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REVIEW ARTICLE
Kang Liu and Long Wang: Optogenetics and neuropathic pain

Optogenetics: Therapeutic spark in neuropathic pain

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ABSTRACT
Optogenetics is an emerging field, which uses light and molecular genetics to manipulate the activity of live cells by expressing light sensitive proteins. With the discovery of bacteriorhodopsin, a light-sensitive bacterial protein, in 1971 Oesterhelt and Stoeckenius laid the pavement of optogenetics. However, the cross-integration of different disciplines is a little more than a decade old. The toolbox contains fluorescent sensors and optogenetic actuators which enable visualization of signaling events and manipulation of cellular activities, respectively. Neuropathic pain is a pain caused either by damage or disease that affects the somatosensory system. The exact mechanism for the neuropathic pain is not known, however proposed mechanisms include immune reactions, ion channel expressions, and inflammation. Current regimen for the disease provides about 50% relief for only 40-60% of patients. Recent in vivo and in vitro studies demonstrate the potential therapeutic applications of optogenetics by manipulating the activity of neurons. This review summarizes the basic concept, therapeutic applications for neuropathy and potential of optogenetics to reach from bench to bedside in the near future.

KEYWORDS: Neuropathic pain; optogenetics; treatment
INTRODUCTION

Neuropathic pain (NP) is a pain caused either by damage or disease that affects the somatosensory nervous system (SNS), either centrally (multiple sclerosis, spinal cord injury, post-stroke, etc) or in the periphery (polyneuropathies related to diabetes, chemotherapy, alcoholism, infection, etc) (1). Patients suffering from neuropathy present with lancinating, shooting and/or burning pain along with tingling sensation which substantially impairs the quality of life (2,3). A single etiology or specific lesion cannot explain these heterogeneous set of conditions. A multidisciplinary approach treatment is often the most successful which include drugs (antidepressant and antiepileptic), anesthetic techniques, and surgical interventions (in patients with refractory NP) (4,5). Opioids were considered a good choice; however, they fail to reduce the pain and are controversial (6). Other drugs include acetyl-L-carnitine (ALC), alpha-lipoic-acid (ALA), cannabis products, botulinum toxin, and angiotensin type 2 receptor antagonists (5,6). A concrete treatment regimen is still not available however, blockers for voltage-gated sodium channel and blockers for the alpha-2-delta-1 subunit of calcium channels demonstrate a significant therapeutic potential (7,8). Moreover, these centrally acting drugs are lipophilic and nonspecific with a narrow therapeutic window, further preventing their prolonged use for pain management. Considering the pitfalls of conventional neuropathic treatments, treatment of such chronic pain requires a novel approach regulating excitability of nociceptors as a final outcome. Importance of nociceptors, immune cells and G protein-coupled receptors (GPCRs) has been well established in the pathology (2,3).

New technical advancements and interdisciplinary treatment methods offer more therapeutic effectiveness. One of such interdisciplinary approaches is optogenetics. Optogenetics is a
versatile tool where engineers, clinicians and the basic research scientists converge their knowledge and thus integrate genetics and optics to achieve gain or loss of function in specific cells of living tissue. Besides in vitro and in vivo manipulation of neuronal excitability and network activity, various optogenetic tools make it possible to target intracellular processes in particular organelles (9). This light-sensitive technology has revolutionized the study of neuroscience with single-cell and millisecond precision control of neurons (9) in the living system. This review article discusses the scientific landscape of the decade-old technique in the neuropathic pain.

**Basic concept of optogenetics**

As the name suggested, optogenetics is the integration of two fields i.e optics and genetics. The optics part is associated with illumination of a specific light spectrum whereas genetics part is associated with the expression of the modified opsin protein in cells. The basic concept of optogenetics is to manipulate the cellular activity by transducing electrical currents generated by light-sensitive proteins (10,11). Figure 1 demonstrates the major optogenetics tools which include: 1) light-activated proteins, 2) light and 3) mode of delivery (important for both of the other tools).

Opsins’ capture light and use it to either actively pump ions across the cell membrane or to open the channels, which allow passive flow of ions across the cell membrane. These proteins are ubiquitously present in all organisms, including eukaryotes and prokaryotes. Engineering and expressing opsins into non-light sensitive cells will enable rapid optical control of specific cellular processes. Therefore, applying optogenetics approach a rapid activation and silencing
can be achieved with no use of chemicals (9,11). Microbial opsins, a significant class of light-sensitive proteins, are classified based on membrane potential. Channelrhodopsins (ChRs) from Chlamydomonas *reinhardtii* depolarize neuronal membrane with light whereas the second class of protein halorhodopsin from Natronomonas *phaarais* (NpHR) causes hyperpolarization. Proton pumps such as bacteriorhodopsin, proteorhodopsin, and archaerhodopsin also affect hyperpolarization by extruding protons from cytoplasm. Co-expression of depolarizing and hyperpolarizing opsins because of varying illumination patterns allows creating a bidirectional channel in cells. (10,12). Chimeric receptors that mimic various signaling cascades represent the another set of light activated opsins. These include light-activated membrane-integrated G protein-coupled receptors (OptoXR) and hybrid receptor with extracellular domain of rhodopsin and intracellular domain of specific adrenergic receptors like optoα1AR (Gq-coupled human a1a-adrenergic receptor) and optoβ2AR (Gs-coupled hamster β2-adrenergic receptor) (10,12,13) . Properties like (i) ion specificity, (ii) subcellular localization, (iii) sensitivity to light, (iv) fast kinetics, (v) possibility for bidirectional control, and (vi) simple structure make opsins as novel and ideal tool in the field of optogenetics.

The second tool in the optogenetic toolbox is the light wavelength to activate a specific opsin. The differential wavelength specificity and sensitivity of opsins allow the coexpression of different proteins in one cell and make simultaneous activation and deactivation feasible. Therefore, two different proteins can be used as antagonistically or synergistically. For example, ChR2 is activated by ~460 nm light and NpHR is activated by ~570 nm light. Engineered opsins with red-shifted activation wavelengths made it easy to deliver the proteins at the challenging sites like deep in the brain with minimal invasiveness. Red-shifted activation wavelengths help in reducing the light scattering and therefore allow deeper light penetration. Channelrhodopsins


(ChRs) are light-gated, non-specific cation channels which are activated by blue-green wavelength from 450 to 545 nm (14). Such blue-green wavelengths fail to penetrate the neural tissue as they are absorbed by flavins, hemoglobin, and melanin. Also blue-green wavelengths scatter more strongly than yellow-red wavelengths. To overcome this red-shifted ChR with spectral peaks near or above 600 nm are engineered which include the cation channels (activation wavelengths): red-activatable ChR (ReaChR) (~590–630); Channelrhodopsin-1/Volvox Channelrhodopsin (C1V1; E122T mutation; ChR1/VChR1 chimera) (~600 nm); Chrimson (~660 nm); fast red-activatable Channelrhodopsin (bReaChES) (~590 nm); Volvox Channelrhodopsin (VChR1; similar to channelrhodopsin-1) (~531 nm) and the chloride pump Jaws (~635 nm). Moreover, the ChR2 gene has been modified to produce a “bistable” channel also known as step-function opsin (SFO). This channel is opened by one wavelength and closed by a different wavelength (9,15).

Due to the existence of the blood brain barrier (BBB), the delivery of the proteins is always a problem. One of the delivery methods is transfection of lentivirus or adeno-associated virus (AAV) packaged with the opsin gene through direct brain infusion. Viral-mediated gene delivery system is one of the most commonly used method. Table 1 summarizes the properties of viruses used in the optogenetics. Viruses are ideal vehicles for delivery as they (i) naturally penetrate into host cells and (ii) exploit the host transcription machinery for the expression of viral genes. Transgenic mice to create a uniform expression of the opsin in a specific group of cells is another method, however limited by weak transcriptional promoter activity in some cells, which makes it difficult to express opsins in a cell-specific manner. Even though noninvasive, genomic alterations are limited by high maintenance cost and significant time for the development of animal models. Transcranial focused ultrasound (FUS) technology, a noninvasive and targeted
BBB is another technology which is used for the delivery of the opsins. This technique involves a systemic injection of a mixture of lipid-based ultrasound contrast agents and opsins (16,17).

Delivery of light in the deep brain regions in a controlled fashion is another challenge which need to be mastered in the optogenetics. For in vitro experiments, a laser or light emitting diode (LED) can be directly coupled to the microscope. Tapered Optical Fibers (TFs) which are smaller compared to flat-cleaved optical fibers are best to deliver light over large brain volumes or spatially confined sub-regions (9). Direct implantation of small LED bulbs on thinned skull above the target region can be used for the stimulation of superficial layers of the cortex. A sensitive method for penetrating the tissues was developed considering the light density drops to as low as 1% with 1mm tissue penetration (18). Fiber-optic-based interface with LED or laser diode systems coupled to lightweight flexible optic fibers which deliver light to deeper brain tissue was developed. Coupled with optogenetics technology, it is possible to simultaneously stimulate and record activity in specific groups of neurons in freely moving animals. Recent advances in light delivery like implementing closed-loop circuits and optordes allow wireless light stimulation, real-time control and therefore simulate more natural environment (19,20).

**Optogenetics in neuropathy**

Optogenetics offers selective activation with a millisecond temporal precision, compared to electrical stimulation which is more generalized and non-selective. Such light-sensitive cellular stimulation eliminates the crucial step of placing the electrodes in brain which has a relatively
homogeneous group of neurons (21). Initial in vivo studies were done in the transgenic mouse models, however, most of them restricted to in vitro preparations and were not applied to freely moving animals. In an early study, Wang et al (2009) have demonstrated the power of optogenetics in causal dissection of pain circuitry (22). They demonstrated the functional connectivity in the substantia gelatinosa layer in a transgenic mouse line that expressed a stimulatory opsin in a the nociceptor subtype expressing Mas-related G-protein-coupled receptor member D (22). In another study, using a binary genetic approach, in Nav1.8-Cre transgenic mouse line to deliver ChR2 channels to peripheral nociceptors, Daou et al (2013) have demonstrated role of optogenetics in pain inhibition. In their experiment, the pain was elicited non-invasively and remotely (without implantation of optical devices) in animals (23). Adeno-associated virus (AAV) serotype 6 expressing ChR protein controlled the neuropathic pain in vivo successfully. AAV virus has advantage compared to other vectors (24). In vivo stimulation of yellow light sensitive third-generation chloride pump halorhodopsin (eNpHR3.0) channel was demonstrated to successfully prevent pain in neuropathic pain model and therefore, highlighted the therapeutic potential of optogenetics (25,26).

The real challenge in this field is to pinpoint the damaged nociceptive neurons. Therefore, understanding the differential genetics in the pathological neurons compared to normal functioning neural circuits become pertinent. A complete transcriptome of the trigeminal ganglia (TG) and dorsal root ganglia (DRG) of adult mice has been analyzed to get an overview of the expression of ion channels and G protein-coupled receptors in physiological and pathophysiological conditions (27–29). This analysis revealed that the conventional strategies which inhibit individual ion channels or inflammatory processes would not be as useful given the complex etiology of the pathological changes.
Because this disease affects not only patients but also their families, researchers are working hard to find the treatment. Researchers are developing alternative approaches for the treatment like Designer Receptors Exclusively Activated by Designer Drug (DREADD). Synthetic ligands such as clozapine N-oxide (CNO) manage the neuropathic by activating these designer receptors. Beside modulated by synthetic chemicals and not by endogenous ligands, DREADDs behave similar to the endogenous GPCRs and therefore interact efficiently with downstream intracellular signaling components (30–32). Because of the similarities to GPCRs, testing of DREADDs on the GPCRs downstream signaling is encouraged.

The light-sensitive proteins are generally introduced to the targeted mammalian neurons via viral gene delivery approaches (33) (for details please refer (12)). Limitations of the viral vector delivery system in humans are well known (33,34). A newer non-invasive technology, transcranial focused ultrasound (FUS), targets blood brain barrier, by systemic injection of lipid-based microbubbles (mixture composed of ultrasound contrast agents) containing molecules of interest (17,35,36). Considering the non-invasive nature of the FUS technique, viral vectors encoding various light-activated protein channels can be delivered which would allow an entirely non-invasive neural stimulation in vivo.

Optogenetics allows us to manipulate and monitor the neuronal circuit functions and has successfully implicated in behavior studies, including sensory perception (31), pain (37), social interactions (38) etc. Recent advancement in the optogenetics have revealed that specific components of higher brain functions can neither be linked to single transmitter systems nor to specific brain locations (1,39), suggesting a multi-tasking optogenetic sensor has to be designed. With these advancements in the field of optogenetics and promising results from recent experiments in non-human primates (40,41), we anticipate that the therapeutic application of
optogenetics will be available in clinics soon.

Spatially targeted, genetically-specific strategies for sustained inhibition of nociceptors is an example where laboratory research to step in the clinics. A recent report demonstrated the advantage of the step-function inhibitory channelrhodopsin, SwiChR, over the normal ChR by overcoming the need of required constant illumination (26). SwiChR inhibit pain for long periods of time with infrequent transdermal delivered light pulses, reducing the required light exposure by >98% and therefore, resolving a long-standing limitation in optogenetic inhibition (26,37,38).

**Optogenetics: beyond neuroscience**

Though, first introduced in neuroscience, optogenetics has been applied to other fields which include cardiovascular diseases, cancer and hepatology. Recent developments in stem cell research demonstrate a potential to have treatment for different diseases. Combinatorial approach of optogenetics and stem cells opened up treatment strategies for neurodegenerative and cardiovascular diseases. Engineered stem cells expressing exogenous light-activated opsins has been used in recent years. Functional analysis of human embryonic stem cells (ESC)-derived neurons in a Parkinson’s disease mouse model that was implanted with a fiber optic cannula revealed that light induces the selective silencing of transplanted neurons in mice (42).

Buegmann et al (2010) have demonstrated the induction of optically derived currents using a genetically modified mouse ECS-derived cardiomyocytes expressing ChR2 variant (43). Some of the studies have also used the hESc for activation of cardiomyocytes by optogenetics (44,45). Recently, optogenetics has been widely studied in the other research fields like diabetes (46), immunotherapy (47), retinal gene therapy and vision (48).
Concluding remarks and future prospective

Chronic pain is a major world-wide health issue. Besides impairing patients normal functionality also impacting the psychology of their families. Therefore, treating such disorders will not only be beneficial to the patients but also to the families. Therefore, selective targeting of neuronal systems would permit inhibition of chronic pain. Future studies should be focused to delineate the influence of the individual genotype on the predisposition to pain chronicity and the response to therapeutic treatments, considering the difference in the sensitivity to painful stimuli and the responses to the drugs varies from person to person. In addition, a better understanding of epigenetic regulation of the disease could also lead to new innovations and therefore should be among the major focus of futuristic therapeutic approaches.

Though, optogenetics is an exciting field for the future treatment of chronic pain, but there are still many questions which researchers have to focus. For example, it is not clear that treatment with the light will be short term or for long term? Also, what would be apt treatment regimen in terms of dosage? Another concern what would be the route and how the light sensitive proteins will be introduced in the human subjects. As stated earlier, the major obstacle is the controlled delivery of light to the cells, however, recent studies have shown some progress to combat this problem using the microscopy-based cell analyses and flow cytometry methods (16,49). In addition, a powerful futuristic approach would be combinatorial approach of optogenetics and clustered regularly interspaced short palindromic repeats (CRISPR)–Cas systems. These technologies will facilitate the targeting of optogenetic tools to attain proof of principle.

RetroSense Therapeutics (now acquired by Allergan) delivered the first in-human optogenetic therapy for advanced retinitis pigmentosa which is followed by RPE65 rAAV a FDA
approved therapy for retinal dystrophy in December 2017 by Spark therapeutics (50). In such conditions, photoreceptors in the retina gradually die off which eventually leads to the blindness. RetroSense’s therapy’, RST-001, delivers an adenovirus virus containing ChR2 gene in the eye of patients to create new photosensors in retinal cells. This clinical trial is still enrolling the subjects and in Phase I/II (https://clinicaltrials.gov/ct2/home). Till date, no results have been revealed from this clinical trial. The exact type of vision which will be generated by modified retinal cells remains unclear because a diverse group of retinal ganglion cells will be stimulating.

Gensight Biologics (https://www.gensight-biologics.com) designed an “biomimetic goggles”, which consist of a camera, a microprocessor and a digital micromirror array. This device stimulates the modified retinal cells by amplifying light signals. This company have recently treated the first subject in the first-in-man PIONEER Phase I/II clinical trial of GS030 at the Moorfields Eye Hospital in London, United Kingdom. GS030 combines a gene therapy (GS030-DP) administered via a single intravitreal injection with a wearable optronic visual stimulation device (GS030-MD) (https://www.gensight-biologics.com). The final results of this study are expected in the fourth quarter of 2020. In addition to this, Gensight Biologics is also developing treatment for the Geographic Atrophy in Dry-AMD using optogenetics approach. Companies like Circuit Therapeutics (http://www.circuittx.com) and Bionic Sight (https://agtc.com/programs/bionic-sight/), are also developing new optogenetic strategies to understand the cause and to treat the different neurological diseases. Recently, optoPAIN, an optogenetic platform to non-invasively assess changes in pain sensitivity has been developed, and used to examine pharmacological and chemogenetic inhibition of pain(48). Such techniques and developments hold the promise to see optogenetics in clinical setting in near future(51,52).
Pathophysiology of the neuropathic pain still remains enigmatic. Structural modifications and activation of nociceptors in neurons generate a high action potential and transmit signals through an already existing pain transmission network. Using optogenetics tools, neuronal circuit function can be manipulated and monitored and therefore a therapeutics approach combing optogenetics and gene therapy can be explored in the near future.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.
REFERENCES


TABLES AND FIGURES

Figure 1. Representation of basic flow chart (A) and description of various tools (B) in optogenetics.
Table 1. Description of commonly used viral delivery system in optogenetics

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