

Advances and applications of peripheral optogenetics in animal models

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ABSTRACT

Peripheral optogenetics is an emerging neuromodulation technique that regulates the activity of the nervous system outside the brain through the expression of photosensitive proteins and the application of photic stimulation. This article reviews recent advances in applying optogenetics to the spinal cord and peripheral nerves, offering a comprehensive understanding of the functions and regulatory mechanisms of the peripheral nervous system through the modulation of specific neuronal activities. By showcasing novel opportunities for disease treatment, this technique opens new avenues in psychophysiological research and neural regulation therapy. Despite current challenges, such as operability, effectiveness, and selective neuron targeting, peripheral optogenetics holds significant potential for advancing neuromodulation. Continued research and technological innovations will further expand its role, offering new possibilities for understanding and treating disorders involving the peripheral nervous system.

Introduction

The application of optogenetics in neuroscience has illuminated the complex neural circuits of the brain, providing profound insights into neural function and dysfunction (Geng et al. 2023). Due to its specificity and stability in regulating neural activity, optogenetics has become a widely used technique for studying neuronal functions. However, relatively little attention has been paid to the application of optogenetics beyond the brain. While the brain is undoubtedly a primary focus of research, other biological systems and peripheral nerves throughout the body also have great potential for optogenetic manipulation. In this review, we aim to bridge this gap by summarizing and discussing existing studies on optogenetic applications in neural systems beyond the brain. Through this effort, we hope to promote a broader and more practical understanding of peripheral optogenetics within the field of neuroscience.

Introduction to optogenetics

Optogenetic technology is a technique that uses photosensitive proteins (e.g., ion channels) and photic stimulation to control and modulate neural activity (Fernandez-Ruiz et al. 2022). Based on the response of photosensitive proteins to photic stimulation, it allows

activation or inhibition of specific neurons. This technology mainly includes two key components: photosensitive proteins and methods for expressing these proteins in target cells. Depending on the experimental purpose, diverse photosensitive proteins could be utilized to control neuronal activity. Researchers need to introduce the photosensitive protein gene into target neurons, enabling them to respond to external light stimulation of specific wavelengths. By delivering precise light stimulation in terms of timing and spatial location, researchers can control neuronal activity with high specificity. Depending on the characteristics of ion channel permeability, photosensitive proteins can be categorized into photoactivation and photoinhibition types (Table 1). With advances in optogenetic technology, researchers have continued to improve these proteins, enhancing their photosensitivity, speed of response, and spectral specificity to meet specific research needs (Berndt et al. 2009, Chow et al. 2010, Gradinaru et al. 2010, Han et al. 2011, Chuong et al. 2014, Klapoetke et al. 2014).

Effective gene delivery and expression of photosensitive proteins are crucial for achieving specific manipulation of cell types with precise spatial and temporal resolution. Commonly used methods in this regard include transfection and transgenic animal models. Transfection involves introducing the gene construct encoding the photosensitive protein (e.g., ion complementary DNA) into target cells, usually using viral vectors or physical methods, such as electroporation and the

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calcium phosphate method, to promote gene expression. Commonly used viral vectors include adeno-associated viruses and retroviral vectors (Boada et al. 2014, Vargas et al. 2016, Beaudry et al. 2017). The gene of the target photosensitive protein is introduced into the animal genome, enabling its expression throughout the organism and creating a transgenic animal model. This method differs from viral transfection in terms of application and effectiveness, as it allows for more stable and long-term expression of the foreign gene (Inutsuka et al. 2022), facilitating more comprehensive studies of the entire organism. However, there are certain limitations associated with using transgenic models for expressing photosensitive proteins. For example, the construction of transgenic animal models requires a relatively long preparation period and incurs higher costs (Bridge et al. 2023). Additionally, different types of cells might respond differently to the expression and function of these proteins (Arenkiel et al. 2007, Kätzel et al. 2011, Madisen et al. 2015, Bridge et al. 2023). Careful selection of promoters and regulatory elements of viral vectors is crucial for improving the expression efficiency and specificity of photosensitive proteins.

A commonly used approach is to combine viral vectors with transgenic animal models, achieving spatially specific expression based on the injection site of the virus and cell type specificity based on the expression of Cre recombinase in transgenic mice. It is also important to check the specificity of the protein after transgenesis and to limit the expression time of the photosensitive protein to avoid interference (Miyashita et al. 2013). In summary, the selection of expression methods for photosensitive proteins depends primarily on the efficacy and specificity of these approaches, as well as the experimental requirements. Optogenetic technology can be effectively applied only when the appropriate photosensitive protein is selected and its expression is precisely and reliably achieved.

Applications of optogenetics in the brain

As a critical organ, the brain’s functional complexity and significance have made it a primary focus for researchers utilizing optogenetic technology to regulate and investigate its activity. With advancements in science and technology, optogenetics has evolved from using viral transfection to activate specific brain regions (Fig. 1A) (Emiliani et al. 2022) to the precise transfer of genes encoding photosensitive proteins (e.g., ChR2) into target cells, enabling the activation of specific cells (Fig. 1B) (Cissé et al. 2018) or neural circuits (Fig. 1C) (Meira et al.

2018).

By establishing causal relationships between neural activity patterns and disease phenotypes, optogenetics has significantly advanced research on neurological disorders, such as Parkinson’s disease (Moon et al. 2018), epilepsy (Wicker and Forcelli 2021), and Alzheimer’s disease (Roy et al. 2016). Its applications also extend to psychiatric disorders, including anxiety, depression (Warden et al. 2012), and substance addiction (de Guglielmo et al. 2019). Beyond investigating the complex functions of neural circuits, optogenetic techniques also offer a promising therapeutic application in diseases, which is the “replacement” technique. For instance, by utilizing light-sensitive proteins (e.g., ion microbial rhodopsins), retinal neurons that have lost photoreceptor function due to hereditary or acquired retinal degenerative diseases can be converted into light-sensitive cells. Visual evoked potentials and photomotor responses indicate a certain degree of recovery in vision (Pan et al. 2015). Furthermore, the real-time and continuous closed-loop control of brain activity in non-human primates demonstrates the promise of combining electrophysiological recordings with optogenetics, which is expected to make significant contributions by monitoring the progression and treatment of neuropsychiatric disorders (Zaaimi et al. 2023).

Applications of optogenetics beyond the brain

In addition to its applications in brain research, optogenetics has also been applied to other areas of the nervous system, including the spinal cord and peripheral nerve terminals. By providing precise spatiotemporal control over cellular activity within the peripheral nervous system, optogenetics offers a powerful tool for investigating the mechanisms underlying various physiological processes. This approach also holds significant potential for advancing innovative therapeutic strategies for treating neurological and systemic disorders (Table 2).

Optogenetics in sensory studies

Optogenetics has proven invaluable in studies aimed at controlling the transmission of sensory signals (Fig. 2A), with a particular focus on pain perception. By utilizing optogenetic techniques to activate specific neuronal populations in the dorsal horn of the spinal cord, nocifensive behavioral responses, such as licking and biting to mechanical and thermal stimuli, can be evoked in animals. These behaviors suggest that

Table 1
Light characteristics of common photosensitive proteins.

Photosensitive proteins	Type	Light	Characteristics	References
Channelrhodopsin-2 (ChR2)	Activation	470 nm blue light	It is possible to selectively activate cation channels, primarily sodium ions, through blue light stimulation, and the activation and deactivation of ChR2 are relatively reversible. However, ChR2 is limited by its shallow light penetration depth, making it unsuitable for deep stimulation.	(Govorunova and Sineshchekov 2023)
Green light-activated Anion Channelrhodopsin 1 (GtACR1)	Inhibition	515 nm green light	Anion channels can be selectively activated by green light, specifically targeting chloride ions, and the activation and deactivation of GtACR1 are relatively reversible. Compared to ChR2, it has better light penetration capabilities.	(Kim et al. 2018)
Channelrhodopsin Crimson (Chrimson)	Activation	590–650 nm red light	Chrimson is sensitive to red light, which possesses strong tissue penetration capability, making it particularly suitable for studying neural activity in deep tissues. Additionally, it can be combined with other wavelength-activated opsins (such as blue light-activated ChR2) to enable multispectral optogenetics.	(Lin et al. 2013, Oda et al. 2018)
Natronomonas pharaonis Halorhodopsin (NpHR)	Inhibition	525 ~ 650 nm (central wavelength is 578 nm) yellow-green light	NpHR, the first effective optogenetic tool for inhibiting neuronal activity, can be selectively activated by yellow-green light, targeting anion channels specific to chloride ions. The activation and deactivation of NpHR are relatively reversible. Additionally, NpHR exhibits good light penetration capabilities.	(Rustichini et al. 2007)
Archaeorhodopsin (Arch)	Inhibition	566 nm yellow light	Arch, an outward-rectifying proton pump, can be selectively activated by yellow laser light. Under specific conditions, it can be used to increase intracellular pH or decrease extracellular matrix pH. In comparison to NpHR, Arch immediately transitions from an open state to a closed state when the laser is turned off.	(Chow et al. 2010)

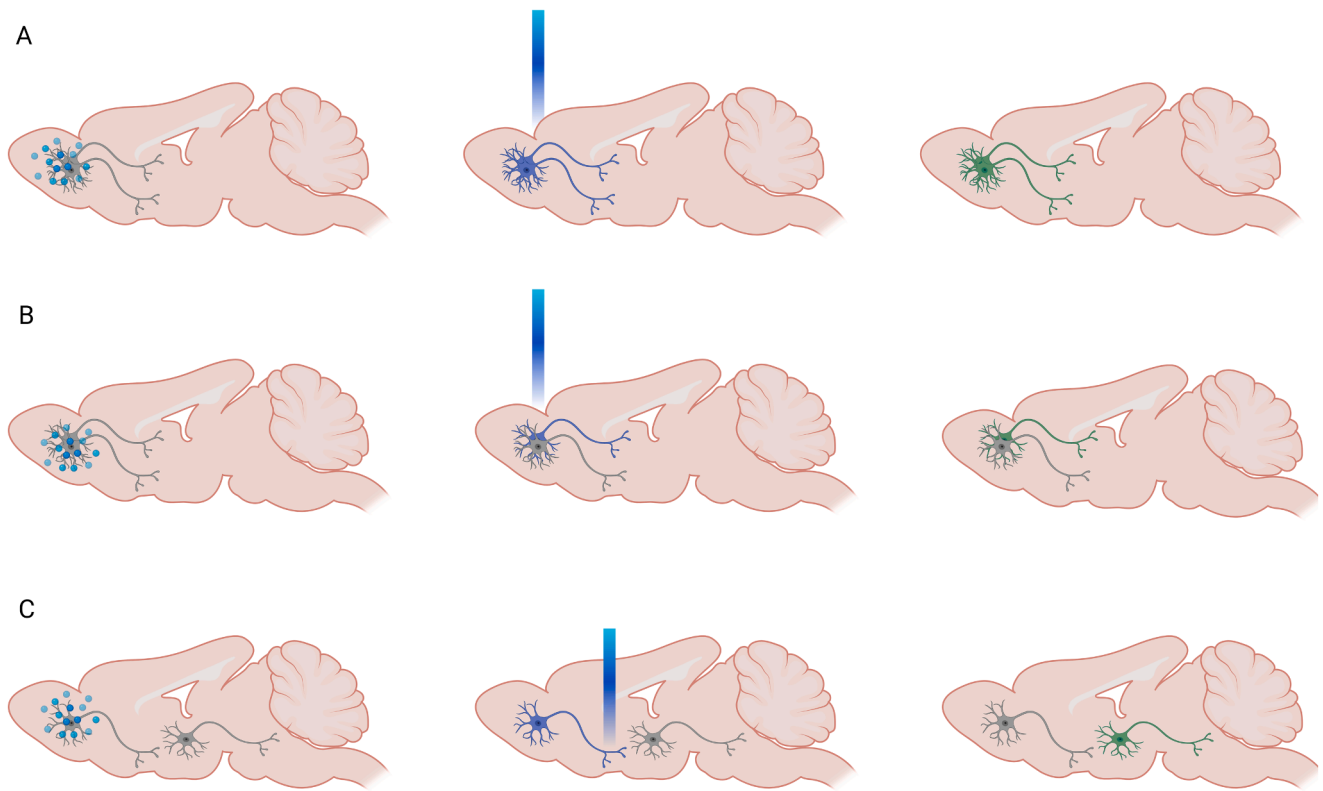


Fig. 1. Commonly used optogenetic methods to modulate the brain. **A. Local Modulation.** Schematic representation of local optogenetic stimulation. Following the expression of photosensitive proteins in a local brain region (*left*), light stimulation (*middle*) could activate the local neurons and their terminals in other brain regions (*right*). **B. Cell Type-Specific Modulation.** Schematic representation of cell type-specific optogenetic stimulation. Using a virus with a cell type-specific promoter to drive the expression of photosensitive proteins (*left*), light stimulation (*middle*) can precisely regulate neurons of specific cell types and their corresponding terminals (*right*), revealing the unique roles of different types of neurons. **C. Neural Pathway Modulation.** Schematic representation of optogenetic stimulation in neural circuits. Light stimulation is applied to the specific terminal (*middle*) originating from a brain region where photosensitive proteins are expressed (*left*), enabling targeted regulation of the specific neural pathway (*right*).

Table 2
Applications of optogenetics in regions outside the brain.

Regions	Research objects	Stimulation targets	Stimulation methods	Research purposes	References
Spinal cord	Spinal cord neurons	Spinal dorsal horn neurons Superior lateral paraspontine nucleus	Local stimulation of Spinal cord Local stimulation of brain	Neural mechanism	(Harding et al., 2020) (Deng et al. 2020)
	Spinal cord injury model DRG	Glutamatergic neurons in area M1 DRG motor neuron subset		Application in diseases	(Deng et al. 2021)
Peripheral nervous system	Nociceptors		Axon in sciatic nerve Epidermal irritation	Neuroregeneration	(Ward et al. 2016) (Daou et al. 2016, Williams et al. 2016, Beaudry et al. 2017)
	Astrocytes, keratinocytes			Neural mechanism	(Baumbauer et al. 2015, Nam et al. 2016)
Body system	Motor nerve endings Vagus nerve	Motor-related neurons Vagal neurons	Axonal stimulation Local activation		(Sharp and Fromherz 2011)
	Cardiovascular System Immune system Digestive system Endocrine System Gastrointestinal System Urinary System	Cardiac sympathetic ganglion CAR-T neurons Neurons within the ENS Parathyroid chief cells Neurons associated with the vagus nerve Peri-bladder neurons	LED irradiation In vivo local activation External red-light irradiation In vitro cell stimulation Local stimulation of vagus nerve trunk Local stimulation of bladder-related neural pathways	Application in diseases	(Chang et al. 2015, Williams et al. 2016, Nonomura et al. 2017) (Yu et al. 2017) (Nguyen et al. 2021) (Pan et al. 2022) (Liu et al. 2022) (Williams et al. 2016) (Kessler et al. 2019)

light stimulation can effectively activate nociceptive fibers, thus eliciting nocifensive behaviors (Harding et al. 2020).
By expressing photosensitive proteins in specific neurons, researchers can use light stimulation to activate or inhibit these neurons, thereby stimulating or alleviating pain sensation and exploring the role

of the peripheral nervous system in pain transmission (Fig. 2C). For example, ChR2-mediated peripheral terminal heterogeneous nociceptors, including mechanoreceptors and thermoreceptors, can be activated or silenced by optogenetics to induce or relieve inflammatory and neuropathic pain (Daou et al. 2016, Beaudry et al. 2017, Warwick

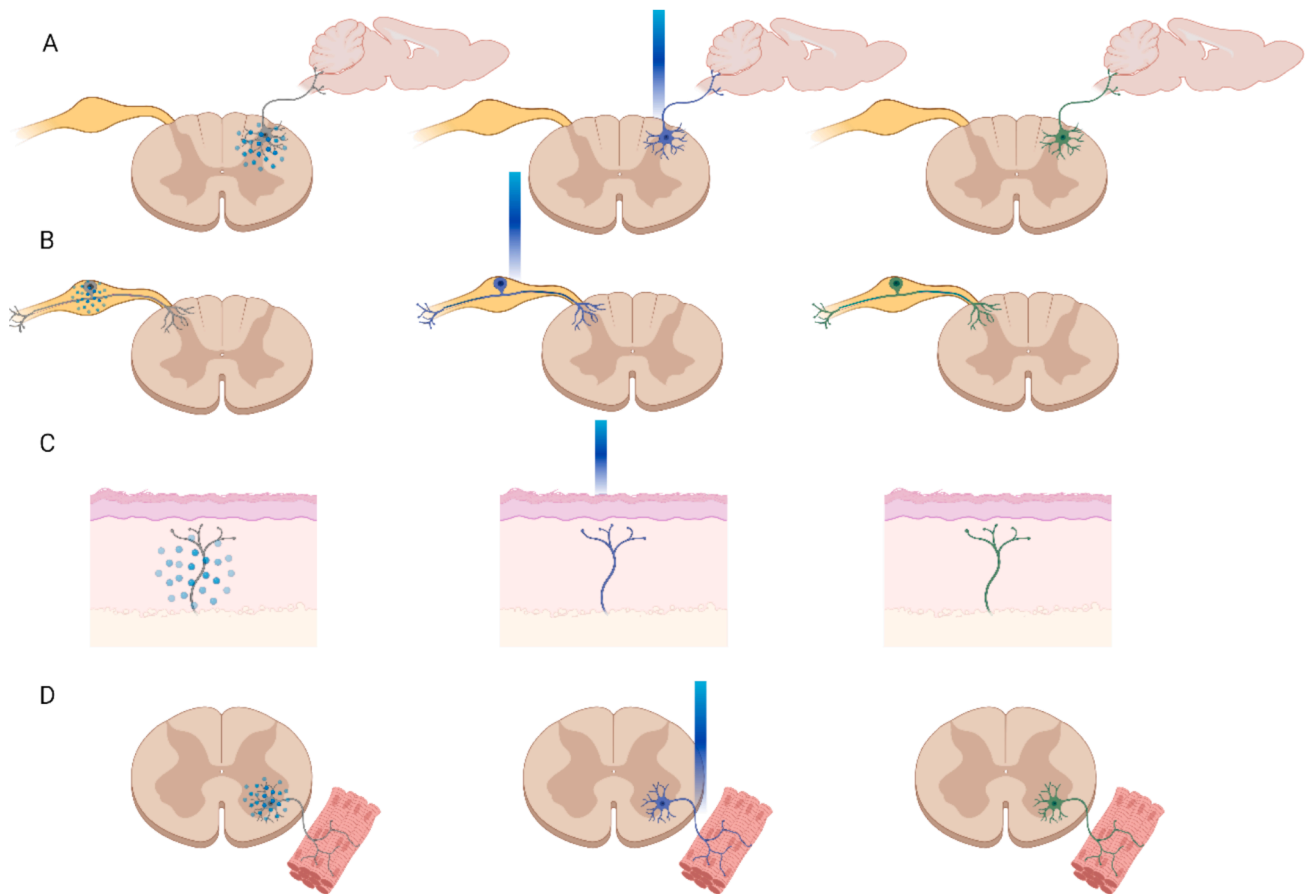


Fig. 2. Optogenetic methods targeting sites outside the brain. **A. Spinal-Cortical Modulations.** Schematic of optogenetic modulation along the pathway from the spinal dorsal horn to the brainstem. In response to the expression of photosensitive proteins in the spinal dorsal horn (left), light stimulation (middle) could activate local neurons and their terminals in the brain (right). **B. Dorsal Root Ganglion Modulations.** Using virus injection or transgenic hybrid mice, the photosensitive proteins were selectively expressed in the dorsal root ganglion (left). Local light stimulation (middle) could precisely regulate the neurons and their terminals in both the spinal dorsal root and peripheral tissues (right). **C. Peripheral Modulations.** Terminals of DRG neurons expressing photosensitive proteins are distributed in the skin (left). Invasive or non-invasive light stimulation to the skin (middle) could directly induce specific sensory inputs (right). **D. Optogenetic Control of Movements.** Photosensitive proteins were expressed in neurons of the spinal anterior horn (left). Optogenetic stimulation of these neurons' axons (middle) could modulate the activity of the corresponding muscles (right).

et al. 2021). Crucially, Iyer and their colleagues achieved bidirectional control of nociceptors in non-transgenic mice (Iyer et al. 2014). By injecting an adeno-associated virus expressing excitatory opsins into the sciatic nerve, they could induce acute pain, trigger aversive responses, and reduce withdrawal thresholds to mechanical and thermal stimuli through light stimulation. On the contrary, they could light-induce the suppression of acute pain, as well as the reversal of mechanical allodynia and thermal hyperalgesia in a neuropathic pain model using the virus expressing inhibitory opsins. This demonstrated the effectiveness of modulations by optogenetics in the peripheral nerve system. Moreover, a study utilizing CGRP-Arch Cre transgenic mice, which carry the Cre-LoxP recombinase system to specifically express inhibitory opsins in DRG neurons, investigated the effects of optogenetic stimulation in a spared nerve injury (SNI) model (Cowie et al. 2018). They found that brief activation of inhibitory opsins reversed mechanical, cold, and thermal hypersensitivity, reduced spontaneous pain, and attenuated the sensitization of primary afferent dendritic currents.

In addition to peripheral neuronal cells, non-neuronal cells in the periphery have emerged as potential targets for studying pain modulation. Cells such as keratinocytes (Baumbauer et al. 2015) and astrocytes (Nam et al. 2016), which express different types of sensory transduction channels, can be modulated optogenetically to explore their roles in pain modulation. Therefore, optogenetics plays a crucial role in pain research, enabling not only the simulation or alleviation of pain but also

significantly enhancing our understanding of the roles of neuronal and non-neuronal cells in pain modulation, thus providing novel tools and insights for advancing pain treatment.

Optogenetics in nerve regeneration studies

The peripheral nervous system has a greater regenerative capacity compared to the central nervous system. However, peripheral nerve regeneration is often incomplete, especially in cases involving severe nerve damage. Traditional approaches, such as growth factor administration (Li et al. 2018) and electrical stimulation (Du et al. 2018), have been used to promote nerve regeneration by stimulating neurons and improving the microenvironment. Despite these efforts, these methods are costly and often yield unsatisfactory outcomes for patients. Recent studies have started to use optogenetic stimulation, which involves expressing photosensitive proteins in specific neurons and applying light stimulation, to facilitate nerve regeneration. For instance, different ChR2 transgenic mouse models have been used to test the effectiveness of optogenetics in promoting the regeneration of motor and sensory neurons (Fig. 2B) (Ward et al. 2016, Ward et al. 2018). The results indicate that more ChR2-positive neurons successfully regenerate axons, while no effect is observed on ChR2-negative neurons, whether motor or sensory. This suggests that selectively enhancing the regeneration of motor or sensory neurons following mixed nerve injury could improve

functional recovery. Optical activation using the light-sensitive protein ChR2 can be employed to precisely control the activation of specific neuron types, providing novel tools for studying how neuronal activity influences axonal regeneration.

In combination with nerve stem/progenitor cell (NSPC)-based therapy programs, optogenetics offers a novel strategy for treating spinal cord injuries (Ahmad et al. 2015). By expressing photosensitive proteins in the lumbosacral spinal cord of rats, NSPC can be activated locally and proliferated at the site of injury, addressing some of the challenges associated with traditional NSPC-based treatment strategies. Moreover, bioluminescence optogenetics (BL-OG) technology has shown potential in promoting the recovery of motor function of lumbar neurons in mice with spinal cord injury expressing luciferase (Petersen et al. 2021). This technology involves the injection of a bioluminescent substrate that reacts with endogenous luciferase to produce light, which subsequently activates neurons expressing photosensitive proteins. Unlike traditional optogenetic techniques, BL-OG overcomes the limitations of insufficient light penetration in tissues, providing a superior, non-invasive method for the study and treatment of neurological disorders. However, the use of chemical injection to generate luciferase results in a loss of some of the advantages of the precise control offered by classical optogenetics.

Optogenetics demonstrates significant potential in the field of nerve regeneration by precisely regulating neuronal activity and promoting axonal growth, offering novel therapeutic strategies for nerve injury repair. In the future, with further optimization of techniques and the development of non-invasive approaches, its clinical applications will become increasingly promising.

Optogenetics in Motion regulation studies

Optogenetic stimulation of the peripheral nervous system can also be used to regulate motor functions. In terms of muscle control, Llewellyn and colleagues have used light-emitting diodes (LEDs) to activate motor axons in the peripheral nerves of mice expressing channelrhodopsin-2 (ChR2), enhancing the performance of some motor units and reducing fatigue (Llewellyn et al. 2010). Specifically, blue light has been shown to activate peripheral motor axons, thereby altering the leg movements of embryonic chicks (Fig. 2D). Sharp and Fromherz further demonstrated that during spontaneous movement, continuous illumination disrupts the full extension of the leg, leading to motor unit recruitment more akin to normal muscle activation (Sharp and Fromherz 2011). These findings suggest that optogenetic methods can be employed to investigate the role of movement and neuronal activity in the development of sensorimotor functions, particularly during the mid-stages of embryonic development. This approach enhances our understanding of the relationship between embryonic movement and nervous system function, while also offering new strategies for regulating motor abilities.

Optogenetics in respiratory system studies

The respiratory system, a complex network of organs and tissues, is responsible for the exchange of gases essential for life: oxygen and carbon dioxide. The vagus nerve plays a crucial role in this system, serving as the primary communication pathway between various organs and the brain. Its branches are widely distributed in the heart, lungs, gastrointestinal tract, liver, pancreas, and other organs (Yuan and Silberstein 2016). It senses changes in organ function and controls autonomic nervous activity. Accordingly, peripheral optogenetics can indirectly regulate human organs by stimulating the vagus nerve. For example, optogenetic activation of Piezo2-expressing vagus sensory neurons has been shown to cause respiratory arrest in adult mice, which suggests that Piezo2, expressed in the vagal sensory neurons of the nodose ganglion, is the primary mechanotransducer required for the Hering-Breuer reflex and respiratory volume control in adult mice (Nonomura et al. 2017). However, not all types of vagus neurons are similarly involved in respiration. Both Purinergic Receptor P2Y1

(P2RY1) and Neuropeptide Y Receptor Y2 (NPY2R) neurons that activate the vagus nerve can produce opposite effects, possibly due to differences in the pulmonary branching patterns and terminal morphology of P2RY1 and NPY2R neurons. These differences influence their distribution patterns and ranges within the lung, as well as their connections and morphological characteristics at the axonal terminals (Chang et al. 2015). These findings highlight the complexity of vagal nerve regulation in respiratory control and suggest that targeting specific vagal neuron subtypes could offer new therapeutic strategies for respiratory disorders. However, since optogenetic activation of nerves will lead to action potentials traveling from the initiation site in both directions (Forcelli 2017), some vagal stimulation may evoke central networks with unexpected, and possibly dangerous, consequences.

Optogenetics in cardiovascular system studies

It has been demonstrated that optogenetics can effectively regulate cardiac sympathetic nerves, significantly suppressing the occurrence of malignant ventricular arrhythmias. In one study, an adeno-associated virus vector was used to deliver the inhibitory photosensitive protein ArchT into the cardiac sympathetic ganglia of experimental dogs, with a miniature LED implanted for photic stimulation (Yu et al. 2017). The results showed that under light exposure, the electrical activity and function of the left stellate ganglion (LSG) nerves were significantly inhibited, leading to a marked reduction in the incidence of ventricular arrhythmias. Additionally, an adaptive cardiac optogenetic device has been developed based on a novel negative stretching-resistive strain sensor array for closed-loop heart rate regulation (Hong et al. 2021). This implantable system features a negative stretch resistance sensor made from carbon nanotubes and natural latex, which changes resistance in response to stretching. The device can continuously monitor heart rate in a closed-loop manner and adaptively control heart rate through the conversion of mechanical, electrical, and optical signals. This approach not only reduces rescue time and improves treatment efficiency, but also operates under low power conditions while achieving high performance. It holds promise as a novel, patient-controlled therapeutic approach for treating cardiovascular diseases associated with excessive cardiac sympathetic nerve activity. In summary, these advancements highlight the potential of optogenetic technology and adaptive cardiac devices in precisely regulating cardiac sympathetic nerve activity, offering innovative therapeutic strategies for cardiovascular diseases.

Optogenetics in Immune system studies

The Stimulator of Interferon Genes (STING) signaling pathway plays a crucial role in antitumor immunity. Based on STING, the collaborative team led by professors Yun Huang and Yubin Zhou developed an ultrasensitive optogenetic tool named LiSmore, enabling precise spatiotemporal control of the STING signaling in live animals (Nguyen et al. 2021). Under blue light irradiation, LiSmore specifically activates the STING signaling pathway, enhancing the maturation and antigen-presenting function of dendritic cells and activating the tumor-killing effect of CD8 + T cells. Compared to small molecule agonists of STING, the application of LiSmore avoids side effects associated with excessive activation of the STING pathway and pan-cell activation due to systemic administration. Moreover, LiSmore has shown significant tumor suppression effects in both in vitro and in vivo models, achieving a safe and precise cellular immunotherapy (Dou et al. 2023). Researchers have also used optogenetic tools to precisely control the activation of CAR-T cells, thus reducing the risk of side effects, such as cytokine storms and off-target effects. This nano-optogenetic immunomodulatory platform provides a unique method for studying CAR-mediated antitumor immunity. However, the toxicity and low yield associated with nanomaterials have limited the widespread application of these technologies (Nguyen et al. 2021). Still and all, optogenetic

technology demonstrates great potential in precisely regulating anti-tumor immunity, offering novel approaches for safe and efficient cellular immunotherapy.

Optogenetics in endocrine system studies

Parathyroid hormone (PTH) plays an essential role in maintaining calcium and phosphorus homeostasis. In the progression of secondary hyperparathyroidism, the expression of the calcium-sensing receptor in the parathyroid gland decreases, leading to excessive secretion of PTH. Researchers have explored the potential of optogenetic technology to introduce a photosensitive protein into the chief cells of the parathyroid gland via a viral vector (Liu et al. 2022). By increasing intracellular calcium, the synthesis and secretion of PTH in human parathyroid cells were inhibited, thereby achieving precise rhythmic regulation of PTH secretion. This study further revealed that, compared to the control group, nude mice transplanted with human parathyroid tissue exhibited a significant reduction in bone mineral density and bone volume. However, in the optogenetic intervention group, both bone mineral density and bone volume were restored to some extent. These findings suggest that optogenetic intervention can effectively mitigate bone loss symptoms caused by secondary hyperparathyroidism.

In addition to the thyroid, optogenetic technology can also regulate other organs or tissues within the endocrine system. For instance, researchers have used optogenetic tools to introduce the photoactivatable adenylyl cyclase (PAC) gene from *Beggiatoa* into mouse pancreatic β -cells (Zhang and Tzanakakis 2017). This PAC can increase intracellular cyclic adenosine monophosphate levels upon light stimulation, thereby enhancing insulin secretion. This technique has significant implications for studying diabetes and related metabolic disorders. Additionally, optogenetic techniques have been employed to specifically activate pancreatic β -cells, α -cells, and δ -cells, allowing researchers to investigate the spatiotemporal relationship between these cell activations and glucagon release (Miranda 2020). In short, optogenetic technology demonstrates significant potential in the study and intervention of endocrine system disorders, offering a novel tool for precise regulation of hormone secretion and exploration of metabolic disease mechanisms.

Optogenetics in gastrointestinal system studies

The gastrointestinal tract is the only internal organ with its own independent nervous system, known as the enteric nervous system (ENS). Spencer and Hu (2020) summarized how neurons within the ENS coordinate sensory and motor functions (Spencer and Hu 2020). Contrary to earlier beliefs, their findings suggested that peristalsis or colonic migrating motor complexes do not require mucosal serotonin. They proposed that chemical sensory stimuli applied to the lumen can release substances from enteroendocrine cells, which subsequently regulate the ENS activity.

In a recent study synthetic biology techniques were used to modify photosensitive *Lactococcus lactis* to secrete specific products in response to blue light stimulation (Pan et al. 2022). By integrating hydrogel delivery technology with optogenetics, precise targeted delivery to the small intestine and controlled in vivo expression of the engineered bacteria were achieved. This approach offers new strategies for the precise regulation of the gut-brain axis and is also helpful for the treatment of neurological diseases. In addition, peripheral optogenetics has been shown to regulate gastrointestinal function: activation of the photosensitive proteins expressed in the neurons expressing the GPR65 of the left stellate ganglion can significantly inhibit gastric contractions (Williams et al. 2016). This could also enhance our understanding of the characteristics of different neuronal populations in the regulation of gastric motility.

Optogenetics in urinary system studies

Park and colleagues found that opsins could be used to specifically activate or inhibit the contraction of smooth muscle cells in the bladder (Kessler and Birder, 2019). Bladder contractions were activated by depolarizing the cells using ChR2, while they were inhibited by hyperpolarizing the cells using halorhodopsin. Park and colleagues highlighted that one unique advantage of this strategy is the targeted delivery of photosensitive membrane proteins (including using viral vectors) to specific cells in the nervous system. In a more recent study, Mickle and colleagues improved the previous technology by developing a closed-loop wireless system that wraps around the entire bladder of rats (Mickle et al. 2019). By measuring changes in resistance during bladder filling and emptying, the system can send a signal to activate small micro inorganic light-emitting diodes when the bladder becomes overactive. This, in turn, activates Arch to selectively reduce neural activity, correcting abnormal bladder function. This approach allows for better regulation based on individual conditions and opens new avenues for restoring lower urinary tract function in humans.

Moreover, researchers have proposed a series of novel therapeutic approaches targeting kidney injury-related diseases based on optogenetics. For instance, Yim et al. developed engineered optogenetic exosomes, called “EXPLORs” (exosomes loaded with proteins through optically reversible protein–protein interactions), as a new drug delivery system for the treatment of acute kidney injury (AKI). In their study, optogenetic techniques were used to load a super-repressor form of I κ B (Exo-srI κ B) into the exosomes to target the nuclear factor- κ B (NF- κ B) pathway, which plays a critical role in the inflammatory response associated with AKI. Administering Exo-srI κ B in mouse models resulted in reduced NF- κ B signaling, decreased systemic inflammation, and improved kidney function after injury (Goggins and Tanaka 2021). This integration of optogenetic technology with exosomes enhances the precision of regulation, allowing for more effective modulation of disease-related signaling pathways, thereby improving therapeutic outcomes.

Conclusions and Outlook on applications of peripheral optogenetics

Peripheral optogenetics offers significant advantages over previous techniques, such as electrical stimulation, pharmacological modulation, and surgery intervention. The key benefit of optogenetics is its superior spatial and temporal precision of stimulation, providing the ability to bidirectionally and selectively modulate specific cells (Musso et al. 2019). In contrast, electrical stimulation lacks specificity, activating all neurons in the vicinity of the electrode rather than targeting specific cell types (Merrill et al. 2005). Pharmacological stimulation, while capable of providing some degree of selectivity, is unable to target specific cells and has low temporal resolution, making it challenging to precisely control concentration levels (Chefer et al. 2009).

Unlike optogenetics applied in the brain, which modulate organism functions primarily through influencing neural circuits within the central nervous system, peripheral optogenetics can directly regulate different tissues according to the projections of peripheral nerve endings. This approach holds promise for identifying and validating neuronal populations in peripheral nerve systems, exploring the neural innervation of organs, and investigating the role of the peripheral nervous system in the occurrence and progression of diseases. For example, the optogenetic tools based on exosomes developed by Goggins and colleagues represent a promising avenue for exploration (Goggins and Tanaka 2021). Labeling disease-specific exosomes could allow for easy, precise, and targeted activation of specific cells, thereby achieving highly selective modulation. It may also be possible to control the degree of cell labeling according to the severity of the disease, enabling a graded effect during activation and thus facilitating more appropriate and individualized stimulation. Moreover, novel experimental models

could be developed based on the controllable and accurate optogenetic modulation of peripheral sensory or motor neurons to induce specific perceptions or movements. A wireless closed-loop stimulation device can be applied to different peripheral nervous systems, such as at particular muscle sites, measuring tension to control its activation and deactivation. This approach is unaffected by the movement of the animal and allows for precise adjustments by adapting to the specific conditions of different animals. Furthermore, wireless control can overcome challenges such as limited light penetration in tissue, paving the way for translating findings from animal models to human applications.

However, several challenges remain in the broad application of peripheral optogenetics. First, the efficient expression of photosensitive proteins in peripheral tissue is challenging. Generally, viral injections must be performed in specific areas with neuronal somata, such as the spinal cord and dorsal root ganglia. However, this injection process presents difficulties mainly related to injection precision and virus expression efficiency. Due to the anatomical complexity of these regions, the injection process requires a high degree of technical expertise. Additionally, the expression efficiency of the virus may be limited, leading to insufficient or uneven expression of the target gene. To address these issues, researchers often rely on transgenic hybrid animals to improve expression efficiency. In addition to the low expression efficiency of photosensitive proteins, there is also a significant gap in the research on bidirectional modulation in regions outside the brain. This is largely related to the fact that neurons in the peripheral nervous system typically transmit signals in relatively fixed patterns.

Second, unlike the brain, which can be stably fixed to the skull, the mobility of peripheral tissues leads to the problem of fixing the end of the optic fiber used for optogenetic stimulation, especially in free-moving animals. To address this issue, portable, wirelessly controlled optical sources have been developed. For example, Liu et al. developed soft and stretchable, fatigue-resistant hydrogel fibers that enable optogenetic stimulation of peripheral nerves in naturally behaving animals during sustained exercise (Liu et al. 2023).

Third, opsins commonly used in optogenetics are activated by visible light, which is easily scattered, reflected, and absorbed by biological molecules or tissues. This means that it can only achieve shallow penetration and can't reach deeper tissues in optogenetic studies. This issue affects both peripheral and brain optogenetics. To address this issue, researchers have started using nanomaterials as a bridge to connect light with opsins, which coined a new term nanophotogenetics (Lu et al. 2024). Combining nanophotogenetics with peripheral optogenetics may yield promising outcomes in exploring neural mechanisms and clinical applications. Indeed, this technology shows great promise in treating diseases (Nguyen et al. 2021) and cell targeting (Zhang et al. 2020). However, it hasn't been widely applied yet, partly due to the toxicity of nanomaterials and low production yields. Replacing nanomaterials with biocompatible options like polylactic acid (PLA) (Singhvi et al. 2019) or polyvinyl alcohol (PVA) (Baker et al. 2012) could improve safety and efficacy. Another potential solution to the issue of insufficient light penetration is in vivo bioluminescence, which involves injecting bioluminescent substrates into the body that react with endogenous luciferases to produce light, thereby activating neurons that express photosensitive proteins. However, the precision of such regulation is likely to decrease. In the future, it may be possible to combine this technology with exosomes to enhance activation accuracy while addressing the issue of inadequate light penetration.

In conclusion, this article has summarized the fundamental principles of optogenetic technology and provided a comprehensive overview of its applications in both central and peripheral regions of animal models. The current challenges associated with peripheral optogenetics have also been critically discussed. Despite these limitations, optogenetics demonstrates substantial promise as a powerful tool for advancing neuroscience research and clinical interventions. Future efforts should prioritize addressing these challenges and exploring

innovative approaches to fully harness the potential of peripheral optogenetics for scientific discovery and clinical applications.

CRedit authorship contribution statement

Wenqian Zhou: Writing – review & editing, Writing – original draft, Conceptualization. **Liping Jia:** Writing – review & editing. **Lupeng Yue:** Writing – review & editing, Conceptualization. **Li Hu:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions.

Wenqian Zhou and Lupeng Yue designed the study, Lupeng Yue and Li Hu administrated the project, Wenqian Zhou wrote the original draft of manuscript, and all authors participated in revising the manuscript. All authors have read and approved the final manuscript.

Ethics statement.

This review article adheres to ethical principles. All sources are appropriately credited, and any potential biases are minimized. The information presented is accurate and objective, ensuring a fair and transparent exploration of the topic.

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Glossary

ACh: Acetylcholine
AKI: Acute kidney injury
Exo-srlkB: A super-repressor form of Ikb
BL-OG: Bioluminescence optogenetics
ChR2: Channelrhodopsin-2
DRG: Dorsal root ganglia
LSG: Left stellate ganglion
LEDs: Light-emitting diodes
NSPC: Nerve stem/progenitor cell
NPY2R: Neuropeptide Y Receptor Y2
NF-kB: Nuclear factor-kB
PAC: Photoactivatable adenyl cyclase
PBN: Parabrachial nucleus
PTH: Parathyroid hormone
BLP: Posterior part of basolateral amygdala
P2RY1: Purinergic Receptor P2Y1
STING: Stimulator of Interferon Genes
vCA1: Ventral hippocampus