Role of Neuromodulation and Optogenetic Manipulation in Pain Treatment

Sufang Liu[1,2], Changsheng Li[3], Ying Xing[2], Yanqing Wang[4] and Feng Tao[1,5,*]

[1]*Department of Biomedical Sciences, Texas A&M University Baylor College of Dentistry, Dallas, Texas, USA;* [2]*Department of Physiology, Zhengzhou University School of Medicine, Zhengzhou, Henan, China;* [3]*Department of Anesthesiology, Henan Anti-cancer Hospital, Zhengzhou, Henan, China;* [4]*Department of Integrative Medicine and Neurobiology, Fudan University Shanghai Medical College, Shanghai, China;* [5]*Center for Craniofacial Research and Diagnosis, Texas A&M University Baylor College of Dentistry, Dallas, Texas, USA*

Feng Tao

Abstract: Neuromodulation, including invasive and non-invasive stimulation, has been used to treat intractable chronic pain. However, the mechanisms by which neuromodulation produces antinociceptive effect still remain uncertain. Optogenetic manipulation, a recently developed novel approach, has already proven its value to clinicians by providing new insights into mechanisms of current clinical neuromodulation methods as well as pathophysiology of nervous system diseases at the circuit level. Here, we discuss the principles of two neuromodulation methods (deep brain stimulation and motor cortex stimulation) and their applications in pain treatment. More important, we summarize the new information from recent studies regarding optogenetic manipulation in neuroscience research and its potential utility in pain study.

I. INTRODUCTION

Pain has been representing the most frequent symptom because it is related to many diseases. Therefore, pain therapy has become a burning problem to be solved. Most pain treatment is dependent on medications, behavior rehabilitation, and neurosurgical methods, such as neurotomy and rhizotomy [1]. However, these approaches cannot target specific area so as not to affect normal neural activity. Electrical stimulation can affect cellular elements only throughout a volume of tissue [2]. Previous studies have demonstrated that neuronal modulation is involved in the analgesia induced by electrical stimulation in the central nervous system (CNS) [3-5]. Effect of electrical stimulation on brain neurophysiology can be evaluated by recording neural activity directly from a specific target area [6, 7]. The main neurostimulation techniques available to date are divided into non-invasive and invasive methods [8-10]. While temporally precise, neurostimulation could not target specific cell populations. In recent years, optogenetic manipulation, which combines the delivery of light of specific wavelengths with gene encoding for light sensitive transmembrane channels, makes it possible to exert spatial and temporal control on specific cell types [2]. This state-of-the-art technology has emerged to provide novel insights into mechanisms of currently used neurostimulation techniques as well as the circuit basis of diseases in the CNS. In this review, we will discuss the principles of neuromodulation and optogenetic manipulation as well as their applications in pain treatment.

*Address correspondence to this author at the Department of Biomedical Sciences at Texas A&M University Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, Texas 75246, USA; Tel: 1-214-828-8272; E-mail: tao@bcd.tamhsc.edu

II. MATERIALS AND METHODS

We searched PubMed using the following keywords in the title or abstract: *optogenetics* and *neuromodulation*, in combination with the keyword *pain*. Additional studies examining analgesic effects of deep brain stimulation (DBS) and motor cortex stimulation (MCS) were identified using the following keywords: *DBS* or *MCS* in combination with the keywords *acute pain* and *chronic pain*. Searches were limited to the papers that were published in English in peer-reviewed journals. The papers regarding other neuromodulation approaches (spinal cord stimulation and transdermal electrical nerve stimulation) have been excluded in the current review.

III. NEUROMODULATION

1. DBS

Electrical stimulation has been used for centuries to treat painful conditions, and direct stimulation of peripheral nerves was firstly used to treat pain [11]. However, electrical stimulation application in the brain for treating refractory pain was not initiated until 1960 [12]. Various deep brain areas are stimulated based on specific target areas, which include sensory thalamus (mainly the ventro-postero-lateral nucleus, VPL nucleus), the posterior limb of the internal capsule (PLIC), and periventricular and periaqueductal gray (PVG/PAG) area [8, 12]. The mechanisms underlying DBS therapy are mainly relevant to the release of endogenous opioids and modulation of sensory afferent information from lemniscal pathways or the transmission of this information from the spinal cord [12, 13].

Successful treatment with DBS depends on accurately placed electrodes into specific brain structures to stimulate the target neurons electrically [14]. The electrode implanted

in the brain is linked to a subcutaneous pulse generator. Currently, the only commercially available DBS medical device were produced by a company located in USA. The stimulation device includes one stimulation lead and four platinumiridium electrodes surrounding the tip of the lead [15]. The electric pulse lasts for 60 to 180 ms and the electric frequency ranges from low-(15–30 Hz) to high-frequency (100–180 Hz). When the DBS device is turned on, an electric pulse will be delivered to the target tissue and it is often biphasic with a waveform composed by a negative phase and a positive phase. The resulting net charge delivered to the target tissue is based on the balance of two phases. Polarization may be produced if the two phases are unbalanced, but it will be null when they are balanced. DBS devices can be either voltage or current controlled so as to keep potential or current difference [6]. DBS may generate an electric field in a three dimensional space, which is linked to the neural processes around the field [15]. It has been shown that electrical stimulation of the subthalamic nucleus with constant current significantly ameliorates symptoms of Parkinson's disease [16]. And local potentials and single-unit activity in the corresponding area are recorded to assess the therapy effects induced by DBS. Single-unit recording provides an available tool to define anatomical, functional, and electrophysiological information [17]. Local field potential recording can examine the synchronous activities of synaptic area in a population of neurons [18].

DBS was initially used for the treatment of a variety of pain syndromes by targeting on the sensory nucleus of the thalamus [19]. DBS is a beneficial method to treat intractable pain especially by stimulating thalamus [20]. The most commonly targeted areas for pain treatment are PVG and the ventralis caudalis of the thalamus [21]. PAG/PVG regions for nociceptive pain therapy and ventroposterolateral/ ventroposteromedial (VPL/VPM) thalamic area for neuropathic pain therapy has been documented [22]. Ray NJ *et al.* found that DBS may produce pain relief by changing the thalamocortical activities [23]. Thalamic stimulation could increase inhibitory neuronal activity while PVG stimulation may decrease excitatory neuronal activity [24]. In addition, when thalamic area is stimulated by DBS for the therapy to chronic pain in patients, the anterior cingulate cortex (ACC) is also activated by the stimulation [25, 26].

2. MCS

The mortor cortex is located in Brodmann area 4, and the primary motor cortex (M1) has a significant identification because of its special location in the brain, thus it may be easier to distinguish for stimulation[8]. Most of the studies involving MCS focus on its use in post-stroke and trigeminal neuropathic pain, for which there are few other treatments [22, 27]. MCS has the following advantages in treating refractory neuropathic pain: (1) MCS appears to be equally effective for both central and peripheral neuropathic pain; (2) MCS seems to have fewer risks and is technically simpler than DBS [26]. Since 1991, MCS has been used to treat chronic neuropathic pain [28]. Electrical stimulation of the M1 has been demonstrated to suppress neuropathic pain in different animal models [29-33]. Currently, MCS has been still used in the clinic to treat intractable chronic pain [34-38].

The mechanisms underlying MCS are complicated. It has been reported that MCS enhances opioids release in various brain structures, thereby inhibiting pain [39]. MCS provides better results than those produced by sensory cortex stimulation [22]. Although the underlying mechanisms are not fully clear, it is indicated that MCS may produce the following effects: i) inhibiting nociceptive inputs in somatosensory areas through neural networks or connections between motor cortex and sensory cortex [40, 41]; ii) increasing cerebral blood flow in the related areas. Thus, pain related syndromes such as inflammatory factors may be reduced during the process of MCS [42]. It is demonstrated that up to 70% of patients could receive MCS again with the similar results [41, 43]. Therefore, MCS may be used as an effective therapy for neuropathic pain. Meanwhile, it has been reported that surgical risks and side effects of MCS show some serious complications, including intracranial bleeding, infection, and permanent neurological deficits [43, 44].

Both excitatory and inhibitory neurons exist in the brain, and activation or inhibition of the different neurons may produce different functional responses. MCS, while temporally precise, indiscriminately affects cellular elements throughout a volume of tissue. The ideal clinical neuro-modulation tool would allow for restoration of physiologic neural activity in a selected pathologic circuit without affecting normal circuits. The recent development of optogenetics, a revolutionary research tool, combines the delivery of light of specific wavelengths (opto) with the introduction of genes encoding for light-sensitive transmembrane channels (genetics) and makes possible highly precise spatial and temporal control of specific neuronal populations [2].

IV. OPTOGENETIC MANIPULATION

The key advantage of optogenetics is to manipulate a single cell, a cell class, a functionally defined cell type, or even subcellular localization like the axon terminals in a given region [45]. Just like other forms of genetic manipulation, optogenetic manipulation can control gain or loss of specific cellular function by light [2]. By combining optical method with genetic technique, the researchers may easily locate and study the genetically targeted neurons in slices, living animals or even freely moving animals [46]. The central components of an optogenetic system in wild type animals involve a light-modulated gene and gene product (opsin), a vector to deliver the opsin, and a light delivery instrument [2].

1. Opsins

Opsins can be divided into ion conducting and G-protein-coupled. The most familiar light-sensitive protein is rhodopsin, a G-protein-coupled receptor in the retina, which includes haloarchaeal proton pump bacteriorhodopsin, chloride pump halorhodopsin, and channelrhodopsin [47]. The first opsin used in mammalian neurons is channelrhodopsin-2 (ChR2), which is derived from the green algae Chlamydomonas. ChR2, a transmembrane protein, can cause the transmembrane cation channel open [48] and depolarize the cellular membrane upon receiving blue light (472 nm) stimulation [2, 49]. The cation conductance appears to depend primarily on the kinetics of channel closure rather than other molecular events [50]. Currently, short timescales are the major kinetic

limitations of optogenetic manipulation. Optical stimulation of ChR2 at frequencies above the gamma (40 Hz) range does not reliably evoke spikes in excitatory neurons [51]. Thus, some mutations were used to alter channel kinetics. For example, mutations of residue glutamate 123 to threonine or alanine (T/A) in ChR2 are created for stimulation above 40 Hz [2]. Since activation is linked to spike pulse and duration, so stimulation at higher light-pulse rates are not only linked to ChR2 photocycle kinetics (light-induced inactivation) but also linked to host cell-specific properties such as potassium and sodium channel activation/inactivation kinetics [51, 52]. However, mutations or chimeras can only improve expression, conductance or activation kinetics, it couldn't increase plateau potentials [51]. Besides ChR2, there are still some other depolarizing opsins, such as archaerhodopsin-3. ChR2 has some variants, including ChR-2/H134R, ChR-2/C128X, ChR-2/E123T, Volvox carteri ChR-1, Chimera EF, Chimera EF with I170V mutation, ChR1, VChR2, and Chimera D [49]. A recent report showed the main important characters of remarkable channelrhodopsin variants, including absorption of the channels like red-shift ChRs, relatively small desensitization, fast kinetics of the channels, and selectively Ca^{2+} permeable phenomena [53]. Thus, broader application would be available according to different ChR variants. Lin JY described how to select ChR variants according to crucial parameters in experiments [54]. The parameters include seven properties: channel conductance, ion selectivity, channel kinetics, desensitization and recovery of the desensitized component in the dark, light sensitivity, spectral response, and membrane trafficking [54]. ChR variants should be cell-type specific because of different membrane properties and different experiment conditions. The features and limitations of currently used ChR variants are discussed in this paper [54].

The most frequently used hyperpolarizing opsins are halorhodopsins (NpHRs), light-activated chloride pumps discovered in archaebacterial. Neuronal spiking rate is reduced by stimulating eNpHR [55]. When NpHRs are activated by yellow light (590 nm), the neuron membrane hyperpolarizes due to chloride ions flowing into the cell so as to inhibit activities of neurons. Currently, three generations of NpHR have been used in the experiments. i) For the first-generation NpHR, export from the endoplasmic reticulum at early trafficking step was found to be impaired, leading to intracellular accumulations [56]; ii)The second-generation NpHR (eNpHR2.0) is specifically for excitatory glutamatergic neurons, and its expression is under control of calcium/calmodulin-dependent protein kinase II promoter [57]; iii) The third-generation NpHR is the most current version (eNpHR3.0). The eNpHR3.0 is highly stable with long time scales [56].

Researchers need to control the expression of the chosen opsin. Overexpression can be useful but high level expression may lead to toxicity, indicating that the lowest possible expression levels should be used for long-term ChR2 expression [58].

2. Viral Vectors

Introducing the viral vectors into the developing brain has been shown to be very useful to achieve widespread gene delivery in cortical neurons [55]. Two categories of nonreplicating viral vectors have been in widespread use in optogenetics: lentiviral vectors and adeno-associated viral (AAV) vectors. By using viral vectors, researchers may get stable long-term expression and high transgene levels in the host-cells so as to achieve specific research purposes. Lentiviral and AAV vectors have different properties: i) Lentiviral vectors are permanently integrated with the genome of host cells, but AAV vectors are mostly expressed in extra-chromosome; ii) AAV vectors can be inserted with constructs up to 5 kb, whereas the inserted length for lentiviral vectors is limited to 10 kb [59]; iii) AAV vectors have a more stable insertion site than lentiviral vectors and may be less likely to induce insertional mutagenesis [2, 60]. By enhancing trafficking, a lentiviral vector, a less strong promoter, may increase the efficiency of delivery into brain [61]. Thus, optogenetics make it possible to manipulate cellular behavior *in vivo*, not requiring the application of exogenous chemical cofactors or reagents [62].

3. Frequency of Optogenetic Stimulation

Stimulation frequency and duration play an important role in optogenetic manipulation. It has been reported that low frequency of optogenetic stimulation induces the releases of amino-acid neurotransmitters; however, higher frequency of optogenetic stimulation induces the releases of both amino-acid neurotransmitters and neuropeptides [63]. It has also been found that dopamine neurons in ventral tegmental area show different release pattern when receiving low (5Hz) or high (50Hz) frequency of optogenetic stimulation [64]. Because too much power released by optogenetic fiber tip may cause tissue damage [65], the lower frequency of optical stimulation would be a better selection.

4. Optopharmacology

Optopharmacology combining optogentics with chemistry gives rise to much more advantages for the treatment of brain diseases compared to conventional pharmacology. There are at least three ways of optopharmaology for control of native neuronal proteins, including endogenous channel plus exogenous caged compound, endogenous channel plus exogenous photoswitch, and genetically tagged channel plus exogenous photoswitch [66]. Since optogenetic technology may stimulate specific neurons as described above, optopharmacology may specifically regulate ion channels and receptors of neurons in the brain. Optopharmacology may produce "brain activity map" by inactivating and activating specific channels or receptors in specific neurons [66]. Moreover, photoswitch may regulate cis and trans isomers of some chemicals.

A light switch has been used as a tool for the remote control of pain [67, 68]. Kokel and colleagues discovered a small molecule called optovin, which can render pain-related neurons responsive to light directly [69]. Optovin is a light-sensitive ligand for TRPA1 [a member of transient receptor potential (TRP) family], which can be reversibly photoactivated by illumination and thus mediates the transduction of pain signals in sensory neurons [69]. Under the condition of light stimulation, optovin seems to form reversible compounds

with cysteine residues of TRPA1, leading to cation ions flowing into cells and resulting in potential changes [69]. Moreover, optovin can also be used in cultured HEK cells expressing human TRPA1 to activate TRPA1 by light switch [67]. In addition, Mourot and colleagues reported another small photoisomerizable molecule, quaternary ammonium-azobenzene-quaternary ammonium (QAQ), which is also sensitive to illumination [68]. QAQ light-dependently inhibits the development of action potential in pain-related neurons by blocking cation channels intracellularly. Therefore, QAQ can reduce nociceptive neuron firing and then inhibit pain signaling [68]. The discovery of optovin and QAQ will help us develop useful optopharmacological approaches for clinical use [67-69]. Taken together, optopharmacological control of channel gating has been used to study functions of different channel subunits [70, 71].

5. Mechanisms Underlying Optogenetic Manipulation

Optogenetic manipulation can produce circuit-specific neuromodulation by overexpressing light-sensitive proteins (opsins) in particular cell types of interest. This is accomplished by the use of viral vectors that infect only certain types of neurons through cell type-specific promoters, such as CaMKIIα, which will localize optogenetic proteins to excitatory neurons [72]. The most extensively used light-sensitive proteins are channelrhodopsins (*e.g.*, ChR2), which are light-gated cation channels that allow positively charged ions (primarily Na^+) to flow into intracellular space. These channels open when activated by blue light (472 nm) and are used to induce neuronal excitation (Fig. **1**). Oppositely,

neuronal inhibition can be achieved via the expression of halorhodopsins (*e.g.*, eNpHR3.0), a chloride pump activated by yellow light (590 nm) [56, 73]. The intracellular flow of negatively charged Cl^- through the chloride pump will induce neuronal inhibition (Fig. **1**). By expressing the two opsins in the same neurons, it is possible for us to study the behavioral consequences of activating or inhibiting the same ensembles of neurons [56].

V. APPLICATIONS OF OPTOGENETIC MANIPULATION IN PAIN STUDY

Currently, optogenetics are mainly used in the following aspects: (1) neurotransmitter specific signaling and function; (2) analysis of function and connection for neuronal networks. Optical stimulation can be used to stimulate the exact layer neurons strictly expressing light-sensitive elements. Thus, optogenetic manipulation can precisely control neuronal activity in the intact network [74]. Because a single neuron can form multiple synapses with various neurons [53], it is possible to analyze the network or neuronal project by stimulate specific neurons with light. A recent study [75] showed the functional connection between mouse rostral forelimb area and caudal forelimb area by combining optogenetics with electrical recording. In this study, *in vivo* ChR2-mediated optogenetic manipulation was used to dissect the functional connections between the two areas, and electrical recording was used to examine the spiking activities induced by light stimulation [75]. Neuronal network activities can also be examined by somatic intracellular calcium level induced by neuronal action potential [76].
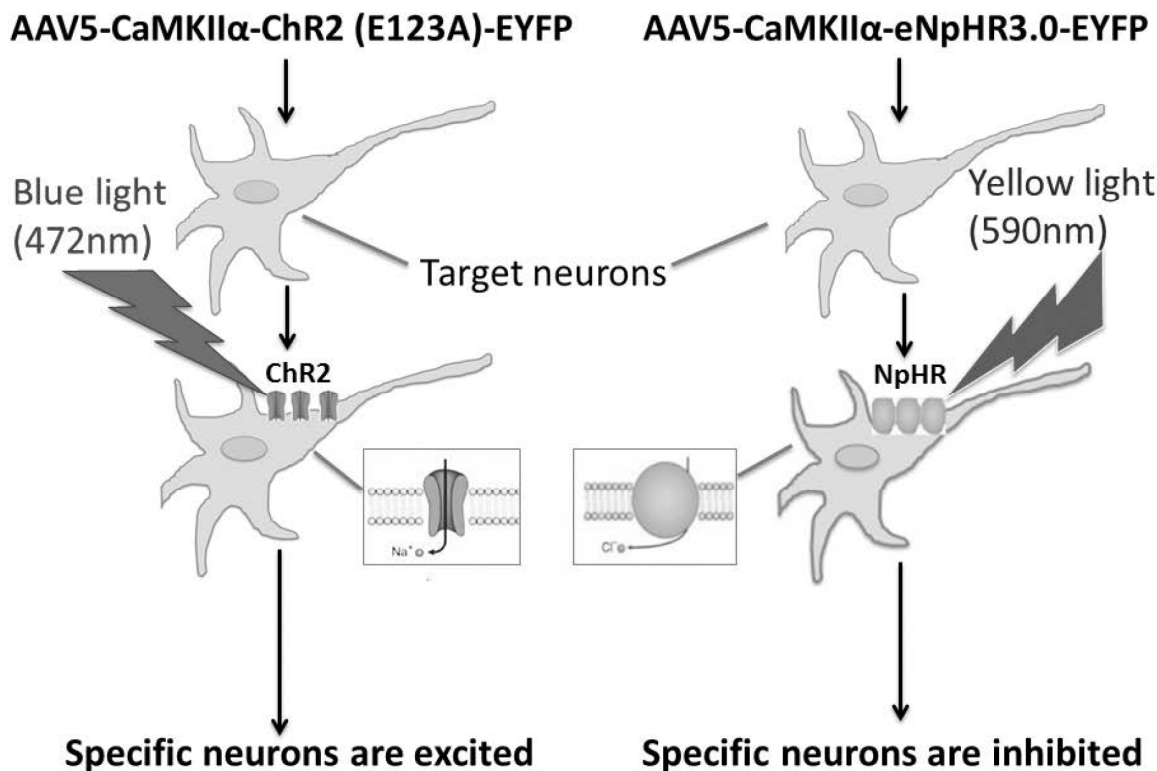

Fig. (1). Mechanisms underlying optogenetic manipulation.

Table 1.   **Advantages and pitfalls of different neuromodulation techniques.**

Neuromodulation Techniques	Advantages		Pitfalls	
DBS	1)	High temporal precision;	1)	Low spatial precision;
	2)	Effective treatment for some neurological disorders;	2)	Cannot induce neuronal inhibition;
	3)	Multiple deep brain nuclei available.	3)	No cell-type specificity.
MCS	1)	High temporal precision;	1)	Low spatial precision;
	2)	Can be used as a non-invasive transcranial magnetic stimulation;	2)	Cannot induce neuronal inhibition;
	3)	Has been used to treat intractable neuropathic pain since 1991.	3)	No cell-type specificity.
Optogenetic manipulation	1)	High temporal precision;	1)	Costly;
	2)	High spatial precision;	2)	Variable opsin expression;
	3)	Can induce both neuronal excitation and inhibition;	3)	Not all target neurons take up virus.
	4)	High cell-type specificity.		

In recent years, optogenetic manipulation has emerged to be used in pain study. It has been reported that activation of metabotropic glutamate receptor 5 activation in the central nucleus of the amygdala (CeA) produces bladder pain and that optogenetic activation of the CeA markedly increases the visceral pain response [77]. It has also been reported that impaired medial prefrontal cortex function contributes to cognitive decision-making deficits under persistent pain [78]. In addition, Barish PA *et al.* [79] designed an optically active μ-opioid receptor by inserting the intracellular domain of the native μ-opioid receptor into the intracellular sequence of rhodopsin. This study applied optogenetic technique to develop a new pain-killer for the treatment of chronic pain [79]. Furthermore, recently optogenetic manipulation has been used to regulate pain pathways in freely moving mice [80, 81]. The optogenetic approach may be employed to help us understand the molecular mechanisms underlying pain processing and may also be used as a novel neuromodulation therapy for intractable chronic pain.

VI. CONCLUSIONS

Different neuromodulation techniques have their advantages and pitfalls (Table **1**). DBS and MCS have been successfully used in the treatment of the CNS diseases including chronic pain, but the mechanisms underlying their analgesic effect remain poorly understood. Both DBS and MCS, while temporally precise, indiscriminately affect cellular elements throughout a volume of tissue. Optogenetic manipulation is uniquely useful in unraveling neuronal circuits in the CNS by enabling reversible gain- or loss-of-function of discrete populations of neurons within restricted brain regions. This revolutionary technology can produce highly precise spatial, temporal, and circuit-specific neuromodulation, thereby enhancing our understanding of the mechanisms underlying DBS and MCS. Optogenetic modulation has some advantages compared to DBS and MCS. For instance, it can spatially activate or inhibit specific neuron populations to control the function of neurons in particular brain area. Moreover, it can also be used to study neural connectivity by combining with electrophysiological recording. However, the optogenetic modulation system is

relatively costly. And it also needs professional training in optical fiber implantation, virus injection, and stereotaxic surgery. The parameters including stimulation frequency and current need to be optimized to obtain the maximal efficacy. Therefore, this new technique still needs more evaluations to fine-tune its parameters before it can be used to treat patients in the clinic.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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