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Review article

Channelrhodopsins—Their potential in gene therapy for neurological disorders

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ABSTRACT

Recently, channelrhodopsins (ChRs) have begun to be used to manipulate the neuronal activity, since they can be targeted to specific neurons or neural circuits using genetic methods. To advance the potential applications in the investigation and treatment of neurological disorders, the following types of basic research should receive extensive financial support. The spectral and kinetic properties of ChRs should be optimized according to the application by generating variants of ChRs or exploring new rhodopsins from other species. These ChRs should be targeted to the specific types of neurons involved in the neurological disorders through a gene expression system using cell- or tissue-specific promoters/enhancers as well as gene delivery systems with modified virus vectors. The methods have to be developed to apply the genes of interest with safety and long-term effectiveness. Sophisticated opto-electrical devices should be developed. Appropriate primate animal model systems should be established to minimize the structural differences between small animals such as rodents and human beings. In this paper, we will review the current progress in the basic research concerned with the potential clinical application of ChRs and discuss the future directions of research on ChRs so that they could be applied for human welfare.

Contents

Abbreviations: AAV, adeno-associated virus; AMD, age-related macular degeneration; ArchT, archaerhodopsin-T from *Halorubrum* strain TP009; ChR, channelrhodopsin; ChR1, channelrhodopsin-1; ChR2, channelrhodopsin-2; ChRFR, ChR-fast receiver; ChRWR, ChR-wide receiver; ChRGR, ChR-green receiver; DBS, deep brain stimulation; GPi, globus pallidus interna; IgG, immunoglobulin G; L-DOPA, L-3, 4-dihydroxyphenylalanine; LED, light-emitting diode; LV, lentiviral vector; Mac, Leptosphaeria maculans opsin; MChR1, channelrhodopsin-1 from *Mesostigma viride*; NpHR, halorhodopsin from *Natronomonas pharaonis*; PD, Parkinson's disease; PPN, pedunculopontine nucleus; RO, raphe obscurus; RP, retinitis pigmentosa; SCI, spinal cord injury; SFO, step-fuction opsin; SSFO, stabilized step-function opsin; STN, subthalamic nucleus; TM, transmembrane; VChR1, channelrhodopsin-1 from *Vovox cateri*.

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1. Introduction

The brain is a network consisting of billions of neurons and their synapses. It is the signal transference from one neuron to another that enables one to feel, move, learn and think. When there is a dysfunction in this system, various neurological diseases can arise. To date, various approaches have been developed to treat people for neurological diseases such as pharmacological, surgical and gene therapies. These methods have improved patients' quality of life, but with some disadvantages. When channelrhodpsins (ChRs) were identified from the *Chlamydomonas* genome by several groups about ten years ago (Nagel et al., 2002, 2003; Sineshchekov et al., 2002; Suzuki et al., 2003), an innovative method referred to as "optogenetics" was developed (Deisseroth et al., 2006; Deisseroth, 2011), which has the potential of leading to new strategies for investigating and treating neurological diseases. In this review, we will discuss potential clinical applications and future research on ChR-based gene therapies for neurological diseases.

2. Current strategies to treat neurological disorders

To date, neurological disorders are commonly treated with pharmacological and surgical approaches, and attempts have been made using gene therapies, as well. Although often effective, they also have shortcomings that need to be overcome. Pharmacological therapy is frequently accompanied by side effects because of the low specificity of the drugs for their target receptors and the undesired spread. For example L-DOPA, one of the most effective drugs currently prescribed for Parkinson's disease (PD), is frequently accompanied by adverse effects such as motor fluctuations and dyskinesia (Rascol et al., 2000; Olanow et al., 2006). These side effects make long term treatment difficult, although recent reports suggest that L-DOPA-induced dyskinesia could be attenuated if L-DOPA is combined with a catechol-O-methyl transferase inhibitor (entacapone) or a monoamine oxidase inhibitor B (selegiline or rasagiline) (Rascol et al., 2002; Miyasaki, 2006). Deep brain stimulation (DBS) is one of the surgical therapies for the treatment of neurological disorders. In the case of PD, DBS has been used to target various regions such as the subthalamic nucleus (STN), globus pallidus interna (GPi), and pedunculopontine nucleus (PPN). Although it relieves patients from some symptoms of PD, the basic mechanisms of DBS remain unclear, though several hypotheses have been described (Limousin et al., 1998; Vitek, 2002; Kringelbach et al., 2007; Montgomery and Gale, 2008). With the development of molecular and genetic techniques, numerous genes that are responsible for various neurological disorders have been identified. However, the mechanisms of neurological disorders are complex and do not always involve only a single gene. For example, epilepsy, which is caused by an imbalance between excitatory and inhibitory neurons, is derived from various genetic abnormalities such as SCN1A (Escayg et al., 2000; Catterall et al., 2010), SCN1B (Wallace et al., 1998), KCNQ2 (Singh et al., 1998; Su et al., 2011), and $GABA_A$ receptor subunit genes (Macdonald et al., 2010). Therefore, gene therapy for epilepsy is still a challenge. However, as ChR2 came to be used to manipulate neural activity in neuroscience research, it has become a candidate for overcoming these limitations.

3. Molecular biology of ChRs

Chlamydomonas reinhardtii is one of unicellular green algae that senses light through an eyespot apparatus. Under electron microscopy, the eyespot is an organelle composed of a plasma membrane, a specialized chloroplast membrane and two layers of highly ordered carotenoid-rich granules. Each granular layer is subtended by a thylakoid membrane. ChRs including ChR1 and ChR2, are generally considered to be localized in the plasma membrane in apposition with the carotenoid granules (Lamb et al., 1999; Suzuki et al., 2003). They are light-activated cation channels and mediate the light-dependent behavior of the algae. ChRs are the members of the microbial (archaeal, type-I) rhodopsin family and consist of a seven-pass transmembrane (TM) apoprotein and a retinal which covalently binds to it. The photoisomerization of all-*trans*-retinal to 13-*cis* configuration is coupled to conformational changes of the apoprotein which gate the channel structure to become open to the permeation of cations such as H^+, Na^+, and K^+. In other words, the light signal is converted into an electrical one by ChRs (Hegemann, 2008). Previously, several groups independently reported that neurons could be endowed with photosensitivity by genetically introducing ChR2 (Boyden et al., 2005; Li et al., 2005; Ishizuka et al., 2006). In doing so, one can manipulate the activity of neurons either *in vitro* or *in vivo* by light with high spatial resolution and millisecond-order precision (Boyden et al., 2005; Ishizuka et al., 2006). Neuronal activity can also be negatively regulated by light (Zhang et al., 2011) if the neurons are engineered to express either a Cl^--transporting rhodopsin, *e.g.* NpHR from *Natronomonas pharaonis* (Han and Boyden, 2007; Zhang et al., 2007) or an H^+-transporting rhodopsin, *e.g.* archaerhodopsin-3 from *Halorubrum sodomense* (Arch), archaerhodopsin from *Halorubrum* strain TP009 (ArchT) and the *Leptosphaeria maculans* opsin (Mac) (Chow et al., 2010). These properties can enable ChR2 and other rhodopsins to be used for manipulating neural circuits and treating neurological diseases. Numerous reports have suggested that optogenetics would have the advantages of safety and targeting specificity when applied to the clinical field.

4. The potential of application of ChRs to neurological disorders

4.1. Retinal prosthesis

Vision is one of the major sources of information about the world. Generally, light entering the eye is absorbed by the photoreceptor cells, the rods and cones, which are the major receptor cells directly sensitive to light. In the photoreceptor cells, light signals are transduced into electrical signals. Then, the information is transmitted through bipolar cells directly or indirectly to retinal ganglion neurons, which generate action potentials. Finally, the signal is sent to the brain by the axons of retinal ganglion cells for further processing, generating visual perception of the external world. However, in cases of retinal degenerative diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD), the patients are seriously impaired in their every-day life. Although light-sensing photoreceptor cells are degenerated in the patients with RP or AMD, other downstream retinal neurons, such as bipolar cells and retinal ganglion cells, are still preserved, with

the function of their optic nerve projections being largely maintained (Humayun et al., 1999). If the remaining neurons could be made light sensitive, the quality of life of such patients could be remarkably improved. Bi et al. expressed ChR2 in inner retinal ganglion neurons of *rd1/rd1* mice, one of the RP animal models, and demonstrated that the mice became sensitive to blue light with the expression of ChR2 (Bi et al., 2006). Meanwhile, Tomita et al. independently delivered ChR2 genes into ganglion neurons of RCS rats, another model of RP, using AAV vector, and demonstrated the recovery of visual-evoked potentials in these rats (Tomita et al., 2007). These pioneering studies using ChR2 open the possibility of treating the loss/weakness of vision with this new strategy. Using a transgenic rat, which expressed ChR2 in retinal ganglion neurons, the optomotor responses, such as moving its head to track objects made up by blue light stripes over a black background, were shown to remain even after the induction of degeneration of photoreceptor cells with toxic light exposure (Tomita et al., 2009) although there remain some debates (Thyagarajan et al., 2010). Lagali et al. expressed ChR2 specifically in the ON-bipolar cells through the mGluR6 promoter. Their behavioral experiment also showed the restoration of vision (Lagali et al., 2008). Recently, the safety and long-term effectiveness in multiple mouse models of loss/weakness of vision have been demonstrated (Sugano et al., 2011; Doroudchi et al., 2011). More importantly, exiting news from Wayne State University reported that RetroSense Therapeutics (a biotechnology company that is developing a game-changing gene therapy to restore vision in patients suffering from loss/weakness of vision) signed a license agreement with Pan's group (Wayne State University) for novel gene-therapy approaches to vision (Navitsky, 2012), which will be an excellent step for ChR-based retinal prosthesis in the clinical setting.

4.2. Spinal cord injury

Spinal cord injury (SCI) includes any damage or trauma to the spinal cord. With acute SCI, such patients can lose the peripheral sense and/or the ability to move the body, in addition to other symptoms such as dysfunction of bladder and bowel, and respiratory insufficiency. Approximately 300,000 people with SCI live in the US (100,000 patients in Japan), and there are about 12,000 new patients every year (Woo et al., 2011). In 2005, Li et al. employed ChR2 to control spinal cord neural activity and offered the possibility that the symptoms of spinal cord injury could be attenuated using ChR2 (Li et al., 2005). Thereafter, as one of the key papers using ChR2 in the treatment for SCI, the report of Alilain et al. (2008) should be noted. They made surgical hemi-section of the spinal cord at the left C2 level in a rat model of SCI, resulting in hemiplegia. As a result, the rats breathed with difficulty due to the half-paralyzed diaphragm. After introducing ChR2 in the phrenic nucleus, blue light illumination triggered the activity of phrenic nerves in the paralyzed diaphragm in a manner synchronous with the respiratory rhythm of the unparalyzed side. More recently, Kiehn's group generated a transgenic mouse line that expressed ChR2 in the hindbrain and spinal cord under the control of the VGluT2 promoter. They were able to control locomotor-like activity of the mice with appropriate left-right and flexor-extensor alternation through blue light illumination. In addition, when they activated glutamatergic neurons in the hindbrain, the neurons provided a direct command that could activate the spinal locomotor network (Hägglund et al., 2010). Guyenet's group employed PRSx8 promoter to express ChR2 selectively in Phox2b-containing neurons, which might be central respiratory chemoreceptors. They firstly showed the crucial evidence that Phox2b-containing neurons play an important role in central respiratory chemoreception (Abbott et al., 2009, 2011). By expressing ChR2 selectively in raphe obscurus (RO) serotonergic neurons, they also showed that RO serotonergic neurons potentiate the central respiratory chemoreflex but do not appear to have a central respiratory chemoreceptor function (Depuy et al., 2011). All these suggested that ChR2-based gene therapy could open up a new strategy for helping SCI patients to walk and respire without external assistance.

4.3. Parkinson's diseases

Parkinson's disease (PD) is one of the most common neurodegenerative disorders affecting mainly the elderly population. It is characterized by the loss of dopaminergic neurons in the substantia nigra, resulting in motor impairments as well as non-motoric symptoms (Thomas and Beal, 2007). Recently, the neural circuitry involved in PD was optogenetically investigated by Gradinaru et al. (2009). They utilized enhanced NpHR (eNpHR) or ChR2, respectively to inhibit or activate the excitatory neurons in STN of hemiparkinsonism rats the substantia nigra of which had been lesioned by a dose of 6-hydoxydopamine. However, neither the eNpHR-mediated inhibition nor the ChR2 mediated excitation of STN neurons relieved the behavioral abnormality of hemiparkinsonism rats, suggesting STN excitatory neurons would not be the targets of DBS. However, using the hemi-parkinsonism mice that expressed ChR2 exclusively in the afferent fibers but not in the cell bodies of STN, they found that high frequency photo-stimulation relieved but low frequency photo-stimulation worsened the PD symptoms. Thus their results suggested the possible involvement of STN afferents in DBS, which is consistent with previous reports that DBS may activate adjacent fiber pathways (Xu et al., 2008). Within the striatum of the basal ganglia, medium spiny neurons can be categorized into at least two types: D1 receptor-expressing (D1) and D2 expressing (D2) neurons. In the classical basal ganglia circuitry, the activation of D1 neurons facilitates movement, whereas that of D2 inhibits movement, and thus they have been termed "direct pathway" and "indirect pathway" neurons, respectively (Albin et al., 1989; Alexander and Crutcher, 1990). However, this idea has never been experimentally tested. Recently Kravitz et al. utilized the Cre-loxP system to express ChR2 in only the direct pathway (D1) or indirect pathway (D2). They presented the first experimental evidence for the classical. More importantly, they could regulate the parkinsonism symptoms by optogenetically activating the D1 neurons (Kravitz et al., 2010).

Optogenetics also appeared promising for the investigation and possible treatment of other neurological disorders such as epilepsy (Tønnesen et al., 2009), schizophreniza (Peled, 2011; Maher and LoTurco, 2012), and autism (Tye and Deisseroth, 2012).

Taken together, all these studies suggested that ChR-based therapy could lead to exciting and promising treatments for neurological disorders.

5. Perspectives

Although ChR-based gene therapy appears promising, for clinical applications, further research concerning the following will be necessary: (1) the development of new ChR variants with various advantages; (2) target selectivity; (3) safety and long-term effectiveness; (4) optimized light delivery systems and (5) the development of non-human primate animal models.

5.1. ChRs variants and new rhodopsins from other species

ChR2 has unique advantages for gene therapy in neurological diseases. However, its clinical application would be limited by several disadvantages such as strong current desensitization, small current amplitude and blue light preference (Nagel et al., 2003; Ishizuka et al., 2006). Gene therapy would be facilitated by variants

of ChRs with various properties generated by targeted mutagenesis, by TM helix shuffling, or by both in combination. Among these, those including the TM1 and 2 of ChR1, for example, ChRFR, ChRWR and ChIEF, generally showed reduced desensitization, improved folding/membrane expression and enhanced photocurrent (Wang et al., 2009; Lin et al., 2009; Yawo, 2009). In addition, ChRGR, a derivative of ChR1 whose TM6 and C-terminal subdomain of TM7 were replaced by the counterparts from ChR2, showed similar wavelength sensitivity as ChR1, but exhibited not only enhanced photocurrents but also almost negligible desensitization (Wen et al., 2010).

The variants derived from the targeted mutation of ChR2 showed certain improvements in various properties. For example, ChR2-T159C exhibited improved membrane protein expression and the largest photocurrent of all available ChR variants (Berndt et al., 2011). ChR2 step-function opsins (SFOs) such as ChR2-C128T, ChR2-C128A, ChR2-C128S and ChR2-D156A, showed extended inward current flowing after the turning off of blue light (Berndt et al., 2009; Bamann et al., 2010). SFOs should be very useful for clinical experiments because of their long-term excitation, reduced light requirements, faster activation and inactivation by light of longer wavelength (Berndt et al., 2009; Yizhar et al., 2011; Tye and Deisseroth, 2012). More recently, stabilized step-function opsins (SSFOs), a double mutant of ChR2, C128S/D156A, was reported to be more sensitive to light and could be activated even in brain tissue as deep as 3 mm (Yizhar et al., 2011).

The counterparts of channelrhodopsin from other species such as VChR1 isolated from *Vovox cateri* and MChR1 from *Mesostigma viride* showed maximal action spectrum at 520 and 528 nm, respectively (Zhang et al., 2008; Govorunova et al., 2011). Activation of ChRs with light of longer wavelength is preferable to minimize light scattering and absorption by tissues, while reducing the photocytotoxicity to the cells. Excitation with red-shifted wavelength is also preferable to avoid strong pupil constriction when applied for retinal prosthesis (Busskamp and Roska, 2011).

We believe more suitable ChR variants will be produced, that, with the aid of structural studies (Kato et al., 2012), will accelerate the development of gene therapy for neurological diseases.

5.2. The target specificity

One of advantages of ChR-based gene therapy is that the gene expression can be regulated in a cell-type-specific manner. For example, the mGluR6 promoter has been employed to drive the ChR2 gene to be expressed specifically in ON-bipolar cells of degenerated retinas in a Pde6b (*rd1*) mice model (Lagali et al., 2008). The CaMKIIα and PRSx8 promoters allow ChR2 to be expressed exclusively in excitatory neurons and Phox2b-containing neurons, respectively (Abbott et al., 2009; Gradinaru et al., 2009). Thus the challenge for future research will be to identify new promoters/enhancers that drive ChR2 gene expression in specific neurons or tissue. To deliver the gene using viral vectors, the cell-specific promoters/enhancers should be small in their size because of the packaging capacity of the virus. For example, the maximal packaging capacity of adeno-associated viruses (AAVs) is 4.1–4.9 kb (Dong et al., 1996), although it was increased in some AAV mutants (Grieger and Samulski, 2005; Allocca et al., 2008). To increase the packaging capacity, studies on developing dual vector expression of the transgene have appeared promising. On the basis of the property of AAV, Duan et al. developed a dual-vector system with which they coinfected the reporter vector containing the transgene and the driver vector encoding multiple enhancer sequences (Duan et al., 2000). Several reports suggested that the limited packaging capacity has been overcome by the two-vector system (Duan et al., 2001; Goncalves et al., 2005; Palfi et al., 2012).

Alternatively, ChR2 could be expressed in a specific type of neuron by relying on the tropism of the viral vectors. Previous studies reported that targeting a gene to a specific cell type can be made by modifying the tropism of viruses, such as the adenovirus (AD) and the AAV (Nicklin et al., 2005; Betley and Sternson, 2011). Based on different capsid proteins, which are responsible for the tropism of viruses, various serotypes of AAV and mutations have been identified (Rutledge et al., 1998; Daya and Berns, 2008; Petrs-Silva et al., 2009). Some of them have been used for gene targeting. For example, in the visual system, AAV2/2 has been demonstrated to drive gene expression specifically in retinal ganglion cells and optic nerve fibers after intra-vitreal delivery (Auricchio et al., 2001). In contrast, AAV6 is favored exclusively by astrocytes (Betley and Sternson, 2011), and ShH10, a new AAV variant, mainly by Müller cells (Klimczak et al., 2009). Another promising way to target ChRs to specific neurons is employing antibody-displaying viral vectors, which not only target the transgene to a specific cell type but also overcome the limited packaging capacity of traditional viral vectors. Recently, Konno et al. reported that they employed the ZZ Sindbis virus vector to specifically infect pyramidal neurons in CA1 and granule cells in the dentate gyrus in an antibody-specific manner in mice (Konno et al., 2011). Also, Ried et al. inserted the Z34C, an immunoglobulin G (IgG) binding domain of protein A, into the AAV2 capsid and made an AAV2-Z34C vector in order to target the vectors to specific cell surface receptors (Ried et al., 2002).

5.3. Safety and long-term effectiveness

Safety and long-term effectiveness are important issues to be solved for gene therapy. The genes of interest have been delivered using either viral vectors or non-viral vectors. Because of the rapidity of experimental implementation, most of the animal experiments of ChR optogenetics have been carried out using virus vectors such as AAVs and lentiviral vectors (LVs). AAV-mediated gene delivery has the advantages of wide tissue tropism, efficient transduction of non-dividing cells and long-term effectiveness. Over 100 variants with diverse cell tropisms have been identified and some of them have been used in the clinical field (Daya and Berns, 2008). It has been reported that AAV-mediated gene expression is safe and effective in the primate brain for at least 8 years (Hadaczek et al., 2010). LV-mediated gene delivery has the advantages of stable gene integration in both non-dividing and dividing cells as well as long-term expression. Numerous animal experiments have shown the safety and long-term effectiveness of these virus vectors for delivering ChR2. However, one has to address the issue of determining the viral titers that are both safe and effective before applying viral vectors for ChR-based gene therapy in a clinical setting, since the issue is still unsolved even for experiments using animal models. Ideally, the dose of virus should be minimal to avoid the immune response of host. To this end, expression cassettes have been used to express higher levels of the transgene with lower doses of the vector. The expression cassette is the part of the vector DNA that is used for cloning and transformation. It usually includes three parts: promoter sequence, open reading frame and 3′-untranslated region. One example is the self-complementary AAV vector which has been demonstrated to significantly enhance the expression level of the transgene with minimal reduction of the packaging capacity (McCarty et al., 2001; McCarty, 2008; Koilkonda et al., 2009).

5.4. Optimized light delivery

To deliver light to the targeted neurons or regions, several issues have to be addressed. For example, what are the optical properties of different tissues in the human brain? What is a more suitable light source, laser or LED? What are the optimal devices to deliver

the light to the experimental or therapeutic system? The former two issues have been extensively reviewed previously (Carter and de Lecea, 2011; Yizhar et al., 2011). Here, we highlight the recent advances regarding the light delivery system.

At present, *in vivo*, most experiments have been performed with light sources such as LED and laser that are connected to the targeting regions through an optic cable. The optic cables and the large LED or laser are inconvenient for experiments with free-moving animals or to deliver the light as a medical treatment. Therefore, one challenge is to invent smart wireless implants. Recently, prototypes of wireless lighting devices have been developed (Iwai et al., 2011; Wentz et al., 2011). Iwai et al. (2011) implanted a battery-powered fiber optics system in the primary motor cortex of freely moving transgenic mice that express ChR2 in the layer V neurons and showed that the device could remotely induced a behavior. Wentz et al. (2011) made a head-borne device powered by a low-strength oscillating magnetic field, thus, successfully reduced its weight to as little as 2 g. They employed this device to control the activities of the ChR2-expressing pyramidal cells in the motor cortex of freely-moving transgenic mice. Such wireless devices could become smaller and more powerful with the development of optoelectronics if only the problem of cost could be solved.

Previously, most the devices delivered monochromatic light on a single spot. However, it has become increasingly necessary to develop multicolored and spatio-temporally patterned irradiating devices for both basic research and clinical applications. For example, to control the ON-OFF responses in the retina, one needs to deliver different wavelengths to activate depolarizing rhodopsins (eg, ChR2) and hyperpolarizing rhodopsins (eg, NpHR) (Zhang et al., 2009). Alternative irradiation of blue and yellow light is necessary to exploit the full potential of SFO and SSFO, which are expected to be applied clinically as well as for basic experiments (Berndt et al., 2009; Yizhar et al., 2011; Tye and Deisseroth, 2012). It is also important to stimulate multiple sites in a spatially patterned fashion for the research of some neurological disorders such as epilepsy. Previously, an implantable probe having multiple waveguides was devised to deliver different wavelengths of light to multiple sites (Zorzos et al., 2010). The probe consisted of a parallel array of waveguide cores each of which conducts light from a laser at either 473 or 632 nm. Recently, an image projector was applied to deliver multicolored light with a pre-programmed spatio-temporal pattern (Sakai et al., 2012; Stirman et al., 2012). Using these systems, the depolarizing rhodopsins (*e.g.* ChR2) and the hyperpolarizing rhodopsins (*e.g.* ArchT and Mac) can be differentially activated. These devices, in combination with microendoscopy technique (Hayashi et al., 2012), would enable one to deliver multicolored and patterned light in the brain of *in vivo* animals.

In the future, the researchers may devise light-emitting nanoparticles that could be controlled and charged by magnetic fields or near-infrared light to generate specific wavelengths of light. If successful, it would no longer be necessary to consider the problem of inserting many optic fibers into the brain.

5.5. The development of non-human primate models

The current investigations of ChR optogenetics have been extensively promoted using rat and mouse model systems, and only a limited number of studies have been done using non-human primates (Han et al., 2009; Diester et al., 2011; Gerits et al., 2012). Although the rodent models are convenient, gene therapy investigations in rodents have not always predicted the results of higher-order models (Gagliardi and Bunnell, 2009). One of the reasons may be the difference in the size of the brain between rodents and primates. For example, the human brain is 650–700 times greater in the weight than the rodent brain (Mink et al., 1981). Such size differences have to be taken into consideration to determine the titer of the virus vector to guarantee the safety and effectiveness in the clinical setting. Secondly, the nervous system of rodents is structurally different from those of primates. In the case of the eye, different from human beings and non-human primates, rodents usually lack the fovea. Thus, before the introduction of ChR-based gene therapy to the clinical field, it would be necessary to evaluate the safety and effectiveness in non-human primates that have anatomical and electrophysiological properties relatively similar to those of a human being. There would be additional benefits with non-human primates as an experimental model system. For example, although blind rats or mice can become sensitive to light with the retinal expression of ChRs, it remains unclear whether they can correctly integrate the signals generated by ChRs to recognize form and color. This question could be addressed by experiments using non-human primates with the appropriate training paradigms. Recently, the safety and effectiveness of ChR2 have been investigated using non-human primates (Diester et al., 2011), but the long-term stability of ChR2 expression in the non-human primate brains remains to be tested.

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