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Optogenetic approaches for controlling seizure activity

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Abstract

Optogenetics, a technique that utilizes light-sensitive ion channels or pumps to activate or inhibit neurons, has allowed scientists unprecedented precision and control for manipulating neuronal activity. With a clinical need to develop more precise and effective therapies for patients with drug-resistant epilepsy, these tools have recently been explored as a novel treatment for halting seizure activity in various animal models. In this review, we provide a detailed and current summary of these optogenetic approaches and provide a perspective on their future clinical application as a potential neuromodulatory therapy.

Keywords

Optogenetics; epilepsy; neuromodulation

1. Introduction

Epilepsy is characterized by aberrant neural activity in the brain that ultimately leads to spontaneous, recurrent seizures. Currently 3 million people in the U.S. and 65 million people worldwide, or roughly 1% of the population, are affected by epilepsy, accounting for a significant worldwide health burden. Approximately 150,000 patients in the U.S. and 2.4 million worldwide are diagnosed every year, and annual epilepsy related medical expenditures are close to $10 billion in the U.S. alone[1] and up to $4748 in direct costs per patient in other countries[2]. Moreover, epilepsy was responsible for approximately 20.6 million disability-adjusted life years lost in 2012[3]. Disability arises from a variety of factors: the behavioral manifestations of seizures and their characteristic unpredictability are very disruptive to the performance of activities of daily living; injuries (in up to 30% of patients) and even death (2–17% of deaths in patients with epilepsy) are not uncommon; and cognitive decline is frequent. All these factors contribute to an ineluctable downward socioeconomic spiral[4].

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Although most patients are able to control their seizures with anti-epileptic drugs, they are generally required for the lifetime of the patient and are commonly associated with side effects that are poorly tolerated in some patients, especially those requiring multiple medications [5]. The 30% of patients who do not become seizure free with anti-epileptic drugs, [6] may be candidates for a variety of surgical options, broadly categorized as resective surgery (*e.g.* temporal lobectomy, lesionectomy), disconnection surgery (*e.g.* corpus callosotomy, functional hemispherotomy), and neuromodulation (*e.g.* vagus nerve stimulation, responsive neurostimulation). Resective and disconnection surgery yield the highest rates for seizure freedom – the goal of all epilepsy patients and their providers - 60–70% [7,8]. However, these procedures are associated with a risk for neurological deficits such as memory, speech, motor and visual impairments, especially if the area of resection lies in eloquent brain areas. Indeed for this reason and others such as risk-aversion and lack of referrals or access to care, only about 3000 patients in the U.S. actually receive surgery each year[9].

Neuromodulatory approaches to countering abnormal brain activity have shown great promise in reducing seizures in patients with intractable epilepsy; the various approaches in current usage are reviewed by Fisher et al[10]. The most successful therapies include vagus nerve stimulation[11–13], stimulation of the anterior nucleus of the thalamus (SANTE) [14–16], and responsive neurostimulation (RNS) of epileptic foci [17,18]. Clinical trials using these devices have demonstrated that 56–68% of patients were able to reduce their seizure frequency by more than 50% at their last visit (the average reduction of seizures in these patients was 48–76%)[19]. Although electrical stimulation allows more targeted and reversible therapy to the brain compared to pharmacotherapy or surgical resection, it is still a challenge to specifically and effectively target only pathological circuits while leaving healthy tissue undisturbed (Figure 1a), which can result in undesired side effects such as memory impairment, worsening of depression, or exacerbation of seizures[15]. This is primarily due to the fact that the effects of electrical stimulation on the surrounding tissue are not cell-type specific and are highly dependent on patient-specific neuroanatomy (*e.g.* it remains difficult to effectively reach seizure onset zones located in deep sulci) and parameters of stimulation, which can be hard to predict. Given the potential side effects, suboptimal response rates, and selective inclusion criteria for surgery, new therapies for epilepsy patients are in dire need.

In this review, we provide an up-to-date overview of the various studies utilizing optogenetics to control seizure activity *in vitro* and *in vivo*. Although this topic has been discussed in the past [20–24], we aim to provide a more detailed summary of key findings and implications to serve as a primer for those interested in knowing the state of the art. In addition to covering several new studies in the field, we will also review key *in vitro* studies that have provided important insight and rationale for the various experimental approaches taken *in vivo*. Finally, the future prospects and limitations to future translation will be considered.

2. Optogenetics: a precise method to control neuronal activity with light

Optogenetics offers an unprecedented ability to alter neuronal activity with very high spatial and temporal accuracy. By utilizing cell-specific expression of light-sensitive ion channels and pumps (opsins) in the brain, specific cell populations can be selectively activated (Figure 1b) or inhibited (Figure 1c) in the context of complex neural circuitry. Compared to electrical stimulation, opsin expression can achieve higher spatial precision due to the possibility of cell-type specific expression, although deactivation of certain opsins after light cessation imposes slower off kinetics. This degree of control of cell-type specific physiology has proven to be an invaluable research tool enabling neuroscientists to study a wide variety of topics such as brain circuitry, synaptic plasticity, and various behaviors in unprecedented ways.

Two of the most widely used opsins in neuroscience research are channelrhodopsin (ChR) and halorhodopsin (NpHR) for neuronal excitation and inhibition, respectively. ChR is a non-selective cation channel that becomes activated in the presence of blue light. Blue light is therefore capable of depolarizing the membrane potential of neurons expressing ChR and driving action potential firing[25]. In contrast, NpHR is a chloride pump that is activated in the presence of yellow light[26]. When neurons expressing NpHR are illuminated by yellow light, chloride is transported into the cytosol, hyperpolarizing the membrane potential and decreasing the likelihood of action potential firing. Both of these opsins have been utilized for seizure control in the studies reviewed here.

Expressing opsins specifically and efficiently is an important goal for optogenetic applications *in vivo*. There are several methods for expressing opsins in the rodent brain, each with their own advantages and disadvantages. One common method utilizes viral vectors, which allow for efficient long-term expression of transgenes. Viral vectors encoding opsin genes are produced *in vitro* and are subsequently injected into the brain where they can transduce target cells. The cell tropism of these viral vectors can vary depending on the class or serotype of the vector and should thus be chosen carefully [27–31]. Cell-type specific expression of opsins can be further achieved by utilizing an appropriate promoter driving transgene expression. Another method used to achieve cell-type specific expression of opsins uses Cre-recombinase driver animals, in which floxed or double-inverted vectors containing opsin genes can be specifically expressed in Cre-recombinase positive cells, driven by cell-specific promotors[32–34]. Cre-driver mouse lines are widely available[35,36], and rat lines are increasingly being produced as well[36,37]. Various CRE-independent transgenic mouse lines endogenously expressing opsins have also been developed and are widely available[35,36,38].

3. Optogenetic approaches to halting epileptiform activity *in vitro*

In vitro brain slice preparations have offered unique opportunities to study molecular, cellular, and pharmacological mechanisms of epilepsy because they allow easier access for both manipulation and recording of activity while preserving intrinsic neural circuitry. Both acute hippocampal brain slices and organotypic slice cultures have been utilized to study epilepsy *in vitro*. Even when prepared from naïve rodents, depolarization-inducing

manipulations, such as trains of electrical stimulation or pharmacological blockage of hyperpolarizing conductance (*i.e.* $GABA_A$ receptors or K^+ channels) can cause seizure-like activity in these preparations. Organotypic slice cultures can also exhibit spontaneous epileptiform activity, which can be useful for studying the development and morphological changes associated with seizure activity. Several important studies described below have utilized optogenetics to study mechanisms of epileptogenesis as well as to stop epileptiform activity in these *in vitro* preparations.

Tønnesen et al. [39] first demonstrated the potential of using optogenetics for halting epileptiform activity when they utilized NpHR to hyperpolarize principal neurons in organotypic hippocampal slice cultures. From whole cell patch-clamp recordings, the group first demonstrated that expression of the opsin itself does not alter the intrinsic firing properties of transduced neurons and that prolonged activation of the light-driven chloride pump does not accumulate enough intracellular chloride to affect normal GABAergic synaptic transmission. After these careful control experiments, the group demonstrated the first proof-in-principle that activation of NpHR in pyramidal cells could hyperpolarize them and suppress epileptic activity. More recently, Ledri et al. [40] explored targeting interneurons instead of principal cells and showed that optogenetic activation of interneurons was able to suppress ongoing epileptiform activity in acute hippocampal slices. They further compared activation of multiple vs. single interneuron subtypes by genetic targeting and found that the former was more effective at suppressing epileptiform activity due to a more generalized GABA release. These two groups therefore tested two key alternative optogenetic approaches to halting seizure activity and provided a rational framework for future *in vivo* studies described below.

In vitro preparations can also be used to elucidate mechanisms of epilepsy that would otherwise be technically challenging to study *in vivo*. For example, Ellender et al.[41] performed patch recordings in organotypic hippocampal slices to investigate the role of inhibitory interneurons in the dynamics during the clonic phase of a seizure. The authors found that chloride accumulation during the initial tonic phase of seizures converts the effects of somatic-targeting interneurons from inhibitory to excitatory, perpetuating afterdischarges during the clonic phase of seizures. Optogenetic silencing of these interneurons thereby resulted in a reduction of afterdischarges. With the ability to manipulate and record local cellular activity *in vitro*, Ellender et al. was therefore able to discern a new role of inhibitory interneurons in the propagation of epileptic activity that complemented previous findings by Ledri et al.

4. Optogenetic approaches to treating epilepsy *in vivo*

Various brain structures involved in generating or propagating seizure activity have been targeted with electrical stimulation in patients with epilepsy (see review by Fisher et al[10]). Similar anatomical approaches have been taken with optogenetics in the rodent, where seizure foci as well as structures remote from the seizure foci have been targeted (Figure 2a). With the ability to selectively excite or inhibit neuronal activity in a cell type-specific fashion, optogenetics offers additional precision compared to electrical stimulation in dissecting and modulating circuitry involved in seizure generation and propagation. In this

review, we therefore further classified different anatomical approaches into three optogenetic approaches based on the type of opsin and cell types targeted: nonspecific inhibition of neurons, inhibition of excitatory (*e.g.* glutamatergic) neurons, and excitation of inhibitory (*e.g.* GABAergic) neurons (Figure 2b).

In addition to targeting various structures and cell types, the method of light delivery can also influence the efficacy of the approach (Figure 3). A major clinical goal of neuromodulatory devices is to be able to deliver therapy only when it is needed (*i.e.* only in response to a seizure or a seizure-prone state). The Responsive Neurostimulation System (RNS) (NeuroPace, Inc.)[18] is a good example of an implantable device used in humans where electrical stimulation is delivered in response to detection of a seizure event (closed-loop) as opposed to delivering electrical stimulation either continuously or at pre-determined intervals (open-loop). Devices that are able to deliver therapy in a closed-loop fashion not only reduce side-effects related to stimulation, but also improve the efficiency and longevity of these devices by conserving battery power. Development of devices which can deliver light in response to seizure activity in real time can help overcome the current limitations of open-loop optogenetic stimulation (*e.g.* power requirements, possible deleterious effects of heat from continuous light delivery, and side effects of prolonged opsin activation) and bring these therapies closer to clinical application in humans *e.g.*. The methods, principles, and recent advances in closed-loop optogenetic neuromodulation have been recently reviewed by Grosenick et al[42].

The various approaches and key results of the studies utilizing optogenetics to suppress seizure activity *in vivo* are summarized in Table 1.

4.1 Direct modulation of the seizure focus

The main goal of direct modulation of epileptic foci is to interrupt ongoing seizure activity. The rationale for directly stimulating the activity of seizure foci with electricity largely stemmed from computational modeling that showed that excitation of neurons could interrupt synchronized bursting activity [43,44]. Indeed, electrical stimulation has been successfully utilized to modulate oscillatory activity [45,46]and seizures [10,47] in patients with epilepsy. The advent of cell-type specific tools such as optogenetics has allowed further dissection of the complex circuitry involved in seizure generation to identify important loci for direct therapeutic intervention.

Nonspecific inhibition of neurons—The notion that seizures are a manifestation of hyperactivity of pathological circuits naturally suggested the strategy of decreasing excitability of these circuits using NpHR. This approach was demonstrated in hyperactive hippocampal circuits byBerglind et al.[48], where an adeno-associated virus (AAV) encoding enhanced NpHR (eNpHR3.0) under control of the human synapsin promoter (hSyn) was utilized to express NpHR ubiquitously in neurons of the mouse hippocampus. Epileptiform bursting was induced in these animals by compromising inhibitory drive with an acute injection of bicuculline into the hippocampus. Upon illumination with yellow light, epileptiform burst rate was decreased by 17%. Although the reduction in burst rate was

significant, the rather modest effect size was attributed to the diffusion of bicuculline being too widespread compared to the area of optogenetic inhibition.

It is difficult to interpret effects of optogenetic manipulation when opsin expression occurs in multiple cell types in the area of illumination (*e.g.* with a CMV, EF1α, or hSyn promoter). The hSyn promoter used in these experiments drives expression of NpHR not only in excitatory pyramidal cells, but also in interneurons, the inhibition of which could potentially offset the inhibitory effects of silencing principal cells as they become disinhibited by decreased inputs from interneurons. Although $GABA_A$ transmission was blocked with bicuculline in these experiments, there still may have been other inhibitory circuits that were not affected by bicuculline (such as $GABA_B$ expressing or glycinergic neurons) that could have been subsequently turned off by optogenetic inhibition. Achieving more cell-type specific expression may thus help to clarify underlying mechanisms.

Inhibition of principal glutamatergic neurons—Tønnesen et al.[39] first demonstrated that specific inhibition of principal cortical neurons via a lentivirus carrying NpHR under control of the calcium/calmodulin-dependent kinase II isoform α (CaMKIIα) promoter was sufficient in reducing epileptiform activity in organotypic hippocampal brain slices, as detailed in the previous section. The same virus was used in a chronic tetanus toxin model of epilepsy in rats by Wykes et al [49], where NpHR activation in the motor cortex of awake animals was shown to attenuate epileptic activity induced by tetanus toxin. In this study, rats were photostimulated 7–10 days after co-injection of tetanus toxin and a CaMKIIα-NpHR2.0 lentivirus in the motor cortex. Although photostimulation was able to significantly reduce epileptic activity in the electroencephalogram (EEG), it was not sufficient to completely prevent all epileptic EEG events or produce any behavioral effects. This could be partially attributed to suboptimal expression levels of NpHR in the brain. For example, the copy number of NpHR delivered to neurons in the brain may be relatively small due to the fact that it is often difficult to obtain high-titer lentivirus compared to adeno-associated viral vectors (AAV). Furthermore, trafficking of NpHR to the cell membrane has been shown to be rather inefficient and may thus lead to suboptimal hyperpolarization of membrane potential upon photoactivation. Membrane trafficking of NpHR has been subsequently improved with addition of trafficking and endoplasmic reticulum-export sequences to eNpHR3.0, improving NpHR photocurrent dramatically[50].

This improved eNphR3.0 was utilized by Sukhotinsky et al. [51] for delaying seizure activity in an acute model of epilepsy in rats. In this study, an AAV encoding CaMKIIα-eNphR3.0 was injected into the hippocampus. Photoactivation (both continuous and pulsed illumination) in these animals was able to significantly delay the onset of status epilepticus (by 6 minutes) following systemic pilocarpine injection in awake behaving animals. Although both electrographic and behavioral manifestations of seizures were able to be delayed in these animals, they were not completely abolished. Similar to Berglind et al.[48] discussed above, the modest scale of seizure attenuation was attributed to the fact that the seizure model they used was not focal, resulting in a large number of potentially epileptogenic neurons residing outside the range of light delivery and expression of opsin.

Krook-Magnuson et al.[52] took a more comprehensive approach of utilizing a closed-loop seizure detection algorithm to silence excitatory principal cells in the mouse hippocampus. In this study, transgenic mice expressing eNpHR3.0 in principal cells (CaMKIIα-NpHR) were generated. These mice were then injected with kainic acid into the dorsal hippocampus to generate a chronic model of temporal lobe epilepsy. The authors were able to detect subsequent spontaneous seizures using custom-built software that utilized features such as signal power, spike waveform, and frequency. Upon detection of seizures in CaMKIIα-NpHR mice, optogenetic inhibition of principal cells ipsilateral to the kainate injection site stopped 57% of seizures within 1s of light illumination and reduced the mean seizure duration by 70%. Across all animals, optogenetic intervention reduced behavioral seizures by 29.6%. This same approach was utilized to specifically inhibit granule cells of the dentate gyrus of the hippocampus in transgenic mice expressing NpHR, which was shown to stop 75% of seizures within 5s of light delivery and reduce the seizure duration by 66%[53].

Excitation of interneurons—Chiang et al.[54] found that 50-Hz photostimulation of the hippocampus of transgenic mice expressing ChR2 under control of the Thy1 promoter was able to suppress seizures evoked by intracerebral injection of 4-aminopyridine by 82.4%. Although the Thy1 promoter in general drives expression of transgenes in various types of neurons in the brain, the authors found that ChR2 expression was mostly localized to GABAergic interneurons in the hippocampus, suggesting that seizure suppression was achieved by enhancing inhibitory GABAergic inputs that counteracted over-excitation seen in the hippocampal circuitry. The same group went on to show that low frequency (1 Hz) optogenetic stimulation of hippocampal interneurons was able to entrain and suppress hyperactivity induced by 4-aminopyridine [55]. Synchronous activation of GABAergic interneurons resulted in synchronous pyramidal cell firing, which acted to reduce the overall hyperactivity of the hippocampus from prolonged afterhyperpolarizations after stimulation was stopped. Different stimulation parameters can therefore lead to similar effects on seizure activity via very different mechanisms.

Excitation of inhibitory interneurons was achieved with greater cell-type specificity in a closed-loop fashion by Krook-Magnuson et al.[52]. In this study, transgenic mice expressing ChR in GABAergic interneurons (Parvalbumin-ChR) were generated and the effect of on-demand optogenetic stimulation was found to stop 59% of seizures within 5s of light illumination and reduced the mean seizure duration by 43%.

Targeting interneurons with ChR2 seems especially appropriate for epilepsy models that result from a loss of inhibitory activity. The advantage of this approach is that GABAergic interneurons widely innervate multiple pyramidal cells in the hippocampus and could thus exert very strong effects on the epileptic network[56,57]. However, as discussed above, GABAergic interneurons have also been directly implicated in synchronizing epileptic activity[58–60] and conceivably could potentiate seizure activity following photoactivation [61]. Stimulation parameters must therefore be carefully determined to ensure that proper inhibitory drive is delivered to the circuit in question. Simultaneous monitoring of optogenetic drive on the circuit by multielectrode recordings, voltage sensitive dyes, or genetically encoded indicators may therefore be advantageous in this context.

4.2 Modulation of structures remote from the epileptic origin

The studies described above all focused on modulating seizure activity directly at the epileptic focus or site of injury. Another approach is to target remote structures projecting to areas involved in seizure genesis and propagation. This approach has unique advantages since seizures may arise from multiple structures or discrete networks in the brain[62]. Indeed, electrical stimulation of structures remote from epileptic foci such as the cerebellum[63–67], thalamus[15,68–73,16], subthalamic nucleus[74–76], and caudate[77–79] have been found to be beneficial in reducing seizures in human patients. Optogenetic control of some of these remote structures have also been evaluated in various rodent models.

Krook-Magnuson et al. investigated the role of the cerebellum in controlling spontaneous temporal lobe seizures by optogenetically targeting parvalbumin-expressing neurons (*e.g.* Purkinje cells) in the medial (vermis) and lateral (lobulus simplex) cerebellar cortex of transgenic mice[80]. They found that interruption of cerebellar activity (either by excitation with ChR2 or inhibition with NpHR) in the lateral or medial cerebellum was able to reduce seizure duration. In contrast, only excitation of PV+ Purkinje cells in the medial cerebellum was able to reduce seizure frequency in addition to duration. Kros et al. [81] also targeted cerebellar nuclei with ChR2 to modulate generalized spike-and-wave discharges (GSWDs) seen in various animal models of absence epilepsy. They found that optogenetic stimulation of the cerebellar nuclei was able to stop GSWDs when stimulation was delivered either bilaterally or unilaterally. The authors implemented this stimulation paradigm in a closed-loop fashion with real-time detection of GSWDs.

The thalamus was targeted by Paz et al. with an optogenetic strategy that interrupted both electrographic and behavioral seizures in a rat cortical seizure model [82]. In this study, rats were given a focal cortical stroke that resulted in hyperexcitability of thalamocortical neurons projecting to the stroke area. Optogenetic inhibition of glutamatergic thalamocortical neurons via an AAV encoding CaMKIIα-eNphR3.0 was subsequently shown to interrupt electrographic and behavioral seizures when light illumination was manually switched on during seizure. By modulating the activity of thalamocortical neurons, the authors were able to demonstrate the potential benefits of targeting structures that lie upstream from areas of seizure activity. They expanded upon their open-loop approach for interrupting thalamocortical seizures by developing a method to automatically trigger optical stimulation when the EEG line-length crossed a certain threshold that was indicative of a seizure event. Using this closed-loop approach, seizures were detected and silenced within 1s of seizure onsets.

Soper et al. subsequently demonstrated that activation of the superior colliculus via ChR2 was able to attenuate seizure activity in various rat models of epilepsy[83]. These effects were seen on seizures involving the forebrain and brainstem (induced by systemic pentylenetetrazol administration), complex partial seizures (induced by focal bicuculline injection in the area tempestas), absence seizures (induced by systemic gamma butyrolactone administration), and brainstem seizures (in genetically epilepsy prone rats).

The fact that modulation of a single area in the brain could reduce seizure activity found in different models of epilepsy illustrates the potential of targeting remote structures that have broad influence on various networks. The cell-type specificity of optogenetic approaches can further refine the effects that these remote structures have on the areas to which they project.

5. Future considerations

5.1 Basic considerations

Optogenetics has become an extremely versatile technique due to the numerous possible combinations of opsin, cell-type expression, and stimulation parameters available. Given this rich toolbox, the appropriate optogenetic probes must be carefully considered since subtle differences can lead to very different physiological outcomes. For instance, non-specific expression of opsin could potentially lead to activation of competing circuitry, masking potentially therapeutic targets. Appropriate light delivery and stimulation parameters must also be carefully explored as inappropriate stimulation can lead to undesired effects. For example, NpHR is known to cause synchronous rebound excitation after prolonged illumination[84], which can potentially exacerbate seizure activity. This rebound firing can potentially be avoided by a more sophisticated illumination regimen or with a different silencer (*e.g.* proton pumps such as Arch)[85].

New optogenetic tools may offer several advantages over the ones used in the studies described above. For example, *Maculans* opsin (Mac) and *Halorubrum* archaerhodopsin (Arch and ArchT) are proton pumps that have been successfully used to inhibit neural activity *in vitro* and *in vivo*[86], and are arguably more effective than NpHR in terms of photocurrent response without risking chloride loading[84]. However, Arch may also have undesired effects of its own with prolonged activation, such as intracellular acidification [87]. Step function opsins (SFOs) are another alternative that may be more suitable for *in vivo* use because they have prolonged deactivation kinetics (potentially leading to longer lasting effects), they can be turned on and off with light of different wavelengths, and they have greater light sensitivity[88]. Several new inhibitory SFOs, namely slow ChloC[89] and SwiChR[90], have also been recently described in the literature.

5.2 Translational considerations

Several important challenges facing the use of optogenetics *in vivo* still need to be addressed before it can be effectively translated into more clinically relevant contexts (Figure 4). First, optogenetics requires gene therapy to deliver and express opsins in the brain, and safety and effectiveness of using viral vectors in humans must be addressed. Although there are currently no fully FDA-approved viral vector mediated gene therapies, several clinical trials have been conducted or are currently underway under investigational new drug (IND) exemptions [91–97]. The pros and cons of utilizing a gene therapy in humans must be carefully assessed. For patients that have limited treatment options (*e.g.* those with intractable epilepsy that are not candidates for resective or ablative surgery, or who are not comfortable with the potential risks), gene therapy remains a promising alternative treatment option. This would be particularly suitable for patients with focal epilepsy because these areas in the brain could be subsequently resected in some patients if gene therapy failed.

Second, viral vector technology must be improved in order to achieve efficient widespread cell-specific expression in humans. The number of cell-type specific promoters capable of being packaged into a viral vector is relatively few due to their large size and expression patterns remain hard to predict. Utilizing lentiviral vectors may offer a means to deliver large genetic payloads containing cell-specific promoters. A variety of available virus serotypes also offer different properties in regards to cell-type specificity, spread, and transduction efficiency[29]. Third, the volume of influence with optogenetic techniques must be improved. Methods to improve viral spread such as delivery to the cerebrospinal fluid[98,99], convection-enhanced delivery[100,101], or utilizing particular viral serotypes[102–104] would allow larger structures to be targeted. This is particularly relevant in gene therapeutic approaches to epilepsy targeting the seizure focus, which can be quite extended as in the case of malformation of cortical development (*e.g.* focal cortical dysplasias[105]), or where larger subcortical nuclei are targeted (*e.g.* anterior nucleus of thalamus). Fourth, light delivery into the brain must also be improved because it is relatively inefficient due to tissue absorption and light scattering. Indeed, one reason why optogenetic approaches have not been able to produce very robust behavioral responses in primates is hypothesized to be because volume of illumination is too small[88]. A potential solution to this problem is to utilize red-shifted opsins (*e.g. Volvox* channelrhodopsin 1,[106] C1V1[107], or ReaChR[108] for excitation; red-shifted Halorhodopsin, Jaws, for inhibition[109]) since longer wavelength light is able to penetrate tissue better. Several groups have also designed multi-fiber arrays that deliver light through multiple fiber optics[110,111]. Although these approaches have helped improve the potential volume of tissue illumination, the scalability to human brain and hardware dependency is still unclear or impractical for clinical application. One approach that may address both of these challenges is the use of luminopsins[112–115], whereby photoactivation of optogenetic channels is conferred by bioluminescent proteins (*e.g.* luciferase) delivered in the same gene payload, thus tracking exactly with the expressed channels. However, this approach is presently limited by the need to deliver the substrate for the bioluminescent proteins as well as its limited temporal control and therefore warrants more extensive safety, pharmacodynamic, and pharmacokinetic studies.

The use of different optogenetic tools and approaches to successfully halt seizure activity in a variety of animal models indicates that no single intervention is ideal for treating all types of seizure activity seen in human patients. In contrast to electrical brain neuromodulation, *e.g.* RNS or thalamic deep brain stimulation, optogenetics offers the power to tailor the therapy, activating or inhibiting as needed, in specified cell types while sparing others in the same vicinity, as indicated by the particular patient-specific epileptic network. Optogenetic therapies will therefore need to be tailored to the needs of the patient in regards to cell-type expression, target structures, and stimulation parameters. Information from pre-surgical workups (*e.g.* EEG, surface, and depth electrode recordings) can be utilized to determine the optimal optogenetic strategy for modulating pathological cellular targets for each patient[116]. Real-time detection and analysis of epileptic biomarkers may help in triggering customized stimulation paradigms for suppressing seizure activity in a closed-loop fashion[42]. Early detection and intervention of seizure activity has been particularly valuable for patients with epilepsy because epileptic foci could be targeted to limit seizure

generalization and altered levels of consciousness. With the ability to modulate circuits with the cell type specificity of optogenetics, these closed-loop approaches to seizure suppression can potentially achieve even greater efficacy with reduced side effects.

6. Conclusion

Optogenetics has proven to be a powerful technique for studying neural circuitry in the epileptic brain. With the ability to perturb specific circuitry and determine the effects on seizure states, optogenetic techniques have proven to be invaluable in studying seizure dynamics, dissecting the circuitry involved in seizure generation, and identifying new therapeutic targets. Optogenetic techniques can also be utilized to generate new epilepsy models: for example, ChR2 was used to induce seizure activity in several animal models[53,117]. While lacking construct validity as models for human epilepsy, these approaches in turn can be used to better understand specific cell types and their roles in epileptogenesis and to develop therapeutic avenues.

Initially a useful research tool, optogenetics has progressed to becoming a promising neuromodulatory therapy. These initial studies have demonstrated that targeted control of neural activity is capable of reducing both electrographic and behavioral seizures in various animal models of epilepsy. It is difficult to assess the efficacy of these approaches due to the limited number of studies, but optogenetic interventions were able to inhibit 60–100% of electrographic and 30–100% of behavioral seizures arising spontaneously in various animal models of epilepsy, which is comparable or perhaps superior to existing neurostimulation therapies in patients. These effects could potentially be increased with the use of stronger[90,118], more responsive[106,119], and longer-lasting opsins[88]. The cell-type specificity and known mechanism of action makes optogenetics a particularly attractive neuromodulatory tool since both of these features are not as refined for existing electrical stimulation modalities.

Despite the promising clinical potential for optogenetics, significant progress still needs to be made in terms of technical scalability and evaluating long term safety for these approaches to become more translatable for clinical use in humans. We are optimistic that these challenges are not insurmountable and that optogenetics will move towards a more translational path with the continued open collaboration of engineers, neuroscientists, clinicians, and regulators.

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Highlights

- Optogenetics shows great promise as a direct neuromodulatory therapy for halting seizure activity.
- The ability to activate or inhibit specific cell types has resulted in various approaches for halting seizure activity in rodent models of epilepsy.
- These approaches offer a promising means of controlling seizure activity, but several challenges still need to be addressed before they can be effectively translated into viable therapies for patients with epilepsy.

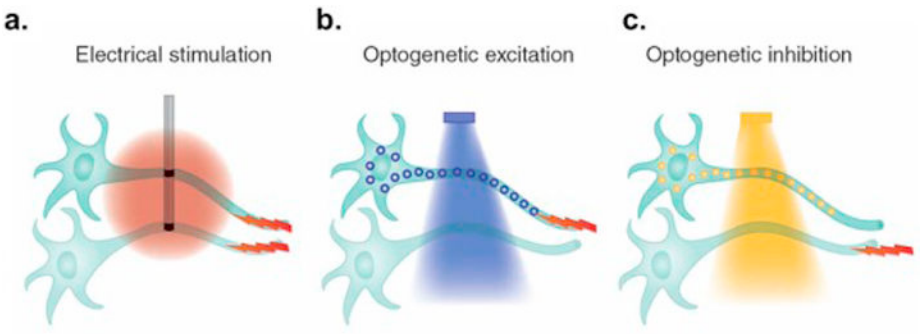

Figure 1. Optogenetic vs. electrical stimulation approaches to neuromodulation

(A) The effects of electrical stimulation is highly dependent on stimulation parameters and acts nonspecifically on cells around the stimulating electrode. The effects of optogenetic excitation **(B)** or inhibition **(C)** can be cell-type specific. Figure reprinted from Nature Methods 8, 26–29 (2011).

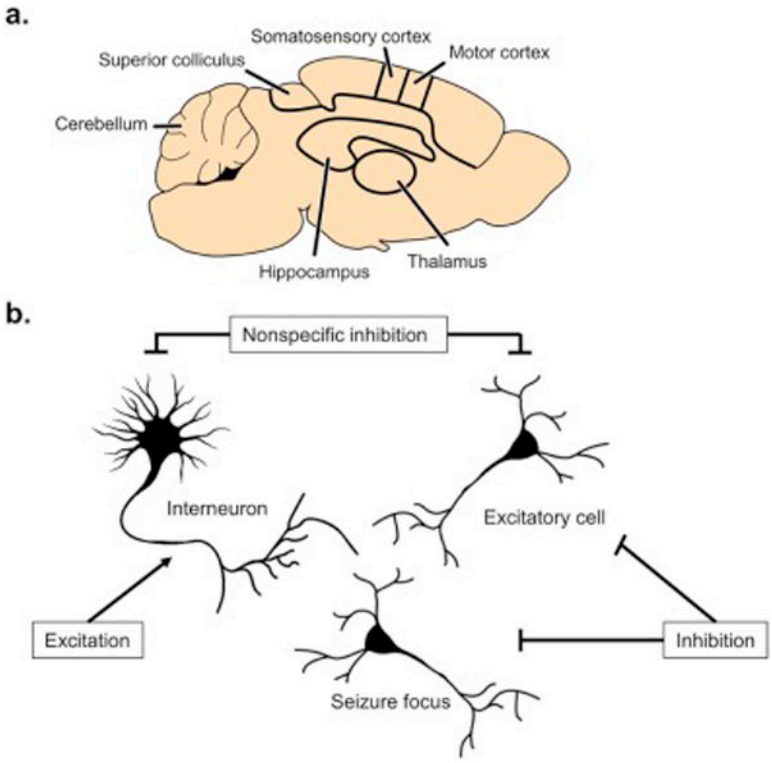

Figure 2. Anatomical and cell-type specific approaches to inhibit seizure activity

The various optogenetic approaches utilized to halt seizure activity can be grouped by structure **(a)** or cell types **(b)**.

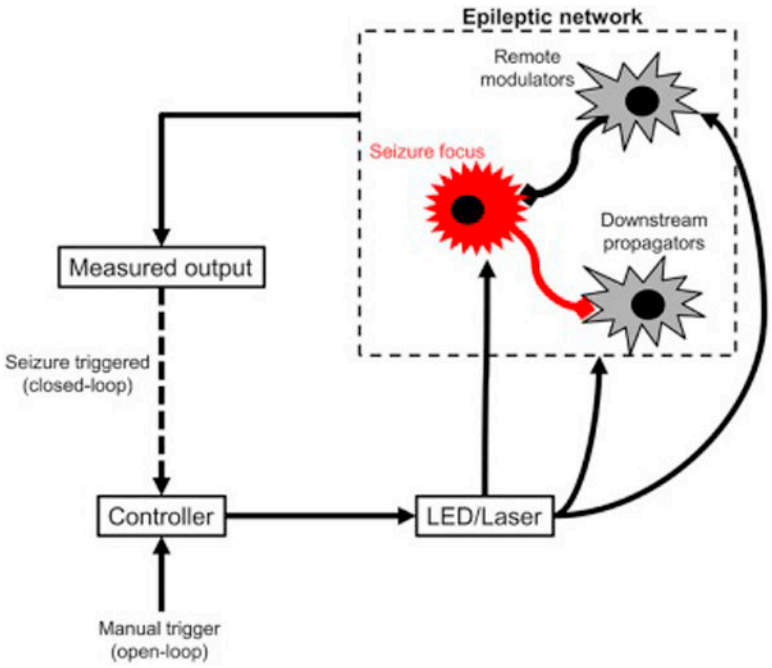

Figure 3. Closed-loop vs. open-loop approaches to light delivery

Optogenetics can be utilized to target specific cell types in the epileptic network at the seizure focus, remote modulators of the seizure focus, or downstream propagators of seizure activity. Light can either be delivered manually in an open-loop fashion, or automatically in response to a detected seizure (closed-loop).

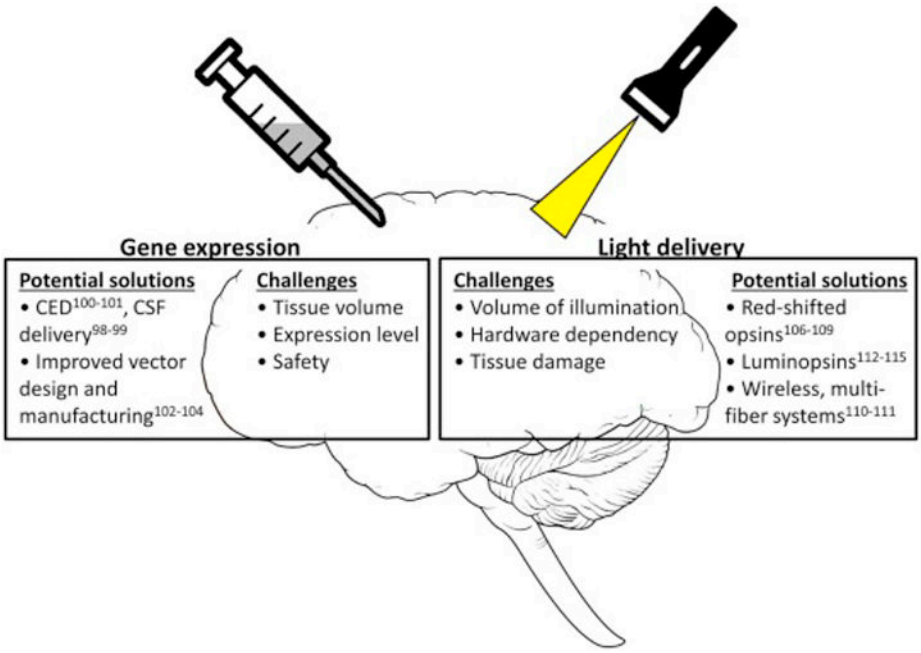

Figure 4. Translational challenges for optogenetics

Several challenges with gene expression and light delivery need to be addressed to facilitate effective translation of optogenetic techniques into a clinical setting. Some potential solutions to these challenges are listed. Abbreviations: CED: convection-enhanced delivery; CSF: cerebrospinal fluid.

Table 1

		Reference	Opsin/Expression system	Animal model	Seizure model	Stimulation location/parameters	Electrographic response	Behavior response
Remote structures	Thalamus	Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury[82].	AAV-CAMKIIα-NpHR3.0	Rat	• Focal cortical seizures by photothro mbotic stroke in somatose nsory cortex (chronic)	• Ventrobasal thalamus. • On-demand continuous illumination for 0.5 or 10s.	• Interruption of epileptic activity. • Reduction of RMS power in cortex (bilateral) and thalamus	• Interruption of all behavioral seizures.
	Cerebellum	Cerebellar directed optogenetic intervention inhibits spontaneous hippocampal seizures in a mouse model of temporal lobe epilepsy[80].	Transgenics: • PV-ChR2 • PV-NpHR • Pcp2-ChR2	Mouse	• Kainic acid injected to left dorsal hippocampus (chronic)	• Lateral cerebellar cortex, vermis • On-demand 3s short (50ms on/ 100ms off) and long (1000ms on/ 50ms off) pulses.	PV-ChR2: • Vermis: 39% duration reduction, 175% increase in time to next seizure. • Lateral: 30–35% duration reduction PV-NpHR: • Lateral: 32–44% duration reduction Pcp-ChR2: • Lateral: 46–49% duration reduction • Vermis: reduction in seizure frequency.	• Not reported
		Cerebellar output controls generalized spike-and-wave discharge occurrence[81].	AAV-hSyn-ChR2	Mouse	• natural mutant *tg* • C3H/HeOuJ	• Cerebellar nuclei (unilateral or bilateral) • Single 30–300 ms pulse manually triggered or on-demand.	*Tg* mice: • 88% (bilateral stim) and 94% (unilateral stim) of GSWDs stopped on average • Significant reduction in GSWD power (6–9 Hz) for all stimulation parameters. C3H/HeOuJ mice: • 87% of GSWDs stopped on average • Significant reduction in GSWD power.	• Not reported
	Superior colliculus	Optogenetic activation of superior colliculus neurons suppresses seizures originating in diverse brain networks[83].	AAV-hSyn-ChR2	Rat	• Systemic PTZ • Focal BM injection • GEPRs • Systemic GB	• Superior colliculus (bilateral) • Continuous, 5Hz, 100Hz pulses with 50% duty cycle before seizure onset.	• PTZ: attenuation of seizures. • BM: attenuation of seizures. • GB: decrease frequency and duration of discharges	• PTZ: decrease in severity. • BM: decrease in severity and frequency. • GEPR: decrease in severity and

		Reference	Opsin/Expression system	Animal model	Seizure model	Stimulation location/parameters	Electrographic response	Behavior response
								latency to onset. • GB: decrease in severity.
Seizure foci	Neocortical	Optogenetic and Potassium Channel Gene Therapy in a Rodent Model of Focal Neocortical Epilepsy[49].	Lenti-CAMKIIα-NpHR2.0	Rat	• Tetanus toxin (chronic)	• Motor cortex • 20s on/20-s off duty cycle	• Reduction of high-frequency power, EEG coastline, and epileptiform events.	• None
	Temporal lobe	Optogenetic inhibition of chemically induced hypersynchronized bursting in mice[48].	AAV-hSyn-NpHR3.0	Mouse	• Bicuculline Methiodid e (BM) infusion by chemical optrode to CA1/CA2 (acute)	• Ventro-posterior hippocampus • 40s continuous illumination	• Reduction of BM induced bursting by 17%.	• Not reported
		Optogenetic delay of status epilepticus onset in an in vivo rodent epilepsy model[51].	AAV-CAMKIIα-NpHR3.0	Rat	• Li-pilocarpin e IP injection (acute)	• Hippocampus • Continuous illumination, 1–2min on/1min off	• Both stimulation protocols delayed onset of status by 6 min on average.	• Delayed onset of status by 6 min on average.
		Seizure Suppression by High Frequency Optogenetic Stimulation Using In Vitro and In Vivo Animal Models of Epilepsy[54].	Thy1-ChR2 transgenic	Mouse	• Intracereb ral 4-AP (acute)	• Hippocampus • 5ms pulses at 20Hz and 50Hz	50Hz stim: • 82.4% ipsi, 66.7% contra suppression of power. 20Hz stim: • 80.2% ipsi, 61.2% contra suppression of power.	• Not reported
		Seizure reduction through interneuron-mediated entrainment using low frequency optical stimulation[55].	VGAT-ChR2-YFP transgenic	Mouse	• Intracereb ral 4-AP (acute)	• Hippocampus • 5ms pulses at 1 Hz	• Ipsilateral: 68% suppression of power. • Contralateral: 59% suppression of power.	• Not reported
		On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy[52].	Transgenics: -PV-ChR2 -CAMKIIa-NpHR3.0	Mouse	• Kainic acid (chronic)	• Dorsal hippocampus • 50ms on/100ms off • 2,000ms on/50ms off	CAMK-HR: • 58% seizures stopped within 1s of light delivery. • seizure duration reduced by 70% PV-ChR: • 59% seizures stopped within 5s of light delivery. • seizure duration reduced by 43%	• 29.6% reduction in behavioral seizures overall

		Reference	Opsin/Expression system	Animal model	Seizure model	Stimulation location/parameters	Electrographic response	Behavior response
		In vivo evaluation of the dentate gate theory in epilepsy[53].	Transgenics: -GC-NpHR -CAMKIIa-NpHR3.0	Mouse	• Kainic acid (chronic)	• Dorsal hippocampus On demand: • 473nm: 30s illumination, 50ms on/100ms off • 589nm: 30s illumination, 2,000ms on/50ms off	GC-NpHR: • 75 ± 7% of seizures stopping within 5 s of light delivery. • 66 ± 4% reduction in seizure duration CAMKIIa-NpHR: • 72 ± 6% reduction in seizure duration	• Not reported

Abbreviations: GC: granule cells; NpHR: Halorhodopsin; ChR2: Channelrhodopsin; BM: bicuculline methiodide; Pcp2: Purkinje cell protein 2; CaMKIIα: calmodulin-dependent protein kinase isoform IIα; hSyn: human synapsin; Thy1: thymocyte antigen 1; VGAT: vesicular GABA transporter; PV: parvalbumin; GEPR: genetically epilepsy prone rats; GB: gamma butyrolactone; 4-AP: 4-aminopyridine.