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Optogenetics Research and the Recent Development in Pain Mechanisms

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ABSTRACT

Optogenetics refers to the use of genetically encoded opsins to specifically activate or inhibit of specific types of neuronal activity and circuits with high spatiotemporal precision control over living tissues and animal models by the development and convergence of natural or transformed opsins tools. Due to the highly temporal and spatial resolution of optogenetics with specificity for tissue and cell type, optogenetic technique system combined with imaging/recording system have now illuminated the causal role of defined cell types and projections ranging from the most basic physiological function to disease-related physiology and behaviors. With the drastic development of science and technology over time, here we provide a brief overview of the recent breakthroughs based on the application of optogenetic tools to look insights into neuroscience, especially the mechanisms of pain in recent years. As for optogenetics is widely used in the field of neuroscience, physiology, and pathology, optogenetic strategies have been applied to pain processing and perception at the Peripheral Nervous System (PNS) and Central Nervous System (CNS) levels to modulate the vast complexity of excitatory and inhibitory neural networks. The regulation of pain incorporates multiple brain regions underlying complicated cellular and circuitry mechanisms in the CNS. Mutated optogenetic techniques make it possible that consistent and robust genetically restricted expression of opsins in the PNS neurons, and wireless μLEDs enable the restricted delivery of light to the neurons. On the promising future, the optogenetics toolsets will not only more mature but also combine more closely with other techniques including neural recording and imaging tools, as well as neural connectivity and cell phenotyping tools.

Keywords: Optogenetics, Opsins, Pain, Peripheral nervous system, Central nervous system.

INTRODUCTION

Optogenetics refers to the use of genetically encoded opsins to specifically activate or inhibit specific cellular functions living tissues and animal models [1]. In comparison to pharmacological manipulations and electrical stimulations, optogenetics provides a high spatial and temporal control selectivity over cellular activity. Though there are numerous reviews published on optogenetics, there is drastic development of science and technology over time. Here we provide a brief overview of the recent breakthroughs based on the application of optogenetic tools to look insights into neuroscience, especially the mechanisms of pain in recent years.

MATERIALS AND METHODS

Optogenetics

The development and convergence of optogenetic tools and the use of genetically encoded opsins to control neuronal circuits [2], enables spatial and temporal control of neuronal activity.

Optogenetics was not established until the algal opsin channelrhodopsin-2 (ChR2) and its application to control the activity of mammalian neurons with high spatiotemporal precision in heterologous expression was identified. The initial evidence by Oesterhelt and Stoeckenius in 1971 of rhodopsin-like proteins from microbial organisms was surprising and intriguing [3]. Normally, it takes many years for a high-resolution crystal structure of channelrhodopsin to be observed, and in 2014 through structure-guided engineering of the opsin channel pore, inhibitory chloride-conducting channels were created in 2014, followed by identification of a natural chloride-conducting channelrhodopsin in 2015. Over the years, more variants with faster kinetics, bistable properties, altered ion conductance and shifted color-response properties in these opsin families have been discovered in nature or engineered in the laboratory.

Selective excitation or inhibition in cells is achieved by changing the cell membrane potential. Taking ChR as a classical example, its protein crystal structure is combined with 7 transmembrane helices, an all-trans-retinal and three pores. The main principle of optogenetics is the use of viral vectors and gene editing techniques to carry different promoter to genetically introduce light-sensitive proteins into specific types of neurons (such as glutamatergic neurons, GABAergic neurons, etc). The light-sensitive ion channels expressed in the neuron membrane absorb the photon, change its conformation, and consequently cause discontinuous inflow [4]. This means that the opsins can possess a different spectral sensitivity, mechanism of function, and a net effect on the cell [4]. Therefore, changing the residue of the mutated pore portion can positively change the ion selectivity by the opsin from a cation to an anion.

Opsins

Due to the high temporal and spatial resolution of optogenetics with specificity for tissue and cell type, one-component optogenetic tools have a major impact on neuroscience. They allow particular cells in complex neural tissues to be specifically regulated [5]. The natural or transformed opsins typically allow light to either excite or inhibit neurons directly or allow light-activated modulation of subcellular functions. There are three major classes of microbial proteins used for single-component optogenetics: channelrhodopsin (ChR), bacteriorhodopsins (BR), and halorhodopsin (HR) [6,7]. ChR is a subgroup of rhodopsins family, which is a photoreceptor in unicellular green algae and is a 7-transmembrane non-selective cation channel protein with a maximum absorption wavelength of 470 nm, that mediates intracellular signaling via a long C-terminal extension [7]. Ion conductance can largely be described by a four-step photocycle, where there are two open and two closed states. Upon absorption of a photon, the retinal chromophore isomerizes, i.e. all-trans retinal to 13-cis retinal, triggering a series of structural changes leading to channel opening, extracellular cations influx, and this causes depolarization of neuronal membrane potential and therefore action potential burst.

On the other side, the maximum absorption wavelength of BR is yellow light 560 nm. After absorption of the yellow light photons, there is a change in retinal isomerization of BR conformation hence pumping protons out of cells, thus inhibiting neurons. Earlier it was discovered that HR elicited a polymerized state in cells when expressed at high levels, affecting the function of the protein (Figure 1). The BR pump protons out of the cell while HR pumps chloride ions into the cell, which typically inhibit neurons activity. Photons of a specific wavelength can temporarily turn on ion channels to the photosensitive state, which quickly turns off when the light is removed. Both *in vivo* and *in vitro* experiments have shown that the light-sensitive inflow current can follow up the photon illumination of 20 Hz or even higher frequency, exhibiting the high temporal and spatial resolution.

With the growth and diversification of the content of the optical gene toolbox, more opportunities for advancing the field of neuroscience have increased. Apart from these three common opsins, OptoXRs is a chimera receptor between a rhodopsin and G protein-coupled receptor (GPCR) [8], which can specifically control GPCR signaling pathway in vivo. In intracellular circulation, when activated by 500 nm green light, OptoXRs further activate Gq, Gs or Gi protein signaling in cells. Amongst them, Gq activates downstream IP3 and DAG, or Gs and Gi may activate downstream cAMP, affecting the excitability and function of glutamatergic or monoamine neurons. And, optoactive -opioid receptor (optoMOR) decreased the Ca^{2+} influx and inhibited the forskolin-induced cAMP generation, activation of CREB, and BDNF levels in optoMOR-expressing cells [9].

Additionally, researchers have engineered optogenetic inhibitors based on high-resolution crystal structures. By completely excluding protons and larger cations the Anion Channel Rhodopsins (ACRs) hyperpolarized the membrane of cultured animal cells with less than one-thousandth kinetics of the light intensity required by most efficient currently available opsins. Therefore, it provided highly sensitive and efficient membrane hyperpolarization hence neural silencing through light-gated chloride conduction [10]. The ACRs specifically conducted anions via six conserved carboxylates having a neutral residue (i.e. E9Q, E56Q, E64Q, E159Q, E219Q and D230N), and anion

transport activity was measured using E. coli expression system. Among them, mutated E159Q and D230N exhibited significantly lower anion transport activity compared to wild-type ACR2, suggesting that E159 and D230 play important roles in anion transport [11]. Moreover, replacing of E90 in the central gate of ChR with positively charged residues produces chloride-conducting channelrhodopsin (ChloCs) with negligible cation conductivity. And, stabilizing the open state dramatically increased the operational light sensitivity of expressing cells (slow ChloC) [12]. Both engineered channelrhodopsins, the inhibitory C1C1 (iC1C1) [13] and slow ChloC, exclude sodium and potassium ions, but conduct chloride, effectively inhibiting action potentials in cultured neurons [14].

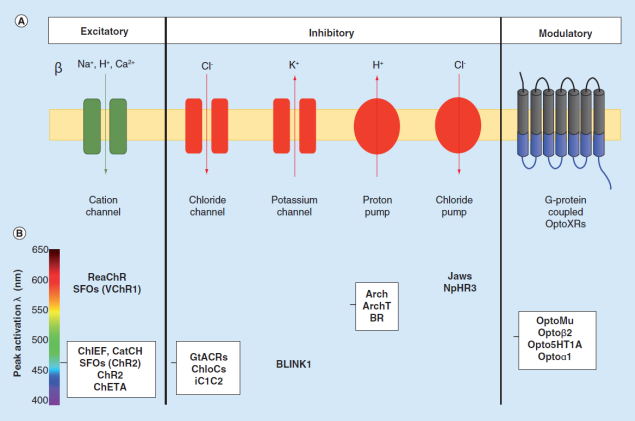


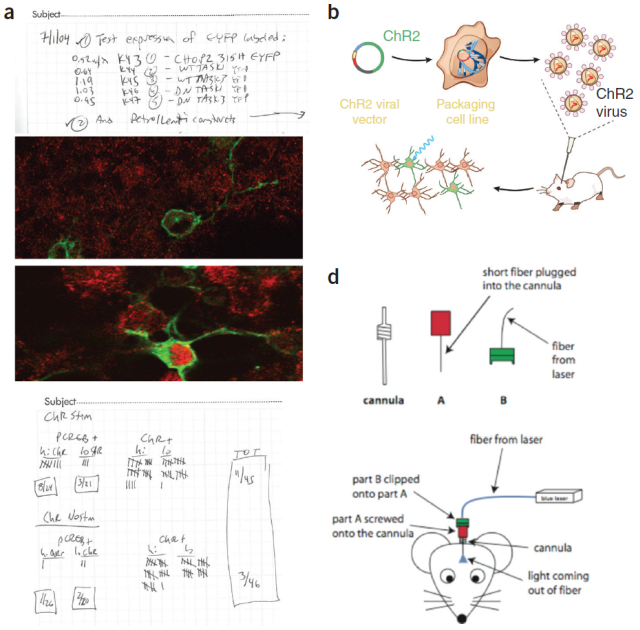
Figure 1: Classification of major optogenetic tools [2]. (A) Opsins are optogenetic actuators that can be categorized into activators, inhibitors or modulators. Most excitatory opsins are nonselective cation channels whereas inhibitory opsins are hyperpolarizing channels (chloride or potassium) or pumps (proton or chloride). Optical activation of OptoXRs recruits specific modulatory G protein-dependent and/or G protein-independent intracellular signaling pathways. (B) Main opsins in each category extensively used this review and their activation peak wavelength (λ).

In addition, when two components of photoswitch ligands and the complementary genetically modified GABAA receptor subunits were conjugated, a comprehensive optogenetic toolkit for controlling GABAA receptor-mediated inhibition in the brain was generated [15]. Optrodes for ChR2-tagged ventral medulla GABAergic neurons showed that they were most active during REM sleep, and least active during eating and grooming [16]. Moreover, some inhibitory opsins have been identified from nature, including the widely used Arch3 [17] and the recently discovered Jaws. Furthermore, an all-optical strategy for simultaneously manipulating and recording the activity of multiple neurons with a cellular resolution by co-expression of a red-shifted opsin and a genetically encoded calcium indicator *in vivo*[18].

Optogenetics techniques and application

In order to improve the use of optogenetics techniques for scientific research, one should first select light-sensitive proteins virus carriers, promoters gene, gene expression regulation strategies (such as Cre/loxP recombination system [20]), and establish transgenic animal lines [21] (Figure 2). If a reporter gene (such as a fluorescent protein) is attached in virus injection region, one can affirm that the target genes have expressed in the specific cell population (excitatory or inhibitory neurons and glial cells). In addition to that, the parameters of the light stimulation (wavelength, intensity, stimulation frequency and duty cycle), and the types of neurons or neural circuits for light delivery are set. Cell type-specific activation using optogenetics is a powerful way to investigate this important circuitry [22,23]. The characteristics of neuronal activity in the physiological and pathological activities, combined with optogenetic intervention for animal behavior and neurophysiological activity, can be specifically explored by recording systems such as patch clamps, fMRI and PET for targeting specific neuronal projections in order to discover neural activity and map neural pathways in the brain [24]. In addition, imaging/recording neuronal wireless systems activity in untethered and freely behaving animals have broad application in neuroscience research. A thin flexible probe that combines light sources and photodetectors to submillimeter dimensions, can provide wireless drug delivery and optical stimulation in freely behaving animals for spatiotemporal control of the targeted neuronal activity [25] and circuit [26]. Current wireless optogenetic systems are based on waveguide-coupled LED and implanted microLEDs (LEDs) [27-29] for wide volume delivery of light [30], and are able to optogenetically modulate neuronal networks thus reducing light requirement [31].

Optogenetics is not only applied in the function of specific neurons and neural networks, but also on the neural network corresponding to the behaviors. Various ChR2 transgenic animal models have been generated and are playing important roles in revealing the mechanisms of neural activities, neural circuits mapping [10], controlling the behaviors of animals as well as exploring a new strategy for treating the neurological diseases in the nervous system [32]. Optogenetics is also widely used in the field of sensory information transmission, including olfactory, auditory, visual and tactile [33] treatment, motivation, rewards, and memory in the brain.



types and

Figure 2: Experimental design of optogenetics technique [**19**]. (A): Host neurons differentiated from adult-derived mammalian CNS progenitors. (B): Design and introduction of high-titer opsin virus and focal illumination of transduced brain regions. (C): Initial engineering sketch of the fiber-optic neural interface for spatially registering viral transduction with focal high-intensity illumination.

For instance, chronic exposure to stress, linked to the manifestation and pathophysiology of neuropsychiatric illness, causes cognitive deficits, anxiety, and depression. Earlier studies have suggested that the Prefrontal Cortex (PFC) and Basolateral Amygdala (BLA) can differentially modulate the stress-induced alterations either by their action on HPA axis or via direct reciprocal connections between them. Optogenetic stimulation of medial Prefrontal Cortex (mPFC) terminals in the BLA produces rapid and long-lasting antidepressant effects [34]. Selective inactivation the hippocampus (HPC)/mPFC pathway [35] or inactivation the mPFC [36] by optogenetics completely reverse depressive behavior. On the other hand, the BLA is connected through projection neurons to the mPFC, HPC and various other regions involved in higher-order behavioral processes [37]. And, the projections from the Ventral Tegmental Area (VTA) to the Nucleus Accumbens (NAc) and the mPFC involve in eliciting depressive-like behaviors in stress-resilient mice [38]. Experimental enhancement of hyperpolarization-activated current or optogenetically increasing the hyperactivity of VTA dopamine (DA) neurons in social defeat stress model of depressed mice completely reversed depression-related behaviors, an antidepressant effect achieved through resilience-like, projection-specific homeostatic plasticity [39]. In addition, optogenetic inhibition of the dorsal raphe nucleus (DRN)-lateral habenula (LHb) circuit results in activation of LHb and depression-like behaviors in chronic unpredictable mild stress models [40]. Moreover, optogenetic cortical spreading depression induction has significant advantages over current models in that multiple cortical spreading depression events can be elicited in a non-invasive and cell type-selective fashion [41].

What is more, optogenetically depotentiation of the conditioned stimulus (CS)-specific auditory pathways to the BLA suppressed conditioned fear responses to the CS [42]. While temporally-specific optogenetic inhibition of dopamine neurons in the VTA during the unconditioned stimulus (US) omission to prevent fear extinction [43]. So, optogenetic high-frequency stimulation of BLA inputs to mPFC interfered with retention of cued associations, attenuated previously acquired cue-associated responses in mPFC neurons and facilitated extinction [44].

Besides, when these neurons are optogenetically inhibited, the activity of these neuroendocrine axes are suppressed and anxiety-like behavior in the elevated plus maze is dampened [45]. Optogenetic activation of contralateral PL

excitatory neurons exerts analgesic and anxiolytic effects in mice subjected to chronic pain, whereas inhibition is anxiogenic in naive mice [46]. Photostimulation of LC-NE fibers in the BLA evokes norepinephrine release in the BLA, alters BLA neuronal activity, and increases anxiety-like behavior [47]. Optogenetic activation of the BLA-PFC circuit in unstressed mice produced persistent increased anxiety-like behavior and hyperactivity in the elevated plus-maze profile [48]. Optogenetic stimulation of the ACC was sufficient to induce anxiety and depressive-like behaviors in naïve animals [49]. Optogenetic silencing of the LHb during uncontrollable stress blocked the typical anxiety-like behaviors produced in male rats [50].

Time-locked slow-wave sleep was induced in mice by optogenetically stimulating GABAergic neurons in the parafacial zone, providing a direct approach to analyze the influences of slow-wave sleep on learning and memory with a clear-cut time-windows [51]. Brief, peri-shock, optogenetic inhibition of glutamatergic cells in the BLA can also alter the effects of fear memory on Rapid Eye Movement sleep (REM) without altering the stress response or behavioral fear [52].

And then, optogenetic stimulation of DG neural ensembles representing a contextual fear conditioning memory increased memory retrieval in the appropriate context in Alzheimer disease (AD) mice Moreover, optogenetic stimulation facilitated reactivation of the neural ensembles that were previously activated during memory encoding. These data suggest that DG manipulation as a potential target to treat memory loss commonly seen in AD [53].

Optogenetic stimulation could inspire/replace Deep Brain Stimulation (DBS) for novel treatments of behavioral diseases [54]. Once the in vitro emulation of the successful optogenetic protocol with DBS validated in animal models, DBS protocols can be designed for human applications [55].

Moreover, activation of co-expressed light-activated ion translocators, ChR2 and Arch, after tumor formation significantly increases the frequency with which the tumors regress in a process called normalization [56]. Optogenetic activation of VTA dopaminergic neurons microinjected with Adeno-Associated Virus (AAV) to express ChR2 produce a significant but transient anti-allodynic effect in nerve-injured or tumor-bearing mice without increasing thermal stimulation response thresholds in sham-operated animals [57].

The application of optogenetics in pain research

As for optogenetics is widely used in the field of sensory information transmission, in the brain, pain incorporates multiple components of the nervous system, at the Peripheral Nervous System (PNS) and Central Nervous System (CNS) levels, unlike some neurobiological disorders which can be attributed to specific brain regions. Chronic pain is an indication of a variety of different underlying pathologies including arthritis, nerve injury, depression, and cancer. An understanding of nociceptive mechanisms and the neurobiology of pain perception is essential to relief both chronic pain and severe side effects. Here the studies that have applied optogenetic tools as an alternative strategy to relief pain by directly modulating somatosensory pathways [21,58,59] are reviewed.

Pain signal transmission is regulated by the vast complexity of excitatory and inhibitory neural networks, so it is necessary for the high temporal and spatial resolution of the optogenetic application in pain research. Therefore, optogenetic strategies have been applied to a number of brain regions that modulate pain processing and perception [60]. For instance, stretchable, multi resonance antennas and battery-free schemes for multichannel wireless operation of independently addressable, multicolor μLEDs are used in the studies of the brain [61]. To successfully carry out the protocol, researchers should have basic skill sets in photolithography and soft lithography, as well as experience with stereotaxic surgery and behavioral neuroscience practices [62].

Numerous clinical and preclinical evidence for the application of optogenetic in CNS suggests that the mPFC-amygdala circuitry dysfunctions trigger pain-related deficits. Optogenetic activation of the PFC not only produces strong antinociceptive effects in a rat model of persistent neuropathic pain but also reduces the affective symptoms of pain [63]. The infralimbic (IL) of the mPFC inputs evokes stronger synaptic inhibition of neurons in the latero-capsular division of the central amygdala nucleus (CeL) than prelimbic (PL) of the mPFC inputs. Optical activation of IL pyramidal cells also inhibited PL pyramidal cells, suggesting that IL output controls PL output [64]. And, selective activation of mPFC cells projected to the brainstem Dorsal Raphe Nucleus (DRN), a serotonergic nucleus implicated in the major depressive disorder, induces a profound, rapid and reversible effect on the selection of the active behavioral state [65].

Further, optogenetic stimulation of the GABAergic inhibitory neurons in the ACC can greatly alleviate pain-associated behavior and decrease abnormal thalamic sensory neuron activity in the trigeminal neuropathic rat model [66,67]. while optogenetic inhibition of ACC excitatory neuronal activity induces conditioned place preference in a

mouse model of chronic inflammatory pain [68]. But optogenetic activation of excitatory pyramidal neurons significantly increased mechanical sensitivity [66]. Optogenetics selective activation of ACC-spinal cord projecting neurons caused behavioral pain sensitization while inhibiting the projection induced analgesic effects [69]. Additionally, Optogenetics activation the cingulate cortex (MCC)-posterior insula pathway can induce and maintain nociceptive hypersensitivity in the absence of conditioned peripheral noxious drive [70].

Similarly, optogenetic activation of the CeA neurons leads to increased visceral pain in the mouse model [60]. In addition, optogenetic activation on the CeL-projecting paraventricular nucleus of the thalamus (PVT) neurons increases optogenetic induced Long-Term Depression (LTD) in the PVT-CeL pathway, as well as a conditioned fear expression [71]. Furthermore, the inhibition-excitation ratio in BLA neurons is increased in the pain model in the IL pathway but not in the PL pathway [72].

The rostral ventromedial medulla (RVM) GABAergic neurons facilitate mechanical pain by inhibiting dorsal horn enkephalinergic/GABAergic interneurons via in vivo opto/chemogenetic manipulations. These interneurons gate sensory inputs and control pain through temporally coordinated enkephalin- and GABA-mediated presynaptic inhibition of somatosensory neurons [73]. Further, the majority of ON- and OFF-cells exert net pronociceptive and antinociceptive effects, responded to optogenetic activation of ChR2-expressing terminals in the RVM, confirming a direct parabrachial nucleus (PB) influence on RVM pain-modulating neurons [74]. Behaviorally, optogenetic stimulation of 5-HT cells in the RVM decreased both mechanical and thermal pain threshold in an intensity-dependent manner, with repeated stimulation producing sensitized pain behavior for up to two weeks [75].

Moreover, activation of parvalbumin (PV) neurons in the Thalamic Reticular Nucleus (TRN) GABAergic neurons promotes sensitivity to pain in mice [76]. Optogenetic activation of GABAergic transmission in the ventrobasal thalamus (VB) attenuated CFA-induced thermal hyperalgesia [77]. In addition, optogenetic activation of the monosynaptic projection on lateral PB induces robust escape/avoidance behaviors, whereas optogenetic silencing specifically reduces facial nociception [78].

However, widespread implementation of optogenetics in studies of the PNS presents two major obstacles, consistent and robust genetically restricted expression of opsins in the PNS neurons, as well as restricted delivery of light to the neurons expressing these opsins as compared to CNS applications (Figure 3) [79].

In order to solve these obstacles, new technologies have been designed to manipulate primary somatosensory neurons and peripheral primary afferents optogenetically. And different serotypes of AAV and various injection methods are used to transduce neurons in Dorsal Root Ganglia (DRG). For example, the LEDs implanted in DRG allow focal DRG-specific control of visceral and/or somatic afferents in conscious mice. Optogenetic increased the action potential in the spinal cord and activated the calcium channel in DRG neurons to improve treatment for neuropathic pain [80]. Viral delivery in DRG of an inhibitory opsin enabled light-inducible inhibition of acute pain perception, and reversed mechanical allodynia and thermal hyperalgesia in a model of neuropathic pain [81]. And, both the mechanical paw withdrawal threshold and the radiant heat evoked paw withdrawal latency were significantly increased upon illumination by green light inhibit the paw of a viral-vector injected in DRG mice [82].

In addition, optogenetic activation of PV-ChR2 neurons in the spinal dorsal horn induces GABA release from presynaptic terminals [83]. The optogenetic inhibition of GABAergic interneurons in the spinal cord dorsal horn produced a transient, but selective induction of mechanical hypersensitivity [84]. Although optogenetic stimulation of spinal astrocytes induces pain hypersensitivity [85]. Optogenetics stimulation of primary somatosensory cortex strongly suppresses spinal trigeminal nucleus caudalis (SpVc) responses to noxious stimuli thereby producing behavioral hypoalgesia [86]. On the contrary, application of optogenetics triggered motor cortex stimulation which provided relief of chronic neuropathic pain [87]. Moreover, illuminating light to the hind paw skin of rat increased the number of spinal dorsal horn Lamina I active neurons, as well as number of activated CeA neurons, and produced an aversion to illumination [88].

Each W-TChR2V4 transgenic lines of rat, coding an algal photoreceptor molecule, shows a sensory-evoked behavior in response to the blue LED flashes on the plantar skin [89]. This can inadvertently activate other closely apposed organs, or co-activate different classes of axons in the same organ to selectively control nociceptive and/or non-nociceptive pathways to specific visceral organs *in vivo* [90,91]. The acute and repeated optogenetic activation of low threshold nociceptive afferents produced robust nocifensive behavior and mechanical sensitization in freely behaving mice, respectively [83].

Furthermore, Arch-driven hyperpolarization of nociceptive terminals was sufficient to prevent ChR2-mediated mechanical and thermal hypersensitivity, and prolonged optical silencing of peripheral afferents led to

poststimulation analgesia with a significant decrease in mechanical and thermal hypersensitivity under inflammatory and neuropathic conditions [92]. Transdermal blue light stimulation of the hind paws of transgenic mice expressing ChR2 in TRPV1+ neurons generated nocifensive behaviors consisting mainly of paw withdrawal and paw licking, while paw lifting occurrence was limited [93]. Additionally, ChR2 has been expressed in Nav1.8+ primary sensory neurons, which includes most nociceptors and some low threshold mechanoreceptors [94].

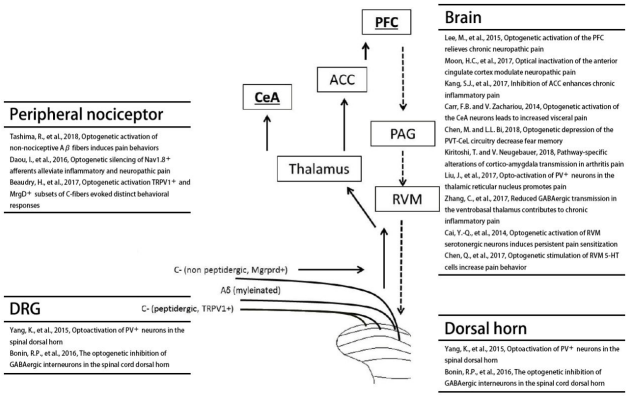


Figure 3: Overview of the pain pathway, and a list of optogenetic studies performed to explore each level. The complete arrows indicate the ascending pain pathway, while the descending pathway is represented by dashed arrows. Different categories of nociceptor, including unmyelinated C-fibers and myelinated A-fibers, are shown. For clarity, only specific brain regions activated during nociception are shown: Prefrontal Cortex (PFC), Anterior Cingulate Cortex (ACC), Central Nucleus of the amygdala (CeA), Parabrachial Area (PB), Periaqueductal Gray (PAG), Rostral Ventromedial Medulla (RVM) and Dorsal Root Ganglia (DRG)[60].

Together, these results illustrate that the application of optogenetics to CNS and PNS studies may increase our understanding of the contributions of specific populations of sensory neurons in pain processing. The use of cell biological principles to enable expansion of the optogenetic technologies suitable for intact-systems biology and behaviors is also explicit from this.

With limitations in reducing the suffering of chronic pain patients in our aging society, a novel therapeutic approach such as optogenetics is more than welcome to give hope for treatment and nursing providers. The latter tools are rapidly improving, in part because optogenetics has helped to galvanize broad interest in neurotechnology development [95]. On the promising future, the optogenetics toolsets will not only be more mature but also combine closely with other techniques such as neural recording and imaging tools, as well as neural connectivity and cell phenotyping tools.

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