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·Review·

Optogenetics in neuroscience: what we gain from studies in mammals

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Abstract: Optogenetics is a newly-introduced technology in the life sciences and is gaining increasing attention. It refers to the combination of optical technologies and genetic methods to control the activity of specific cell groups in living tissue, during which high-resolution spatial and temporal manipulation of cells is achieved. Optogenetics has been applied to numerous regions, including cerebral cortex, hippocampus, ventral tegmental area, nucleus accumbens, striatum, spinal cord, and retina, and has revealed new directions of research in neuroscience and the treatment of related diseases. Since optogenetic tools are controllable at high spatial and temporal resolution, we discuss its applications in these regions in detail and the recent understanding of higher brain functions, such as reward-seeking, learning and memory, and sleep. Further, the possibilities of improved utility of this newly-emerging technology are discussed. We intend to provide a paradigm of the latest advances in neuroscience using optogenetics.

Keywords: optogenetics; channnelrhodopsin; halorhodopsin; mammals

1 Introduction

Since Fork *et al.* (1971) first activated abdominal ganglion neurons with bacteriorhodopsin (BR), a light-sensitive protein[1], numerous researchers have tried hard to explore better solutions for the optical control of neuronal activity. In 1983, Farber and Grinvald successfully initiated action potentials in neurons using a fluorescent dye and light[2]. Later, Schmucker *et al.* combined a laser and a chromophore, and elicited inhibition of cells in larval *Drosophila*[3]. These preliminary efforts accelerated the subsequent generation of multi-component optical tools with higher efficiency. Zemelman and colleagues created an explosive combination termed "chARGe", which is composed of arrestin-2, rhodopsin, and the α-subunit of a heterotrimeric G protein, to influence neuronal activity with light[4]. The strategy of caged compounds was also effective in binding to ectopically-expressed ligand-gated ion channels in neurons by the photolysis of specific proteins, thus interfering with neuronal activity[5] while the diffusible uncaged ligand and other concomitant products could give rise to unexpected interruptions[6]. Moreover, Matthew Banghart and colleagues developed a new strategy for engineering optical control directly into ion channels by tethering the photoisomerizable synthetic molecule MAL-AZO-QA (i.e. maleimide-azobenzene-quaternary ammonium) to a modified Shaker K^+ channel protein. Before treatment with MAL-AZO-QA, the Shaker K^+ channel was mutated to ensure that light was the principle modulator of gating[7]. This alternative strategy for controlling neuronal activity was a great progress in terms of cell specificity and avoidance of interference during manipulation. But its multi-component nature hinders its application as an optical tool for controlling particular neurons. Eventually, Karl

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Deisseroth's laboratory in 2005 first developed the use of a single component, channelrhodopsin-2 (ChR2), to drive hippocampal neurons[8].

An optimal optogenetic design includes four steps[9]. First, appropriate optogenetic tools (proteins) are needed to control neuronal activity. These proteins, which are light-activated, allow diverse interventions in neuronal activity for different purposes (Table 1). The original microbial opsins, ChRs, are used to activate neurons, while halorhodopsins (NpHR) and BR are inhibitory optogenetic tools. However, these proteins are inefficient in terms of stable expression and the control of spiking properties and photocurrent. In order to optimize these optogenetic tools, many modified strategies have been offered, including genetic variants[10], chimeras[11], and structural engineering[12]. Recently, researchers have developed new optogenetic tools to regulate cellular biochemical signaling[13]. They combined the light-activated opsins and G protein-coupled receptors or GTPase with engineering approaches and found that this is efficient in controlling cellular signal transduction. Second, optogenetic tools can be transfected to targeted neuronal groups by specific vectors. The most widely used vectors are viruses, which are capable of fast targeting and constant load[14,15]. Lenti- and adeno-associated (AAV) viral families, together with other viruses such as *Herpes simplex* virus (HSV) families and pseudotyped lentiviruses, are the traditional candidates for delivering optogenetic tools to neuronal clusters with fewer cellular interruptions, but the genetic length of the optogenetic tools delivered is not long enough. However, Cre-dependent animal lines are helpful in solving this problem[16]. They are able to carry large genetic fragments, or even a whole genome. Third, light is the operator of neuronal activity. Since different opto-

Table 1. Optogenetic tools and related parameters

Optogenetic tools	Effects	Deactivation time-constant	Peak activation wavelength (nm)
ChR2	Excitatory	10 ms	470
ChR2-H134R	Excitatory	18 ms	470
ChETA	Excitatory	E123A 4 ms	470
		E123T 4.4 ms	500
		T159C/E123T 8 ms	500
		C1V1 120 ms	540
		C1V1(E162T) 50 ms	535
		C1V1(E162T/122T) 38 ms	545
ChIEF	Excitatory	10 ms	450
C128S	Excitatory	1.7 min	470/560
D156A	Excitatory	6.9 min	470/590
VChR1	Excitatory	133 ms	545
NpHR 3.0	Inhibitory	4.2 ms	590
Proton pumps	Inhibitory	18.9 ms	560
Arch	Inhibitory	9 ms	566
β2AR	Biochemical signaling	500 ms	500
α1AR	Biochemical signaling	3 s	500
Rh-CT	Biochemical signaling	3 s	485
b-PAC	Biochemical signaling	20 s	441

genetic tools are sensitive to light of specific wavelengths, the delivery of light determines the activation or inhibition of neurons. Lasers, mercury arc lamps, and light-emitting diodes are successfully used to stimulate specific optogenetic tools in targeted neurons, thus achieving control of cell groups. Finally, readout systems with high resolution are essential to investigate or analyze the collected data. The first used device was the optrode, which combines a fiber-optic cable with a tungsten electrode[9]. Subsequent attempts focused on the combination of silicone multi-site electrodes with optical fibers[17] or movable tetrode arrays[18]. Recently, the two-photon microscope was used to optimize the readout system for investigation of neuronal activity in real-time[19]. Thus, a perfect readout system is helpful in making better use of optogenetic technology.

2 Application of optogenetics in mammals

The rapid development of optogenetics has provided considerable data for understanding the complicated mechanisms of behavioral responses. In the past seven years, the application of optogenetics was focused on learning and memory, sleep, addiction, Parkinson disease, and retinal diseases, among others (Table 2). Here, we discuss the recent progress in these fields in terms of different brain regions.

2.1 Cerebral cortex ChR2 and enhanced *Natronomonas pharaonis* halorhodopsin (eNpHR) are the most widely used optogenetic tools to control neuronal activity in the cerebral cortex. Combined with the transgenic animal lines noted above and AAV vectors, optogenetics has been applied to reveal the mechanisms of generation of the cortical γ-rhythm. Optogenetic activation or inhibition of fast-spiking neurons by photostimulation of ChR2 or eNpHR initiates or abolishes γ-oscillations, respectively[20,21], during which NMDA receptor activity is essential[43]. Since the γ-rhythm has the characteristics of resonant circuitry, it may influence cortical information-processing[21]. During the activation of fast-spiking neurons that initiates γ-oscillations, sensory responses are inhibited and learning and memory are impaired. Research on higher cortical functions, such as depression and learning and memory, has received much attention recently. In 2010, Herbert and colleagues used ChR2 in the medial prefrontal cortex (mPFC) to study depression. They demonstrated that optogenetic stimulation significantly relieves depressive behavior, without any influence on motor activity or social memory[44]. Previous researchers failed to distinguish the precise neuronal groups associated with learning and memory, while optogenetics provided this possibility. Huber *et al.* (2008) successfully calculated the number of

Table 2. Application of optogenetics in different regions

Brain regions	Optogenetic tools	Vectors	Subjects	Featured publications
Cerebral cortex	ChR2/eNpHR	AAV	γ-rhythm; associative learning	[20, 21, 22, 23]
Thalamus	ChR2	Lentiviruses	Thalamocortical interactions	[24, 25]
Hippocampus	ChR2	AAV	Synaptic plasticity	[26, 27]
Ventral tegmental area	ChR2	Cre recombinase/AAV	Addiction	[28, 29]
Nucleus accumbens	ChR2/eNpHR	Cre recombinase/AAV	Addiction	[30, 31]
Striatum	ChR2	AAV	Parkinson disease	[32, 33]
Amygdala	ChR2	AAV	Fear response	[34, 35]
Hypothalamus	ChR2/eNpHR	Lentivirus	Sleep-wake cycles	[36, 37]
Brainstem and spinal cord	ChR2/eNpHR	Lentivirus/AAV	Sleep; central chemoreceptors	[38, 39, 40]
Retina	ChR2/NpHR	AAV	Treatment of retinal diseases	[41, 42]

neurons necessary for perception by using ChR2 in mouse primary somatosensory cortex and found that optogenetic activation of the targeted neurons apparently elicits associative learning[22]. Interestingly, the same clusters of neurons may display different responses with the same micro-stimulation in different temporal patterns. Activation of one ensemble induces an aversive response while stimulation of another cluster of neurons elicits appetitive effects. Opposite responses can be elicited even in the same ensemble, depending on the learning sequences[45]. Associative fear conditioning is partly attributed to the disinhibition of pyramidal cells in auditory cortex. Optoactivation of parvalbumin-positive interneurons impairs fear learning. Pyramidal disinhibition results from activation of interneurons of layer I by cholinergic inputs, and then inhibits parvalbumin-positive interneurons in layers II/III[23]. Since higher cortical functions are integrated in complicated networks, it is attractive to take advantage of optogenetics to target specific neurons so that the roles of individual parts can be uncovered, then the whole network can be clarified.

2.2 Thalamus Optogenetics has also been used to investigate thalamocortical interactions. Lentiviruses loaded with ChR2 and fluorescent protein genes have been used to specifically target thalamus. Since dense reciprocal projections between thalamus and cortex constitute one of the most essential tracts for processing sensory cognition, revealing the interactions between these regions is necessary for better understanding of the processing of external signals from the environment. In 2010, Scott *et al.* systematically studied the neuronal pathways between thalamus and cortex by using ChR2[24]. Photoactivation of axons from thalamic relay cells to cortex induce synaptic currents in excitatory neurons and fast-spiking neurons, which results in feed-forward inhibition of cortical circuitry. On the other hand, photostimulation of the corticothalamic projection activates neurons of relay nuclei and the reticular nucleus which inhibit relay cells. They provided direct evidence of the reciprocal projection between thalamus and cortex and the feed-forward pathway within it. But how thalamic signals are transmitted to cortex and influence its activity is still under investigation. Firing of thalamic neurons by photostimulation desynchronizes cortical neurons[25]. This indeed revealed that thalamic activity drives cortical responses but the mechanism of desynchrony needs further elucidation. Recently, Halassa and colleagues found that optogenetic activation of neurons specifically expressing ChR2 in the thalamic reticular nucleus changes the firing patterns in the thalamocortical pathway, that is, from tonic to burst firing. Moreover, it gives rise to spindle waves in the cortex[46]. Thus, in spite of the sparse optogenetic investigations of the thalamocortical pathway presently available, it is promising that much will be learned about the role of this pathway in higher cortical functions in future.

2.3 Hippocampus Since Boyden *et al.* (2005) introduced optogenetics into neuroscience for the first time by controlling hippocampal neurons at high spatial and temporal resolution[8], a great deal of research has followed to investigate neuronal responses in the hippocampus by optogenetics. Goold and Nicoll[26] used pLenti-CaMKIIα-hChR2-mCherry-WPRE in the hippocampus to explore the role of autonomous cell excitation in synaptic plasticity. They found that photostimulation of CA1 pyramidal cells decreases the postsynaptic currents of AMPA and NMDA receptors, that is, they play an inhibitory role. Further studies revealed that chronically increased L-type Ca^{2+} currents and subsequent activation of CaMKK–CaM4 signaling are essential in mediating the synaptic depression noted above. Specifically, the GluA2 AMPA receptor subunit is involved in autonomous cell-excitation-induced depression of AMPA receptor currents, a process which requires the synthesis of novel proteins[26]. The internal circuits of the hippocampus involve the Schaffer collateral (SC)–CA1 pathway, which is also influenced by cholinergic inputs from the septal nucleus. Specifically expressing ChR2 in cholinergic neurons by using AAV made it possible to directly investigate their effects on SC–CA1 synaptic responses. Cholinergic regulation of synaptic plasticity relies on the order and interval between the cholinergic stimulation and SC activation[47]. Interestingly, Kohl *et al.* (2011) found differences between left and right hippocampal CA3–CA1 pathways in synaptic plasticity by application

of ChR2. They revealed that optical activation of left CA3 axons induces more LTP than the right, in which GluN2B plays an important role. This is direct evidence of asymmetry between the hemispheres at the pathway level[27]. Behaviorally, the hippocampus is implicated in learning and memory. Goshen and colleagues demonstrated that photoinactivation of these neurons apparently inhibits both the acquisition and retrieval of remote fear memories while optogenetic reactivation of specific neuronal groups is sufficient to elicit a fear response[48]. On the other hand, optogenetics can be used to control epileptiform activity. Tonnesen *et al.* (2009) successfully decreased the burst firing and epileptiform activity of hippocampal CA3 neurons by activating NpHR[49]. They provided a novel preliminary direction for the optogenetic treatment of epilepsy.

2.4 Ventral tegmental area (VTA) Optogenetics has provided direct evidence of the co-existence of glutamatergic and dopaminergic signaling. Using Cre recombinase and vesicular glutamate transporter 2 knock-out mice, Stuber *et al.* directly revealed glutamate release from VTA dopaminergic axons projecting to the nucleus accumbens (NAc)[50]. Interestingly, photoactivation of VTA dopaminergic neurons elicits the redistribution of AMPA receptors[51]. Thus, this suggests that two mechanisms, dopaminergic and glutamatergic transmission, in VTA dopaminergic neurons are involved in addiction. Since reciprocal projections between VTA and NAc exist and they are influenced by each other, it is necessary to clarify the signal pathways responsible for activation of either nucleus, and how they interact and eventually affect behavior. Also, the firing patterns of VTA dopaminergic neurons, tonic and phasic, influence behavioral responses. In 2009, Tsai and colleagues used AAV to specifically express ChR2 in the VTA dopaminergic neurons of mice. They directly demonstrated for the first time that phasic firing patterns, rather than tonic firing, induced by optogenetic stimulation in these neurons are sufficient to initiate conditioned place preference similar to that of behavioral training[28]. Moreover, phasic activation of VTA dopaminergic neurons leads to a positive reinforcement in food-seeking operant tasks, which is absent when there is no food reward in behavioral training. Importantly, such behaviors can easily be regained by photoactivation of the targeted dopaminergic neurons[29,52]. Recently, Witten *et al.* (2011) developed new recombinase-driver rat lines, tyrosine hydroxylase (*Th*)::*Cre* and choline acetyltransferase (*Chat*)::*Cre* lines, to achieve specific optogenetic stimulation of dopaminergic and noradrenergic neurons. They found that photostimulation of dopaminergic neurons with a phasic mode in the VTA leads to a positive reinforcement in intracranial self-stimulation tests[53]. In contrast, optogenetic activation of VTA GABAergic neurons induces an inhibitory response[54,55].

2.5 NAc Medium spiny neurons (MSNs) in the NAc project directly to VTA non-dopaminergic neurons. Photostimulation of MSNs leads to IPSCs in recorded postsynaptic neurons and these are blocked by a $GABA_A$ receptor antagonist, indicating a role of GABA receptors in this mechanism. Further, photoactivation-induced IPSCs are sensitive to opioid, which plays an important role in addiction[31]. So it is important to distinguish the different roles of various pathways involved in addiction. Interestingly, different kinds of neurons in the NAc play distinct roles. Lobo *et al.* (2010) used ChR2 in the NAc and lateral striatum of D1-Cre or D2-Cre BAC (bacterial artificial chromosome) transgenic mice to explore the signal foundations of reward behavior. They found that optogenetic stimulation of D1-positive MSNs apparently facilitates cocaine-induced conditioned place preference while activation of D2-positive MSNs leads to inhibitory effects. Further, they revealed the signaling pathways underlying this behavioral preference by imitating the deletion of BNDF-TrkB signaling. The results showed a decrease of pERK42 and pERK44 expression when activating D1-positive neurons while no significant changes were found during the activation of D2-positive neurons[56]. Interneurons in the NAc also take part in addiction. Witten and colleagues used ChR2 and eNpHR3.0 to control the activity of cholinergic interneurons in the NAc in order to clarify its role in cocaine reward. Optogenetic activation of cholinergic interneurons by ChR2 leads to increased IPSCs in MSNs. *In vivo* studies showed that the excitability of MSNs increases when the cholinergic interneurons are inhibited by eNpHR3.0. And

this blockade results in the disappearance of cocaine-induced addiction[30,57]. Thus, optogenetics allows the exploration of possible solutions to addiction. Specifically, reward value helps subjects to weigh different options in reward-seeking behaviors. Domingos *et al.* (2011) introduced an assay to investigate the reward-value of different nutrients using optogenetics. They combined water drinking and the optogenetic activation of VTA dopaminergic neurons as the reference and found that mice prefer natural nutrients (for example, sugars) to artificial sweeteners (such as sucralose). And food-deprivation leads to an elevated value of sucrose while leptin plays the reverse role[58].

2.6 Striatum Striatal circuits play an important role in the planning and editing of motor activity, and are involved in degenerative diseases such as Parkinson disease (PD). MSNs are the major components of the striatum, and their connections with other neurons are poorly understood. In 2011, Chuhma and colleagues investigated the connections of MSNs by restricted expression of ChR2 and found that they connect to other MSNs and tonically active neurons in the dorsal striatum, while in the globus pallidus, they mainly make connections with type B/C but not type A neurons. Interestingly, MSNs exclusively connect to GABAergic neurons rather than dopaminergic neurons in the substantia nigra pars reticulata[59]. Functional motor deficiency is notable in PD and one of the primary goals in treating this disease is to improve motor functions. Surprisingly, Kravitz *et al.* specifically expressed ChR2 in striatal MSNs to clarify the role of the direct and indirect pathways of the basal ganglia in a mouse model of PD. They found that photoactivation of the indirect pathway results in a Parkinsonian state while optical stimulation of the direct pathway totally reverses the appearance of PD, a possible strategy for treatment[32]. Since dopamine (DA) deficiency is responsible for PD, attempts to control DA release were made in 2010. Caroline and colleagues used optogenetic parameters in the dorsal striatum by specific expression of ChR2 through AAV transfection. Optogenetic stimulation elicited DA release, as with the electrophysiological method. Although these data facilitated the study of DA-related events, further studies are still needed to illustrate the role of DA release in PD[60]. Another strategy for PD treatment is the transplantation of neuronal stem cells. In 2011, Tonnesen and colleagues assessed the properties of transplanted stem cell-derived and host DA neurons in a mouse PD model through optogenetic control. They found that optical stimulation or inhibition of targeted neurons resulted in complex synaptic connectivity between the grafted and host neurons. Thus, the value of transplantation is still controversial[33].

2.7 Amygdala Optogenetics directly revealed the role of the amygdala in the regulation of emotion. By using ChR2 and AAV, optogenetic stimulation of the lateral amygdala and a sound stimulus were presented to candidate rats. Subsequent encounters with the specific sound were sufficient to induce a fear response[61]. Further, photostimulation of the medial subdivision of the central amygdala (CEm) resulted in fear responses in freely-moving mice[34]. However, little is known about the internal circuits of the central amygdala. Haubensak and colleagues used optogenetics to image the microcircuitry of the amygdala. Photoactivation of PKC-δ^+ neurons in the lateral subdivision of the central amygdala (CEl) induced IPSCs in neurons projecting to the CEm peri-aqueductal grey (PAG); similar results were found for CEl PKC-δ^- neurons. Thus, functional connectivity exists between CEl PKC-δ^+ or PKC-δ^- neurons and neurons projecting to the CEm-PAG, which play an important role in associative fear learning[62]. Interestingly, projections from hypothalamic oxytocin (OT) neurons are essential for fear responses. Knobloch and colleagues specifically expressed ChR2 in hypothalamic OT neurons with rAAV vectors. *In vitro* optoactivation of OT neurons resulted in GABAergic inhibition of central amygdala (CeA) output neurons. Furthermore, *in vivo* photostimulation of OT neurons led to significant relief of fear responses. Thus, the hypothalamic OT neurons–CeA pathway plays an important role in fear responses[35]. The amygdala is also involved in the regulation of anxiety and reward-seeking. Application of optogenetics to basolateral amygdala (BLA) axons projecting to the CeA facilitates the exploration of this circuit in anxiety. Photostimulation of the targeted terminals results in reversible anxiety, while

optical inhibition of these projections leads to enhanced anxiety. Interestingly, optical control of neuronal somata fails to induce these effects[63]. Moreover, photostimulation of glutamatergic fibers in the BLA–NAc pathway elicits reinforcement of reward-seeking while optical inhibition has the opposite effect. D1 receptor signaling is essential for the photoactivation-induced reinforcement. However, photostimulation of glutamatergic fibers in the mPFC–NAc pathway only induces synaptic responses, but not behavioral reinforcement. This strongly suggests an important role of glutamatergic NAc-projecting pathways in reward-seeking[64].

2.8 Hypothalamus The application of optogenetics in the hypothalamus is focused on its role in the sleep-wake cycle. Orexin neurons are a cluster of cells located in the lateral hypothalamus, and project widely through various brain regions. In 2007, Adamantidis *et al.* expressed ChR2 specifically in orexin neurons with lentivirus. Photoactivation of these neurons increased the occurrence of a switch from sleep to wakefulness[36], while photoblockade led to increased slow-wave sleep in the daytime when the mice were inactive, and did not occur at night. Furthermore, acute inhibition of orexin neurons by light decreases the activity of neurons in the dorsal raphe (DR), another important component of the arousal system, while activity of the DR neurons remains unchanged if the loss of orexin innervation is chronic[37]. It is worth noting that the effects of orexinergic activity is in turn influenced by sleep itself. Sleep deprivation diminishes orexinergic effects though the activity of these neurons is still elevated by photostimulation, as well as the activity of neurons in the locus coeruleus and tuberomammillary nucleus, as suggested by c-fos expression. Thus, orexin is an important factor in the regulation of wakefulness through connections with other arousal systems and sleep homeostasis[65]. Adequate sleep is necessary for memory consolidation but direct evidence is still rare. Rolls and colleagues used ChR2 to achieve optogenetic control of orexin neurons in the lateral hypothalamus. Light activation of orexin neurons fragments sleep, but the total amount and intensity of sleep are maintained, and this impairs memory consolidation in a novel object recognition task. Therefore, sleep patterns, together with the amount of sleep, also play an important role in memory consolidation[66]. Interestingly, Lin and colleagues identified the aggression center in the hypothalamus using ChR2 in mice. Photostimulation of the ventrolateral subdivision of the ventromedial hypothalamus elicited attack behavior towards others, regardless of homogeneity, heterology, or just objects like gloves. Nevertheless, the neuronal groups mentioned above are silent during mating. Thus, these data raise the question of how these neurons interact with each other in different behaviors[67].

2.9 Brainstem and spinal cord The locus coeruleus (LC) is also involved in sleep-wake regulation. Carter and colleagues used ChR2 and eNpHR to control the activity of noradrenergic neurons in this nucleus[38]. Since noradrenergic neurons in the LC are wake-active, photoinhibition of these neurons significantly decreases wakefulness while optical stimulation increases sleep-to-wake switching. Importantly, the authors found differences between tonic and phasic long-term stimulation of these neurons. The former pattern induces more wakefulness and locomotion with 1 hour of light but a decrease when the photostimulation period extended to 5 hours. Nevertheless, phasic stimulation elevated the amount of wakefulness and reduced motor activity with both 1 and 5 hours of continual light. Thus, these data directly support the hypothesis that neurons in the LC regulate wake-sleep states[38].

Various central chemoreceptors are located in the brainstem and optogenetics provides tools for the direct exploration of the role of these receptors in the regulation of respiratory and blood-pressure responses. The retrotrapezoid nucleus (RTN) is thought to be involved in sensitization to CO_2, and photostimulation of the RTN increases activity of the phrenic nerve[39]. Moreover, active expiration occurs after photoactivation[68]. On the other hand, photoactivation of C1 neurons in the rostral ventrolateral medulla (RVLM) in which ChR2 is expressed increases sympathetic activity and blood pressure[40,69]. These findings provided the direct evidence that C1 neurons play a role in sympathetic-promoting activity and blood pressure regulation. Interestingly, optogenetics also revealed a role

of astrocytes in the activation of chemoreceptors, during which ATP plays an important role. This was the first direct evidence that astrocytes take part in a respiratory reflex[70]. Intriguingly, optogenetics is also effective in rescue from spinal cord injury (SCI). In 2008, Alilain *et al.* recovered respiratory movements in rats after SCI using ChR2. They provided a new and invasive potential direction for treatment of SCI[71].

2.10 Retina Since the tools used in optogenetics, such as ChR2 and NpHR, are proteins activated by light, we would expect to explore its application in the retina since it also contains light-sensitive proteins. Retinal diseases such as retinitis pigmentosa and photoreceptor degeneration always lead to the death of photoreceptors, which results in deficient light-sensing. Recovery of visual perception is the primary purpose of treatment. Recently, optogenetics has provided new directions. One of the possible means to rescue vision is to reconstruct the visual pathway by activating ganglion cells in the inner retinal layer. Bi *et al.* (2006) used AAV to specifically express ChR2 in inner retinal neurons, mostly ganglion cells, and elicited excitatory responses[72]. However, the two kinds of ganglion cells, ON and OFF neurons, have different properties in response to light. After successfully restoring the ON response, they used the light-activated anion channel, HaloR, to rescue the OFF response. Finally, to exert precise control of neuronal activity, simultaneous expression of ChR2 and HaloR in ganglion cells was achieved[73]. Other studies have also explored the possibility of restoring vision by optogenetic control of ganglion cells and achieved satisfying results[74,75]. Despite these promising data, attention should still be paid to the reliability of this method. Tomita and colleagues found re-processing of visual signals after photoactivation of the exogenously-expressed ChR2[76]. Other possible strategies reconstructing the visual pathway include optocontrol of bipolar cells or photoreceptors. In 2008, Lagali and colleagues specifically expressed ChR2 in ON bipolar neurons by electroporation in the *Pde6b*^{rd1} mouse, a model of retinal degeneration[41]. Stimulation of targeted neurons activated ganglion cells. Consistent with this, the visual signals were also processed to the visual cortex and behavioral changes were easily obtained. Thus, visual restoration by optogenetic reactivation of bipolar cells is effective. But there is still no direct evidence for optogenetic regulation of the OFF response in bipolar cells. Intriguingly, direct transfection of halorhodopsin (eNpHR) into cone cells by AAV is also satisfactory in rescuing vision, as demonstrated by Volker Busskamp and colleagues[42]. They used the mouse model of retinitis pigmentosa and found that photocurrents were recorded after photostimulation. Undoubtedly, visual pathways, including ganglion cells and visual cortex, are activated and behavioral performance is improved with these "artificial photoreceptors".

3 Future directions

We have provided a brief introduction to optogenetics, and illustrated their application in neuroscience in recent years. Optogenetics is a powerful technology for exploring mysterious phenomena in neuroscience, but the information it provides is still relatively limited. Although it has high resolution in the manipulation of neuronal activity, it cannot imitate neuronal activity in accordance with changes in entire neuronal circuits, and is unable to provide essential data on the properties of targeted neurons. On the other hand, it is inefficient to determine the dynamic role of one certain kind of cells with related behavior even it is a perfect choice to reveal the link of these neurons and the behavior. Further, explosive progress will be made if optogenetics is successfully combined with manipulation and monitoring of the activity of neuronal circuits in real time after stimulation. So, optimization of this technology will continue.

The diversity of optogenetic tools still needs to be enriched for more precise control of neuronal groups in terms of temporal and spatial resolution. So far, the application of optogenetic tools such as halorhodopsin and light-activated G protein-coupled receptor, is also limited by the characteristics of the tool itself in some brain regions, although many efforts to improve the genetic or chemical conformation have been made[77]. Efforts to explore new optogenetic tools are still needed. Four aspects should be stressed for a better application of this novel technology.

First, we should explore the optimal proteins that are sensitive to light of specific wavelengths. In order to achieve multi-control of neuronal clusters at the same time, it is important to simultaneously express different kinds of optogenetic tools in one neuron while leaving its physiological activity undisturbed. Then, the selection of vectors and the use of genetically-altered animal lines for safe and stable expression of the light-activated proteins should be attended to. Third, we should seek the appropriate patterns of illumination to precisely imitate the activity of normal neurons. Last but not least, an ideal readout system is necessary for better analysis of the data collected.

Recently, the combination of optogenetics and magnetic resonance imaging (MRI) has increasingly been used to map brain circuits[78,79]. As one of the most important advantages of optogenetics, mapping brain circuitry is particularly important. Comprehension of neuronal connections is essential for a better understanding of the neural mechanisms of behavior. The combination of optogenetics and MRI can achieve more precise and reliable observations. However, whether it is possible to investigate the dynamic connections of neuronal groups in real time in awake animals by optogenetics and MRI is an interesting and important question for exploration.

Although optogenetics is applied to control the activity of targeted neurons or to investigate neuronal circuits in the brain, its value in the treatment of diseases due to neuronal degeneration also cannot be ignored, since initial efforts have paid off[32,80]. These promising methods provide new directions to reverse neuronal degeneration. However, caution and deeper studies are still needed to transfer this technology from animals to humans.

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