*GFAP*-CatCh-EYFP	*In vivo* model	Optogenetic stimulation of Schwann cells (SCs) induces SC proliferation, promotes differentiation, and enhances myelination in both SC monoculture and SC-motor neuron coculture, closely correlating with intracellular Ca^{2+} levels in SCs. In SC monoculture, optogenetic stimulation significantly increased SC proliferation, with the number of $BrdU^{+}$/$S100β^{+}$ SCs rising over time. optogenetic stimulation of SCs also activated the expression of myelin-associated proteins such as Krox20 and MBP in dedifferentiated SCs, significantly upregulating their redifferentiation and remyelination.	Jung et al., 2019; 2020
Neurons; astrocytes; microglia; oligodendrocytes	*CaMKII*-tTA *Orexin*-tTA *Mlc-1*-tTA *Iba1*-tTA *PLP*-tTA transgenic mice	*In vivo* model	Knockin-mediated enhanced gene expression using improved tetracycline-controlled gene induction (KENGE-tet) successfully generated transgenic mice expressing a highly light-sensitive ChR2 mutant at levels sufficient to drive the activity of multiple cell types, guided by cell-specific promoters.	Tanaka et al., 2012

To enhance the activity of neuron-specific promoters, strategies involving the fusion of these promoters with transcriptionally robust promoters, such as the cytomegalovirus (CMV) promoter, have been used. The construction of such enhanced promoters increases the production of the regulated gene but also leads to a significant reduction in neuronal specificity (Hioki et al., 2007). A study examining the activity of various neuron-specific promoters found that the mouse phosphoglycerate kinase-1 (mPGK) and hSyn promoters directed higher levels of transgene expression in neurons compared to the short chicken beta-actin (sCAG) and hCMV promoters. While the hSyn promoter drove neuron-specific transgene expression, the sCAG, hCMV, and mPGK promoters resulted in expression in both neuronal and non-neuronal cells, including oligodendrocytes and astrocytes (Nieuwenhuis et al., 2022).

The mGAD65 (glutamic acid decarboxylase) promoter is used to target GABAergic (gamma-aminobutyric acid) inhibitory neurons, as it is associated with the enzyme GAD65, which, like GAD67, plays a crucial role in the synthesis of the neurotransmitter GABA (Hoshino et al., 2021). The tyrosine hydroxylase (TH) promoter is used to target dopaminergic neurons, as TH is the rate-limiting enzyme in dopamine biosynthesis. This promoter has proven valuable in optogenetic studies investigating dopamine-related processes and in studies focused on enhancing memory retention in animal models (Chohan et al., 2020; Tse et al., 2024).

Cholinergic neurons, which produce the neurotransmitter acetylcholine, can be targeted using the promoter for choline acetyltransferase (Zhou et al., 2023). Promoters for PV, somatostatin, and VIP are commonly used to target specific subclasses of GABAergic interneurons in the cortex and hippocampus. Interneurons expressing PV, somatostatin, and VIP play a unique and critical role in modulating cortical network dynamics (Tsanov et al., 2020; Hoshino et al., 2021). Additionally, targeted expression of transgenes induced by promoters for cholecystokinin, serotonin transporter, vesicular acetylcholine transporter, substance P, and proenkephalin is significantly colocalized with specific markers of target neuron populations (Nagai et al., 2019).

Optogenetic studies on neuronal activation typically rely on a limited range of cell-specific promoters. The choice of promoter often depends on several factors, including the specifics of the experimental design, the required level of specificity, and the scope of targeting. While most of the promoters mentioned above can provide cell-type specificity, they do not guarantee complete isolation, as there may be some expression in other cell types or subtypes regulated by the chosen promoter. The current

repertoire of promoters used in optogenetics is relatively small, but the field has the potential to expand, leading to the development of new tools for more targeted modulation of neuronal activity, both at the vector level and in the regulation of opsin expression.

Modulation of astrocyte activity

The homeostatic functionality of astrocytes is crucial for the normal functioning of the nervous system. Many neurological disorders are characterized by a loss of this homeostatic function or a remodeling of astroglial homeostatic capabilities, which in turn triggers a complex defense mechanism known as reactive astrogliosis. Optogenetic stimulation of astrocytes has the potential to restore their homeostatic functionality and help normalize processes within the nervous system (Wang et al., 2023).

The human glial fibrillary acidic protein (GFAP) promoter is commonly used to target astrocytes. GFAP is an intermediate filament protein primarily expressed in astrocytes, with some expression is also found in Iba1-positive microglia and NeuN-positive neurons. For example, AAV8-ChR2 is the most efficient vector for opsin expression in astrocytes and demonstrates the highest tangential and radial viral spread in the rat brain compared to AAV1 and AAV5 (Balachandar et al., 2022). In a study by Xu et al. (2021) on spinal pain, pain reduction was shown to be mediated, in part, by the influence of the astrocytic environment in neural tissue. Suppressing astrocyte activation through optogenetic manipulation—using AAV1-ChETA injections and transgenic mouse lines with the GFAP-Cre promoter system—reduced pain by stimulating Aβ-fibers and blocking long-term depression induction in neurokinin 1 receptor (NK1R)-positive neurons, thereby exerting non-neuronal control over pain (Nam et al., 2016; Cho et al., 2020; Xu et al., 2021). The short version of the astrocyte-specific GFAP promoter, GfaABC1D or GFAP104, is also used for astrocyte activation. Optogenetic stimulation of hippocampal astrocytes with the GfaABC1D promoter and metabotropic opsin Opto-a1AR allows for the modulation of long-term synaptic plasticity, potentially normalizing synaptic transmission and correcting defects in plasticity in various neurodegenerative diseases (Gerasimov et al., 2021). In another study, melanopsin, a G-protein-coupled photopigment, was fused to mCherry and cloned into AAV particles using the short GFAP promoter (GFAP104). When exposed to blue light, melanopsin induced a significant Ca^{2+} response in small regions of astrocytic outgrowths and transient changes in excitatory postsynaptic currents in hippocampal CA1 neurons. Moreover, melanopsin activated endogenous G-protein and IP3 signaling pathways in astrocytes, promoting the release of ATP/adenosine, which mediates synaptic amplification, and glutamate, which contributes to NMDA-dependent slow inward currents in CA1 neurons. Low-frequency stimulation of melanopsin-expressing astrocytes induced long-term synaptic plasticity. *In vivo* activation of melanopsin-expressing astrocytes using the GFAP104 promoter also improved episodic memory in animals, suggesting that melanopsin can regulate astrocyte-mediated neuronal networks in a variety of ways (Mederos et al., 2019).

Astrocyte optogenetics is less advanced than neuronal optogenetics, and achieving the same level of synchronization accuracy has not yet been possible. Additionally, optogenetic stimulation of astrocytes may inadvertently induce a phenotypic shift toward a pro-inflammatory state, leading to astrogliosis, which could be detrimental *in vivo*. Therefore, the development of more precise optogenetic techniques for the controlled modulation of astrocytes is essential. Despite these challenges, optogenetic stimulation of astrocytes has the potential to regulate synaptic plasticity in neurons and influence the broader network of astrocyte-mediated neuronal interactions. Continued research in this area of optogenetics will undoubtedly deepen our understanding of the mechanisms through which astrocytes influence neurons in the central nervous system.

Modulation of microglia and oligodendrocyte activity

To ensure microglia specificity, the Iba1 promoter is used to target opsins specifically to this cell population (Tanaka et al., 2012). While the Iba1 promoter predominantly functions in the resident microglia of the striatum and cerebellum, its effectiveness may be limited in the cerebral cortex. Furthermore, the co-injection of miR-9 and miR-129-2-3p enhances the specificity of transgene expression under the Iba1 promoter by facilitating non-microglial detargeting (Okada et al., 2022). This proposed transgenic modification could expand the repertoire of optogenetic tools for the targeted expression of light-sensitive proteins.

Another promoter used to influence microglia is the Cx3cr1 promoter (chemokine (C–X3–C motif) receptor 1). The microglial optogenetic approach in transgenic mice expressing CX3CR1 creER/+ has demonstrated a controlled method for the specific activation of the opsin ReaChR, which induces depolarization of spinal microglia. This transient activation of microglia through direct optogenetic stimulation leads to long-term changes in neuronal activity, including the production of IL-1β, which enhances responses elicited by C-fibers and contributes to chronic pain hypersensitivity (Yi et al., 2021).

Optogenetically stimulated Hoxb8 microglia in specific brain regions mediate the activation of neurons and neural circuits, which, in turn, facilitate the induction of specific behaviors, including anxiety, grooming, or both simultaneously (Nagarajan et al., 2023).

Targeting the pro-inflammatory M1 microglia phenotype involves utilizing the specificity of the CD86 promoter. In M1 microglia, the CD86 promoter regulates the expression of an optogenetic Bax construct (Cry2-mCh-BaxS184E) that can be activated by exposure to blue light. Upon activation, Bax induces apoptosis, the release of mitochondrial proteins, cleavage of caspase-3, changes in cell morphology, and ultimately the death of pro-inflammatory microglia, thereby mitigating the cytotoxic effects following SCI (Paschon et al., 2020). Another important component is the CD11b promoter (Cluster of Differentiation 11b), which is expressed in a variety of leukocytes, including monocytes, neutrophils, natural killer cells, granulocytes, and, most notably, microglia within the brain. Cre recombination driven by the CD11b promoter occurs in blood myeloid cells, peritoneal macrophages, and other cell types, which poses a confounding factor for the use of CD11b-Cre mouse lines in precisely targeted microglial manipulation (Wieghofer et al., 2015).

Glial precursor cells also serve as targets for optogenetic manipulation, with their differentiation directed toward the oligodendrocyte lineage. The OS3ChR2 photoreactive glial precursor cells described may act as a therapeutic agent for diseases associated with oligodendrocyte dysfunction (Ono et al., 2017). Furthermore, targeted optogenetic modulation of oligodendrocytes in transgenic proteolipid protein (PLP)-tTa mice—where ChR2 is expressed exclusively in oligodendrocytes under the regulation of the PLP promoter—induces both transient and long-lasting functional plastic changes in the white matter of the hippocampus following cell depolarization. This effect is mediated by early and late changes in the amplitude of compound action potentials (CAPs), which enhance axonal conduction through two distinct mechanisms, allowing for precise control of synaptic activity among the axons myelinated by oligodendrocytes (Yamazaki et al., 2014, 2019).

In another study, transgenic neural/glial antigen 2 (NG2)-tTA mouse lines were generated using the NG2 promoter, leading to the development of unique mouse lines that express ChR2 in NG2 glia and mural cells. This setup facilitated investigations into the functions of these cells *in vivo* (Oishi et al., 2023; Janeckova et al., 2024). Optogenetic activation of mural cells resulted in a decrease in cerebral blood flow, while depolarization of NG2 glia reduced the proportion and density of double-positive GFP/Ki67 cells, indicating a decreased level of NG2 glia proliferation. Additionally, stimulation of NG2 glia correlated with an increase in the density of DM20/GFP double-positive cells, suggesting that NG2 glia differentiate into premyelinating oligodendrocytes.

The number of studies focusing on direct optogenetic modulation of microglia is relatively small, with most involving the use of transgenic Cre animals to manipulate their activity. Furthermore, there has been a lack of direct evidence for the activation of microglia (Hyung et al., 2023). It is also important to note that the processes underlying the phenotypic shift of microglia—specifically, the transition between proinflammatory M1 and antiinflammatory M2 phenotypes—have not been extensively studied in optogenetic stimulation. The shift of microglia toward the pro-inflammatory M1 phenotype may adversely affect active tissue regeneration.

However, with the advancement of this methodology, Yi et al. (2021) reported that microglia, like neurons, can undergo depolarization. This transient activation of microglia through direct optogenetic stimulation results in long-term changes in the activity of neurons and neuronal circuits. The secondary effects of microglia on neurons may fundamentally alter previously established methods of optogenetic modulation, positioning microglia as exceptional target cells that can drive a wide range of changes within the nervous system.

Modulation of Schwann cell activity

Schwann cells have been sparsely studied as optogenetic targets, yet the potential utility of this cellular population continues to grow. Optogenetic modulation has been shown to facilitate the proliferation, differentiation, and myelination of Schwann cells in both monoculture and co-culture with motoneurons, enhancing the expression of myelin-associated proteins, including early growth response protein 2 (Krox20) and myelin basic protein (MBP) (Jung et al., 2019). A comparative analysis of optogenetic stimulation of Schwann cells and motoneurons revealed the importance of selecting appropriate cellular targets for the therapy of various neurodegenerative diseases. Specifically, Schwann cells influenced by the Ca^{2+}-conducting opsin CatCh under the GFAP promoter markedly accelerated myelination, while motoneurons modulated by CamKIIa-hChR2 contributed to myelination only to a limited extent. This underscores the need for a strategic approach in the optogenetic application of distinct cell types (Jung et al., 2020).

Controlling optogenetically modified Schwann cells is crucial for optimizing their growth and function. Incorporating these modified Schwann cells into neuronal cultures, both *in vitro* and *in vivo*, may enhance neuronal growth and improve the connectivity and functionality of neuronal circuits. Given their regenerative and neuroprotective properties, Schwann cells are emerging as promising candidates for therapeutic applications in optogenetics. However, the most effective methods for manipulating optogenetically modified Schwann cells, such as identifying and applying specific promoters that exhibit specialized gene expression profiles (Fogarty et al., 2020), have yet to be determined (Hyung et al., 2023). Furthermore, the functionality and efficacy of optogenetically modified Schwann cells under *in vivo* conditions require further investigation through extended studies.

Limitations

In the last decade, optogenetics has emerged as a powerful and revolutionary technique that allows researchers to control the activity of specific target cells *in vivo* for various neurological diseases, bringing it closer to active clinical use. However, like any technique, optogenetic approaches have several limitations that must be resolved before fully exploiting their therapeutic potential, particularly in clinical practice. One of the main risks associated with optogenetic techniques is the possibility of off-target effects, where light stimulation may inadvertently activate or inhibit neurons outside the intended target population. Systemic or local delivery of opsins cannot effectively limit gene delivery to non-target cells, increasing the risk of overall toxicity and immunogenicity due to off-target effects and intracellular overload (Shen et al., 2020; Stone et al., 2023). Additionally, selecting a suitable delivery vector for light-sensitive opsins presents a challenge, with AAV vectors often considered the most appropriate choice. AAV vectors do not integrate their DNA into the host genome and persist as episomes, resulting in prolonged gene expression with very low toxicity across various cell types, including neurons and glial cells. However, a previous study indicates that transgene expression can last for years (Stone et al., 2023), raising questions about the feasibility of prolonged opsin expression in the body. Conversely, many clinical applications may require lifelong expression of opsins, making the maintenance of transgene expression over time an important consideration (Shen et al., 2020). It is also essential to recognize that the external light stimulation of opsins within brain cells is limited by the depth of light photon penetration. This limitation can complicate the precise targeting of specific neuronal circuits in deep brain regions. Furthermore, the temporal accuracy of optogenetic stimulation is constrained by the time required for light to activate or inhibit opsins and elicit a neuronal response. The use of opsins that are activated by narrow wavelength ranges and require high light doses can reduce the effective light penetration needed for photoactivation of target cells, increasing the likelihood of photodamage to surrounding tissues. Therefore, researchers face the challenge of addressing these issues to improve the effectiveness of optogenetic stimulation in brain structures.

Conclusions

Among the important strategies and methods developed to address these challenges, several promising approaches have emerged (**Figure 5**). If further refined, these strategies could facilitate the rapid application of optogenetic methods in clinical practice, effectively overcoming many of the existing limitations.

Advances in genetic engineering and viral vector technology have enabled more precise targeting of specific cell types within neural circuits, thereby reducing off-target effects. Capsid engineering and directed evolution are promising strategies for further improving AAV tools (Borodinova et al., 2021). Significant advancements in optogenetics, particularly using recombinant AAV vectors that carry opsin genes controlled by cell-specific promoters, have made this technique a promising therapeutic avenue for treating diseases of both the central and peripheral nervous systems. In diseases discussed in this article, targeted optogenetic stimulation—either directly or indirectly via glial cells—has been shown to increase the expression of neuroprotective factors and enhance neuronal plasticity. This results in a complex, coordinated pattern of neuronal circuit activity, ultimately leading to the restoration of nervous tissue innervation. Interestingly, glial

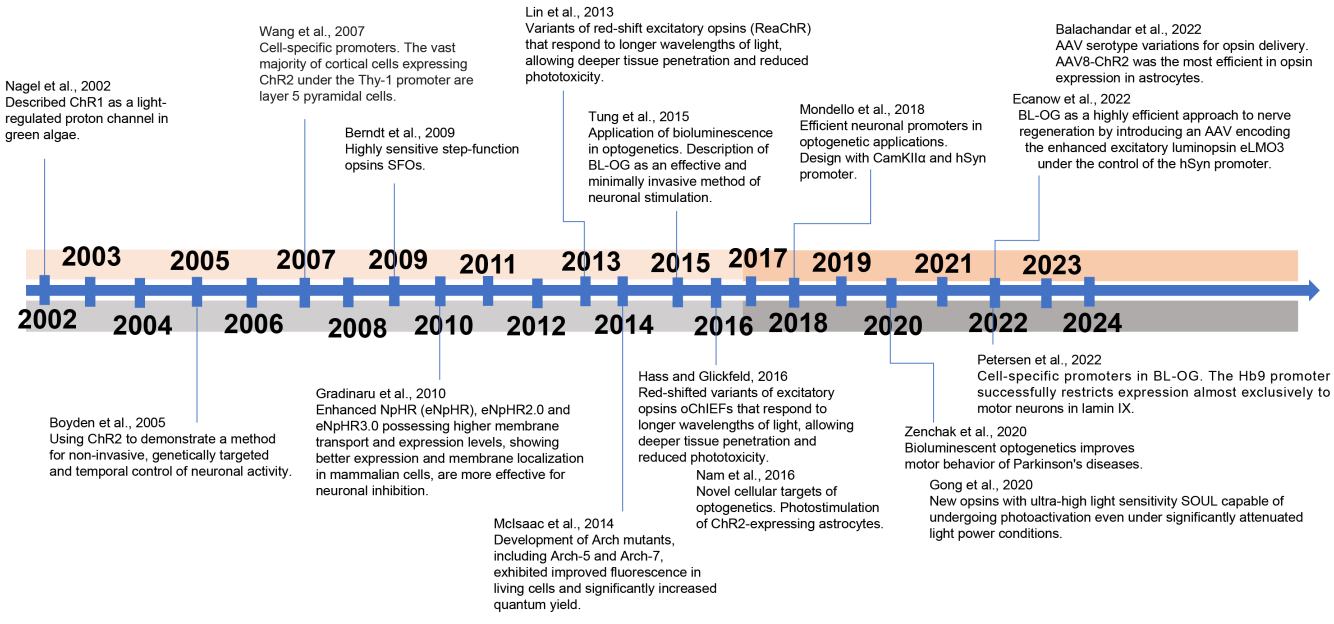

Figure 5 | **Timeline of significant advances in optogenetic tools, their refinement, and future directions to address limitations in applications.**
Arch: Archaerhodopsin; AAV: adeno-associated virus; BL-OG: bioluminescent optogenetics; CamKIIα: calmodulin kinase II alpha; ChR: channelrhodopsins; hSyn: human Synapsin; Hb9: homeobox 9; LMO3: luminopsin; NpHR: halorhodopsins; SFOs: step-function opsins; SOUL: step-function opsins with ultra-high light sensitivity.

cells also act as active target cells; studies have demonstrated that optogenetically activated glial cells can produce secondary effects on neurons that yield similar positive outcomes as direct neuronal stimulation. This insight allows for a re-evaluation of traditional optogenetic modulation methods, positioning glial cells as independent optogenetic targets that can induce a wide range of changes in the nervous system. Moreover, researchers are developing new opsins with improved characteristics, such as faster kinetics and enhanced light sensitivity. For example, the recently developed opsins SOUL can be effectively used to stimulate deep brain sites. Additionally, closed-loop optogenetic systems and non-invasive light delivery methods that dynamically adjust light stimulation in response to neural activity may enhance temporal accuracy, mitigate the risk of hyperstimulation, and reduce tissue damage. Among these innovative systems, bioluminescent optogenetics stands out for its clear advantages over traditional methods. The potential use of bioluminescent optogenetics in clinical practice could enable non-invasive and reversible modulation of neuronal activity. The dose-dependent administration of chemical substrates to activate bioluminescent platforms may facilitate high temporal fidelity in target cell activation (Petersen et al., 2022). This capability could be particularly valuable for managing conditions such as epilepsy, Parkinson's disease, and depression, where precise control of neural circuits is essential for symptom management (Chen et al., 2015; Gittis et al., 2018). Although the field of bioluminescent optogenetics is still in its early stages, ongoing research and development in this area hold promise for significant advancements in innovative therapies for a wide range of neurological disorders in clinical practice.

The versatility of optogenetics, combined with the ability to modulate neuronal activity with spatial and temporal precision, represents a paradigm shift in our approach to treating a variety of diseases, including those affecting neural tissue. Intensive testing and validation of protocols in animal models will be a crucial step toward making optogenetic tools a clinical reality.

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光遗传学方法促进神经组织再生: 对基本光遗传学原理和治疗目标细胞的研究综述
文章特色分析

一、文章重要性

1. 系统性综述价值

本文全面梳理了光遗传学在神经再生领域的研究进展，涵盖了从兴奋性与抑制性视蛋白的演变、生物发光光遗传学、病毒载体递送系统到各类神经细胞的特异性调控，为研究者提供了一个完整的知识框架。

2. 临床转化潜力突出

文章强调光遗传学在脊髓损伤、周围神经损伤、神经退行性疾病等方面的治疗潜力，特别是通过 AAV 载体和细胞特异性启动子实现精准调控，为未来临床治疗提供了理论依据和技术路径。

3. 多细胞靶向策略

不仅关注神经元，还深入探讨了星形胶质细胞、小胶质细胞、少突胶质细胞、雪旺细胞等胶质细胞作为光遗传学靶点的潜力，拓展了传统“神经元中心”的研究视角。

二、创新性特色

1. 生物发光光遗传学的引入

文章重点介绍了 BL-OG 技术，即利用荧光素酶-视蛋白融合系统，通过化学底物（如 CTZ）在细胞内产生光，实现对神经活动的非侵入性、自主性调控，避免了外部光源的局限。

2. 细胞特异性启动子的系统总结

文中提供了详尽的启动子列表，如 hSyn、CaMKIIα、GFAP、Iba1、PLP 等，用于在不同细胞类型中精准表达视蛋白，增强了光遗传学的细胞类型特异性与空间精度。

3. 新型视蛋白工具的发展与应用

包括：

- SFOs 与 SOULs：具有超长开放状态，适用于慢性过程研究；
- 红光/远红光激活视蛋白（如 Chrimson、ReaChR）：提升组织穿透性；
- 阴离子通道视蛋白（如 ACRs）：提供更高效的抑制性调控。

4. 病毒载体工程的进展

强调 AAV 载体在临床前研究中的优势，并通过衣壳工程、启动子优化、血清型选择等手段提升其靶向性与安全性。

三、对学科的启示

1. 从“控制”到“修复”的范式转变

光遗传学不再局限于神经活动的“开/关”控制，而是被赋予促进神经再生、轴突生长、髓鞘修复、突触可塑性等功能，成为神经修复工具。

2. 胶质细胞作为新兴治疗靶点

文章强调胶质细胞在神经回路调控中的主动作用，提示未来研究应更多关注神经元-胶质细胞相互作用在疾病机制与治疗中的意义。

3. 跨学科融合趋势明显

光遗传学已与基因编辑、纳米技术、生物发光成像、合成生物学等领域深度融合，推动神经科学向精准化、可调控、可视化方向发展。

4. 临床转化路径逐渐清晰

通过非侵入性调控、长效表达系统、闭环反馈控制等策略，光遗传学正逐步克服其在安全性、特异性、持久性方面的限制，向临床应用靠拢。

总结

本文不仅是一篇技术性综述，更是一份光遗传学在神经再生领域的路线图。它系统整合了从基础工具开发到临床应用前景的全链条进展，强调了多细胞靶向、非侵入调控、胶质细胞作用等新兴方向，为未来神经再生研究提供了重要的理论支持与技术启示。