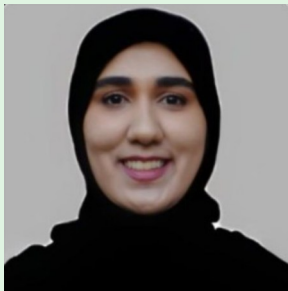


Optogenetics as a Mechanistic Probe and Therapeutic Tool for Axon Guidance in Neural Regeneration

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Abstract

Optogenetic tools have transformed the study of neural circuits, yet their application to axon guidance and neural regeneration remains underexploited. This review critically evaluates how optogenetics has been used and can be further leveraged to dissect and control axonal growth mechanisms. We first condense current knowledge of axon guidance cues and intracellular signaling, emphasizing pathways amenable to optogenetic intervention. Next, we analyze optogenetic tools with respect to their suitability for axon guidance studies, highlighting trade-offs between temporal precision, tissue penetration, and signaling specificity. We then synthesize key *in vitro* and *in vivo* studies, demonstrating what optogenetics has uniquely revealed about growth cone dynamics, receptor signaling, and circuit refinement. Finally, we identify persistent technical barriers, including opsin expression stability, light penetration, and developmental timing, and propose concrete strategies to overcome them. By framing optogenetics not merely as a manipulation tool but as a mechanistic probe, this review aims to accelerate its translation toward regenerative therapies.

Keywords: Optogenetics; Axon guidance; Neural regeneration; Growth cone; Channel rhodopsin; Spinal cord injury

1. Introduction

Neural regeneration following injury or disease fails largely because regenerating axons cannot navigate accurately to their targets. Axon guidance—the process by which growth cones interpret molecular and physical cues to reach appropriate synaptic partners—has been studied for decades using pharmacological, genetic, and lesion approaches (1,2). However, these methods lack the temporal precision,

cell-type specificity, and reversibility needed to dissect the dynamic interplay between neuronal activity and guidance cue sensitivity.

Optogenetics, the use of light-sensitive opsins to control neural activity with millisecond precision, Thesis: We argue that optogenetics is not merely a tool for mimicking or blocking guidance cues, but a unique mechanistic probe capable of revealing causal relationships between neural activity, signaling cascades, and growth cone behavior.

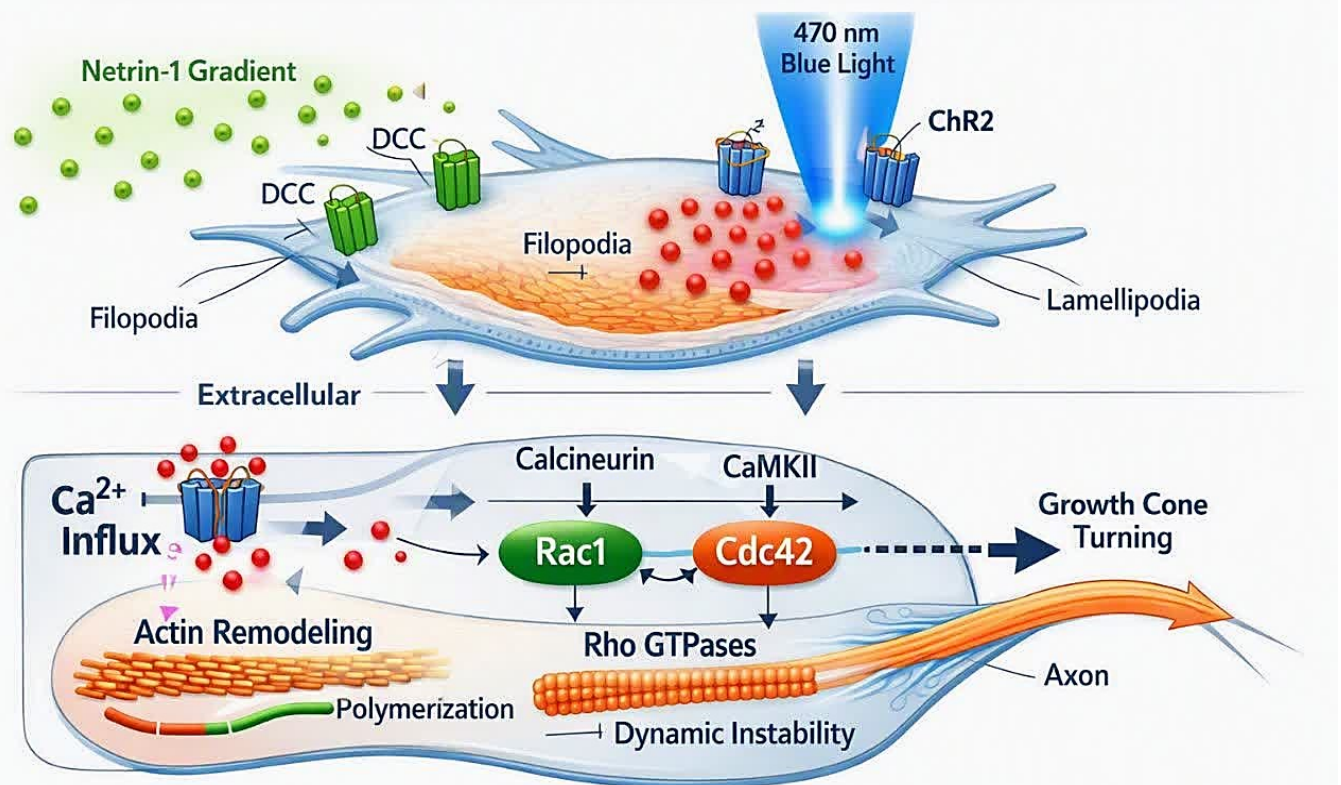


Figure 1. Schematic representation of optogenetic-guided turning in a neuronal growth cone. Blue light (470 nm) activates Channelrhodopsin-2 (ChR2), inducing rapid Ca^{2+} influx and downstream modulation of Calcineurin and CaMKII. Concurrently, Netrin-1 gradients engage DCC receptors, coordinating Rho GTPase-mediated actin and microtubule remodeling to steer the

Realizing this potential requires overcoming specific technical and conceptual challenges, which we outline here.

2. Axon Guidance: A Mechanistic Primer for Optogenetic Intervention

Axon guidance is mediated by four conserved cue families: netrins, slits, semaphorins, and ephrins. These cues bind receptors DCC, Robo, Neuropilin/Plexin, and Eph on the growth cone surface, activating intracellular signaling cascades that remodel the actin and microtubule cytoskeleton (6–8) (Table 1).

Key pathways relevant to optogenetics:

Critical for this review: All major guidance pathways are electrically and activity-sensitive (10,11). Ca^{2+} influx through voltage-gated channels modulates growth cone turning responses to netrin-1

(12). Neuronal firing rate alters surface expression of guidance receptors (13). GPCR signaling directly couples to G α_q and G α_i pathways that regulate cytoskeletal dynamics (9).

This crosstalk between electrical activity and molecular cue sensitivity is the primary mechanistic entry point for optogenetic intervention. Optogenetics is uniquely suited to dissect this crosstalk because it can manipulate activity with millisecond precision while leaving molecular cue presentation intact a capability not possible with pharmacology, lesions, or constitutive genetics (14)

3. Optogenetic Tools for Axon Guidance: Capabilities and Trade-offs

Optogenetic control of axon guidance requires opsins with specific properties. **Table 2** summarizes currently available tools and their suitability for axon guidance studies.

Table 1. Major axon guidance signaling pathways and their optogenetic targetability.

Pathway	Guidance Cue	Receptor	Downstream Effectors	Optogenetic Targetability	Ref
Ca²⁺ signaling	Netrin-1, BDNF	DCC, TrkB	Calcineurin, CaMKII, Rho GTPases	Direct (ChR2, Opto-Stim)	(5,12,16)
cAMP/PKA	Netrin-1, Slit	DCC, Robo	PKA, Epac, cytoskeletal regulators	Indirect (via activity)	(6,10,13)
PI3K/Akt	Neurotrophins	Trk	mTOR, GSK3 β , actin dynamics	Indirect (via Opto-TrkB)	(16,27)
Rho GTPases	Semaphorins, Ephrins	Plexin, Eph	RhoA, Rac1, Cdc42	Direct (Opto-GEF)	(8,28)

Table 2. Optogenetic tools for axon guidance: properties, trade-offs, and key references.

Tool	Wavelength	Effect	Kinetics	Tissue Penetration	Axon Guidance	Limitations	Ref
ChR2	470 nm	Depolarization	Fast (ms)	Poor	High temporal precision	Ca ²⁺ overload, phototoxicity	(5,14,19)
eNpHR	590 nm	Hyperpolarization	Fast (ms)	Moderate	Effective silencing	High light intensity required	(3,14)
ReaChR	590–630 nm	Depolarization	Fast (ms)	Good	Red-shifted, better penetration	Lower expression levels	(20,24)
(SFOs)	470/590 nm	Sustained depolarization	Slow (min)	Moderate	Mimics tonic activity	Limited testing in development	(19,20)
BiPOLES	470/590 nm	Bidirectional	Fast	Moderate	Dual-color control	Complex viral delivery	(15)
Opto-GPCRs	470 nm	G-protein signaling	Variable	Poor	Direct pathway recruitment	No ion flux; limited opsin repertoire	(16)
PiGM-Iq	470 nm	Gaq inhibition	Fast	Poor	Selective GPCR dissection	Inhibitory only; not activating	(9)
Glu-UCNP-ChR1	980 nm (NIR)	Depolarization	Fast	Excellent (mm scale)	Deep brain/spinal cord	Requires nanoparticle delivery	(17)

Critical gap: Most axon guidance studies still use ChR2 and eNpHR, which were not optimized for developmental timescales (18). Prolonged ChR2 activation elevates basal Ca^{2+} desensitizing growth cones to guidance cues (19). Step-function opsins, Opto-GPCRs, and NIR-UCNP systems remain underexplored in this context (20).

4. What Has Optogenetics Revealed About Axon Guidance?

We organize this section not by experimental modality (in vitro vs. in vivo) but by biological insight what optogenetics has uniquely revealed about axon guidance mechanisms.

4.1. Growth Cones Integrate Activity and Molecular Cues in Real Time

Using local ChR2 activation in cultured neurons, Harris et al. demonstrated that depolarization alone is sufficient to induce growth cone turning, but only in the presence of baseline netrin-1 (5). This gating effect whereby activity sets the gain for molecular cue responses could not have been resolved with pharmacology or genetics alone. Notably, this study also showed that optogenetic guidance can override competing endogenous signals and redirect axons across potent repulsive barriers, restoring functional connectivity in zebrafish (5).

Mechanistic insight: Activity does not replace guidance cues; it modulates the sensitivity of the growth cone to those cues.

4.2. Receptor Signaling Dynamics Are Spatiotemporally Restricted

Optogenetic recruitment of TrkB signaling (Opto-TrkB) revealed that sustained, not pulsed, receptor activation is required for BDNF-mediated axon branching (16). This finding challenges the assumption that guidance cues act as simple binary switches.

More recently, Lockyer et al. developed PiGM-lq (Photo-induced Gα Modulator—Inhibition of Gαq), an optogenetic tool that disrupts Gαq signaling via membrane recruitment of a minimal RGS domain (9). Activation of PiGM-lq in cultured dorsal root ganglia

neurons altered axon guidance responses to serotonin, demonstrating that GPCR-Gαq signaling is a direct and manipulable node in guidance cascades (9). This approach is also amenable to Gαi signaling, offering a generalizable optogenetic strategy for dissecting GPCR-mediated axon guidance (9).

Mechanistic insight: GPCR signaling in axon guidance is spatiotemporally dynamic and can be manipulated with pathway-specific optogenetic tools.

4.3. In Vivo, Axon Guidance Errors Are Corrected Dynamically

In zebrafish, Harris et al. used optogenetics to misroute axons during development, then observed error correction upon light cessation (5). This suggests the existence of latent guidance information that optogenetic manipulation can override but not erase a phenomenon invisible to static genetic perturbations. The study demonstrated that axons can be guided over large distances within complex living environments, overriding competing endogenous signals and constructing novel circuitry (5).

Mechanistic insight: The developing nervous system maintains redundant, parallel guidance information that can be unmasked when primary guidance signals are transiently overridden.

4.4. Disease-Relevant Guidance Defects Can Be Phenocopied and Rescued

A landmark study by Ji et al. (2025) applied near-infrared (NIR) optogenetics using glutamate-modified upconversion nanoparticles (Glu-UCNP) to treat spinal cord injury in mice (17). By expressing channelrhodopsin-1 (ChR1) in corticospinal tract (CST) neurons and delivering Glu-UCNP to spinal cord injury sites, NIR light was converted to visible light, precisely guiding regenerating CST axons to synapse with V2a spinal interneurons (17). This approach restored voluntary forelimb function in behavioral assays and was validated by single-cell transcriptomics, which revealed dynamic changes in axon regeneration-associated molecular networks (17). This study provides the first evidence that optogenetic axon guidance can achieve functional circuit repair in a clinically relevant mammalian model. Mechanis-

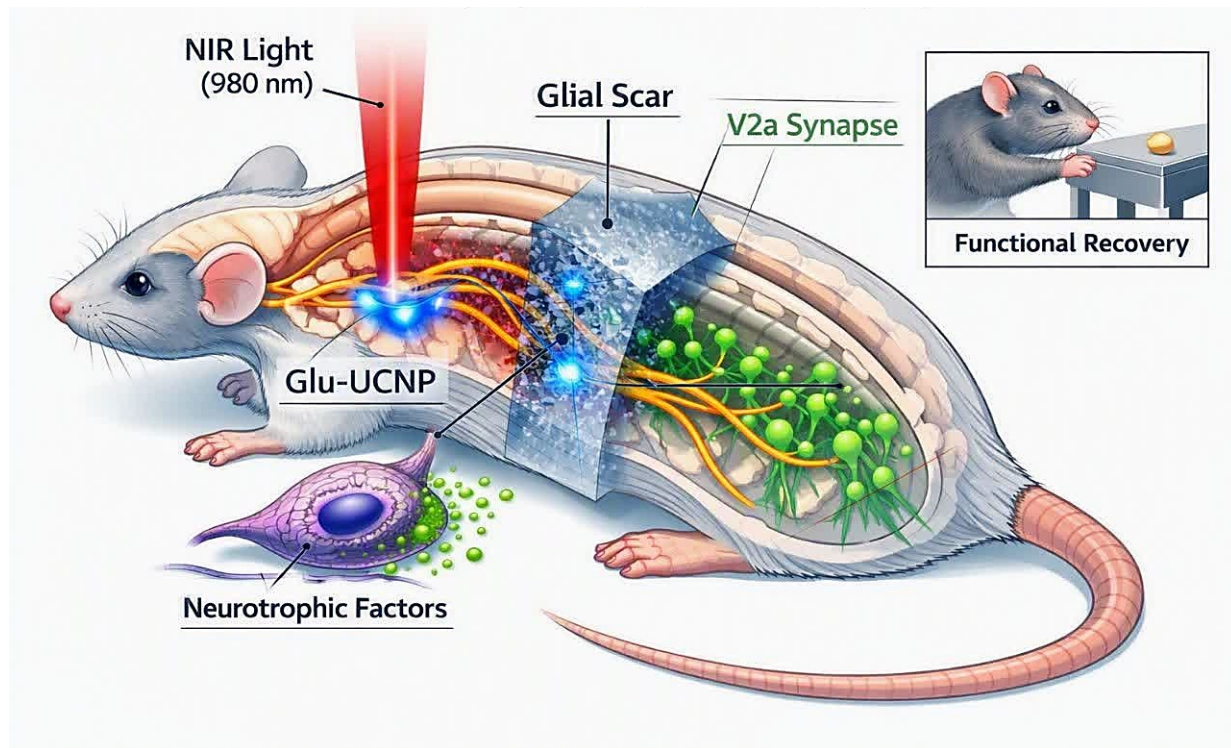


Figure2. Optogenetic spinal cord injury (SCI) repair in a mouse model. A near-infrared (NIR) light beam (980 nm) penetrates the skin and vertebral bone to reach Glutamate-modified Upconversion Nanoparticles (Glu-UCNPs) at the lesion site. The nanoparticles convert NIR light into visible blue light, activating Channelrhodopsin-1 (ChR1) in corticospinal tract (CST) axons. Regenerating CST axons (orange) traverse the glial scar (gray/blue) and form synaptic connections (green puncta) with V2a spinal interneurons (green). A Schwann cell (purple) releases neurotrophic factors upon optogenetic stimulation. The inset shows a mouse paw reaching for a pellet, indicating functional

tic insight: Targeted optogenetic guidance can overcome the glial scar and myelin-associated inhibition in adult mammalian spinal cord, provided that light delivery is non-invasive and sufficiently penetrating.

4.5. Glial Cells Are Emerging as Optogenetic Targets for Axon Guidance

Numata et al. (2025) demonstrated that optogenetic Ca^{2+} activation in Schwann cells (using Cap-ChR2) triggers release of neurotrophic factors that promote axon regeneration via autocrine/paracrine signaling (21). This suggests that combinatorial optogenetic targeting of both neurons and glia may be more effective than neuron-only approaches (18). Mechanistic insight: Glial cells are not passive substrates but active participants in axon guidance; their signaling can be optogenetically harnessed.

5. Challenges

Despite its promise, the application of optogenetics to axon guidance faces several unresolved challenges.

5.1. Opsin Expression Kinetics and Stability

Most opsins (e.g., ChR2, eNpHR) were optimized for acute neuronal silencing or firing, not for sustained modulation over developmental timescales (18). Prolonged opsin expression can alter intrinsic excitability or induce cytotoxicity (19). Davletshin et al. systematically evaluate these limitations, noting that first-generation opsins were not optimized for developmental timescales and that prolonged expression can alter intrinsic excitability (18). They highlight emerging solutions including cell-specific promoters, modified opsin variants, and biolumi-

nescent optogenetics to reduce phototoxicity and improve tissue penetration (18,20).

5.2. Spatiotemporal Precision Versus Developmental Scale

Axon guidance occurs over hours to days, across millimeters. Optogenetic control is typically applied over seconds to minutes, within micrometer-scale fields (5). Bridging this gap requires either chronic illumination strategies or closed-loop systems that adapt stimulation based on growth cone position or activity (22,23).

5.3. Light Delivery in Vivo

In deep brain structures or spinal cord injury sites, light scattering and absorption limit effective opsin activation (24). The Glu-UCNP system developed by Ji et al. directly addresses this challenge, achieving non-invasive deep brain stimulation via NIR light (980 nm) with millimeter-scale tissue penetration (17). However, this technology requires validation across multiple injury models and species.

5.4. Crosstalk with Endogenous Signaling

Optogenetic depolarization or hyperpolarization non-specifically alters intracellular Ca^{2+} , cAMP, and MAPK pathways the very cascades that transduce guidance cues (19). Disentangling direct optogenetic effects from indirect modulation of cue sensitivity remains a major experimental challenge. Pathway-specific tools like PiGM-Iq and Opto-GPCRs offer partial solutions but are not yet widely adopted (9,16).

5.5. Translational Hurdles

Beyond basic science, optogenetic axon guidance faces barriers common to all gene therapies: viral vector immunogenicity, opsin immunogenicity, and ethical considerations surrounding germline modification (18). The RReSTORe Consortium has outlined a roadmap for applying optogenetic axon guidance strategies to human retinal ganglion cell repopulation and optic neuropathy repair, emphasizing that ex vivo applications such as guiding axons in engineered nerve grafts before implantation

are immediately feasible and should be prioritized (25).

6. Conclusion

Optogenetics has moved beyond its origins as a circuit-mapping tool to become a high-precision probe of axon guidance mechanisms. By enabling millisecond control of neuronal activity and receptor signaling, it has revealed how growth cones integrate electrical and molecular information, how guidance errors are corrected, and how disease-associated mutations disrupt these processes (4,5,9,17). Pathway-specific tools like PiGM-Iq and Opto-GPCRs now allow researchers to dissect GPCR signaling with unprecedented selectivity (9,16). Non-invasive deep brain stimulation via NIR-UCNP technology has demonstrated functional circuit repair in mammalian spinal cord injury (17).

However, the field remains in its infancy. Most studies still rely on first-generation opsins optimized for acute circuit manipulation, not developmental timescales (18,20). Few have attempted closed-loop control (22,23). Translation remains distant, but ex vivo applications are within reach (25).

The next decade will require deliberate engineering of opsins for developmental timescales, integration with human neuron models, and realistic appraisal of therapeutic timelines. If these challenges are met, optogenetics may finally deliver on its promise to illuminate and guide the regenerating axon.

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