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Optogenetic approaches to treat epilepsy

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HIGHLIGHTS

- This review highlights the potential use of optogenetics to treat epilepsy.
- We discuss optical manipulation of neuronal activity and recent research where this technique has been used to control seizures in animal models of epilepsy.
- We then discuss the different opsin strategies available and what will be required to translate promising animal research to treatment in the clinic.

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ABSTRACT

Background: Novel treatments for drug-resistant epilepsy are required.
New method: Optogenetics is a combination of optical and genetic methods used to control the activity of specific populations of excitable cells using light with high temporal and spatial resolution. Derived from microbial organisms, 'opsin' genes encode light-activated ion channels and pumps. Opsins can be genetically targeted to well-defined neuronal populations in mammalian brains using viral vectors. When exposed to light of an appropriate wavelength, the excitability of neurons can be increased or decreased optically on a millisecond timescale.
Comparison with existing method(s): Alternative treatments for drug-resistant epilepsy such as vagal, cortical or subcortical stimulation, focal cooling, callosotomy, or ketogenic diet have met with limited success, whereas optogenetic approaches have shown considerable pre-clinical promise.
Conclusions: Several groups have reported that optogenetic approaches successfully attenuated epileptiform activity in different rodent models of epilepsy, providing proof of the principle that this approach may translate to an effective treatment for epilepsy patients. However, further studies are required to determine the optimal opsin, in which types (or subtypes) of neurons it should be expressed, and what are the most efficient temporal profiles of photostimulation. Although invasive due to the need to inject a viral vector into the brain and implant a device to deliver light to opsin-transduced neurons, this approach has the potential to be effective in suppressing spontaneous seizures while avoiding the side-effects of anti-epileptic drugs (AEDs) or the need to permanently excise regions of the brain. Optogenetic approaches may treat drug-refractory epilepsies.

Contents

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1. Introduction

Even with optimal treatment, over 20% of people with epilepsy continue to have seizures (Kwan et al., 2011). Of these, approximately 75% have focal epilepsy, for whom the best prospect of seizure freedom is surgery. However, surgical resection is only appropriate in the minority of cases, where the removal of the epileptogenic zone will not have adverse effects on movement, language, and vision (Schuele and Luders, 2008). New approaches for drug resistant epilepsies are urgently required. Alternative treatments for drug-resistant epilepsies include therapies such as vagal, cortical or subcortical stimulation, focal cooling, callosotomy, or ketogenic diet (Kahane and Depaulis, 2010; Kossoff and Hartman, 2012; Boon et al., 2009). However, these therapies have met with limited success. As the seizure focus can often be precisely defined using MRI and EEG, a promising approach is to modify gene expression locally in neurons contributing to the initiation of seizures. There are several gene therapy approaches that have been experimentally tested in animal models of epilepsy (reviewed in Simonato et al., 2013; Kullmann et al., 2014; Simonato, 2014; Sorensen and Kokaia, 2013). Of these optogenetic strategies have shown considerable pre-clinical promise. Optogenetics, although initially invasive, can (in principle) be permanent and require no further intervention other than light delivery, in contrast with antiepileptic drugs (AEDs) that need to be taken for decades. Therefore, a strategy based on expressing opsins in neurons within an epileptic focus may translate to an effective therapy for epilepsy.

2. Optical manipulation of neuronal activity

Neurons are electrically excitable and maintain a voltage gradient across their membranes using a variety of pumps and ion channels. When positive ions flow into a neuron they depolarise the membrane potential, and if the change in voltage is large enough an action potential is generated. When negative ions flow into the neuron the membrane hyperpolarizes making action potential firing more difficult. As a result, neuronal excitability can be directly controlled by methods that depolarise or hyperpolarise the membrane. There are two general classes of opsins that can facilitate or inhibit action potential firing in neurons by depolarising or hyperpolarising the neuron in response to light of specific wavelengths. The idea of optically controlling neuronal function was first suggested by Francis Crick (Crick, 1999) and the optical manipulation of behaviour was demonstrated more than a decade ago (Zemelman et al., 2002; Lima and Miesenbock, 2005). However, it has only been in recent years that the full potential of this approach has been realized. This is for two main reasons. First, the discovery and bioengineering of opsins with improved biophysical properties. Second, advances in molecular biology that has resulted in the ability to target these opsins to specific types of neurons (Fenno et al., 2011).

Channelrhodopsin-2 (ChR2) is an ion channel derived from the alga *Chlamydomonas reinhardtii*. When activated by blue light, it passes positively charged ions into a cell, depolarizing its membrane (Nagel et al., 2003). In 2005, Ed Boyden and Karl Deisseroth at Stanford University successfully expressed ChR2 in mammalian neurons making them responsive to photostimulation (Boyden et al., 2005) – see Fig. 1.

Since ChR2 is rapidly activated and inactivated when the light is switched on and off, single action potentials can be fired in response to brief (~2 ms) exposures of light allowing for precise temporal light-mediated control of neuronal spiking.

The bioengineering of naturally found opsins has resulted in a variety of chimeric light-sensitive proteins with enhanced expression, trafficking, kinetics and light activation properties (Lin, 2011). These include a variant of ChR2 with red-shifted spectral properties (Yizhar et al., 2011). This and other opsins activated by yellow or green light (Lin et al., 2013) have particular advantages in studies of living animals as light penetration through tissue increases with wavelength. This means that a larger area of brain can be stimulated with the same light intensity. Some versions of ChR2, including the H134R mutation (Nagel et al., 2005) and the (E123T/T159C) (Berndt et al., 2011) can allow cells to be optically driven with spike-timing precision up to frequencies that approach the highest firing rates observed *in vivo*.

Halorhodopsin (NpHR), is a light-driven chloride pump derived from the halobacterium *Natronomonas pharaonis*. It was the first microbial opsin shown to inhibit neuronal activity (Zhang et al., 2007). When expressed in mammalian neurons and exposed to yellow light, halorhodopsin pumps chloride ions into the cell, hyperpolarising the membrane potential and inhibiting action potential firing. Extensive work on mutagenesis of this opsin has resulted in better expression levels, larger photocurrents and more effective membrane hyperpolarization, and currently eNpHR3.0 is the version of the opsin most commonly used (Gradinaru et al., 2010). Appropriate care should be taken to ensure that excessive

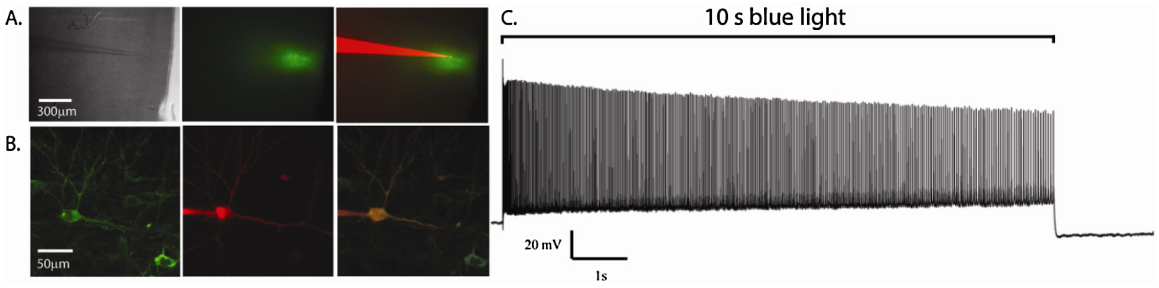

Fig. 1. Channelrhodopsin-mediated firing of action potentials. (A) Viral-mediated expression of channelrhodopsin tagged with a fluorescent protein (green) in a restricted part of rodent cortex. (B) 2-Photon image of a virally transduced neuron (green) patched with a pipette (pipette contained a red dye). (C) Illumination of the brain slice with 10 s of blue light results in robust AP firing (detected electrophysiologically from the patched neuron).
Rob Wykes (unpublished data).

chloride loading does not alter the chloride reversal potential (E_{Cl}), as this could result in depolarising GABA-mediated currents (see later). Light-driven proton pumps – such as those derived from archaeorhodopsin-3 (Chow et al., 2010), are also able to inhibit neuronal firing by pumping protons out of the cell and hyperpolarising the membrane potential. Variants of halorhodopsin and archaeorhodopsin-3 have been found to be very effective at silencing neuronal activity both in tissue samples and in live animals. A red-shifted cruxhalorhodopsin derived from a strain of shark and named 'Jaws' has been engineered to result in red light-induced photocurrents much larger than earlier silencers (Chuong et al., 2014). More recently versions of ChR2 have been engineered to conduct anions rather than cations (Berndt et al., 2014; Wietek et al., 2014), allowing the possibility of quicker inhibition as they rely on the direct activation of an ion channel rather than relatively slow operation of a pump.

Optogenetics has been embraced by neuroscientists. It has already allowed us to identify neuronal circuits involved in information processing and behavioural responses, both in normal and diseased brains (Tye and Deisseroth, 2012). Compared to the methods traditionally used to stimulate or modulate neuronal activity, optogenetic approaches have a far superior selectivity than electrode techniques and better temporal resolution than pharmacological manipulations. Optogenetic techniques have been applied to the study of neuronal circuits in numerous regions of the brain. These include the cerebral cortex, hippocampus, striatum and the nucleus accumbens. Rodent and non-human primate studies have indicated that optogenetic neuromodulation can potentially be used to treat diverse neurological disorders (Tonnesen, 2013), including motor dysfunction in Parkinsonian animals (Kravitz et al., 2010) and permitting simple visual abilities in genetically blind rodents (Lagali et al., 2008; Doroudchi et al., 2011).

3. Optogenetic control of seizures

An ideal therapeutic strategy for epilepsy would target only brain regions responsible for seizure generation, something not possible with systemically delivered anti-epileptic drugs. Additionally this therapy should not interfere with normal physiological function. Because seizures are intermittent, an important advance would be achieved by developing a method for the rapid and reversible suppression of activity in a restricted area of the brain. A way to suppress seizure activity 'on demand' is to photoactivate light-sensitive ion channels and transporters that have been expressed in neurons. There are two general optogenetic approaches one could take to inhibit seizures. The first would be to express an inhibitory opsin (such as halorhodopsin) in excitatory (principal) neurons to suppress their excitability and reduce output. The second approach would be to express an appropriate opsin in populations of interneurons to control their firing in a manner that would result in increased inhibition of principal neurons. Choosing and expressing an appropriate opsin for interneurons is not straightforward and will be addressed in more detail later.

The expression of halorhodopsin in principal neurons has successfully been used to control electrically and chemically induced epileptiform activity in brain slice preparations (Tonnesen et al., 2009). In recent years, several groups have begun to investigate whether the optogenetic control of neurons can attenuate seizure activity *in vivo* in distinct rodent models of epilepsy. Initially these studies were focused on rodent models of neocortical, thalamic and temporal lobe epilepsy and relied on expression of Halorhodopsin (either NpHR2.0 or NpHR3.0) in principal neurons. (Wykes et al., 2012; Paz et al., 2013; Krook-Magnuson et al., 2013).

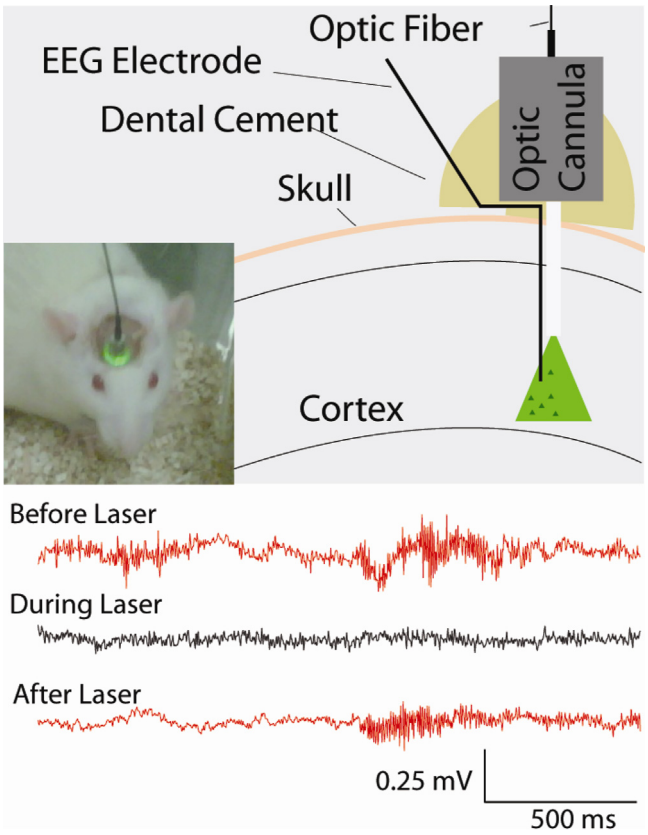

Fig. 2. Optogenetic suppression of epileptiform activity *in vivo*. Acute *in vivo* optogenetic attenuation of epileptic activity. Pyramidal neurons within the epileptic focus were transduced with a virus to express halorhodopsin and could be stimulated with a 561-nm laser *via* an attached fibre optic. Representative EEG traces before, during and after laser illumination. Bursts of pathological high frequency activity were suppressed during laser stimulation.
Adapted from R. Wykes et al. (2012).

3.1. Neocortical epilepsy

To test whether an optogenetic approach would work in awake, freely moving animals, we chose a rodent model of chronic neocortical epilepsy based on tetanus toxin injection into the motor cortex (Nilsen et al., 2005; Wykes et al., 2012). In this model, frequent bursts of high frequency epileptiform EEG activity (70–160 Hz) associated with clonic movements of the contralateral forelimb are observed. Similar to *epilepsia partialis continua* in human patients, these seizures respond poorly to AED therapy (Nilsen et al., 2005). We used a viral vector encoding halorhodopsin to transduce principal neurons within the epileptic focus. We then delivered yellow light using a laser attached to a fibre optic cable targeted to the epileptogenic region of the brain. When we activated halorhodopsin with the laser light, we were able to attenuate pathological high frequency epileptiform events (Wykes et al., 2012) – see Fig. 2.

3.2. Thalamocortical epilepsy

In a model of focal cortical stroke, in which spontaneous seizures develop around one month after insult, the thalamus was identified as being critically involved in the generation of post-stroke seizures (Paz et al., 2013). Halorhodopsin-mediated inhibition of thalamocortical neuronal activity was sufficient to interrupt seizure generation and prevent the epileptogenic activity from spreading throughout the thalamocortical network (Paz et al., 2013).

3.3. Temporal lobe epilepsy

In a model of chronic temporal lobe epilepsy, halorhodopsin-mediated inhibition of principal neurons was effective in attenuating hippocampal spontaneous seizures. Additionally this study demonstrated that channelrhodopsin 2-mediated excitation of a sub-population of GABAergic neurons could also attenuate spontaneous seizures (Krook-Magnuson et al., 2013).

Therefore optogenetic strategies have been investigated in multiple rodent models of epilepsy and despite diverse underlying pathophysiologies and different epileptogenic areas of the brain, these strategies were able to successfully suppress seizure activity.

4. Identification of the best opsin strategy

Most epilepsy-related optogenetic studies have focused on expressing halorhodopsin in principal neurons to suppress seizure activity. However, opsin activation or inhibition of interneurons may also be effective and have started to draw attention in the past few years. Several groups have thus used channelrhodopsin-2 expressed in interneurons to control seizure activity in various *in vitro* and *in vivo* epilepsy model (Krook-Magnuson et al., 2013; Ellender et al., 2014; Ledri et al., 2014; Shiri et al., 2015; Yekhlef et al., 2015). This strategy is, nevertheless, not straightforward and there are several issues that need to be addressed here. The cortical and/or hippocampal inhibitory system consists of numerous different interneuron classes (>20 types) exhibiting diverse firing patterns, morphologies, axonal targets, molecular markers, and functions within the network (Klausberger and Somogyi, 2008; DeFelipe et al., 2013). Hence, we need to precisely identify the contribution of each sub-population of interneurons in seizure generation, maintenance and termination to efficiently employ them during pathological activity.

Although interneuronal classification is complex and is based on multiple parameters, two broadly defined types of interneurons can be distinguished and investigated as a starting point: the peri-somatic targeting interneurons and the dendritic targeting interneurons (Klausberger and Somogyi, 2008). The former are usually fast-spiking cells ideally placed for the rapid control of the spike timing of pyramidal neurons, while the latter often form the feedback inhibitory circuits contributing to dendritic integration and to the routing of information received from different excitatory inputs. Dendritic targeting interneurons can also exert a disinhibitory action in the local networks by suppressing activity of other interneuronal types.

A pioneering study, conducted by the group of Ivan Soltesz, showed that selective optogenetic stimulation of peri-somatic targeting interneurons (expressing parvalbumin (PV)) has an anti-epileptic effect by reducing duration of seizures *in vivo* (Krook-Magnuson et al., 2013). However, several *in vitro* studies have attributed a pro-epileptic role to this neuronal population. Activation of PV interneurons paradoxically can evoke epileptiform discharges under hyperexcitable conditions, and their recruitment during spontaneous seizure-like activity was ineffective to suppress it (Ellender et al., 2014; Shiri et al., 2015; Yekhlef et al., 2015; but see also Ledri et al., 2014, in which optogenetic activation of interneurons suppressed epileptiform activity). Even though these diverse outcomes may partly depend on the experimental model used (*in vivo vs. in vitro*), it is essential to clarify the role of peri-somatic inhibition in the initiation and maintenance of seizure activity in order to develop the appropriate strategy.

Monitoring the spiking pattern of different interneuron classes during seizure-like activity has revealed that some peri-somatic targeting interneurons cease firing and enter into a "depolarization block" state shortly after the epileptiform discharge onset, while dendritic targeting interneurons augment their activity (Timofeev et al., 2002; Ziburkus et al., 2006; Cammarota et al., 2013; Karlocai et al., 2014). In addition, Ellender et al. (2014) have recently reported a selective transient collapse of the chloride gradient (leading to a depolarizing shift of the $GABA_A$ reversal potential) at the somatic region of pyramidal neurons, shifting somatic but not dendritic inhibition towards excitation during seizure activity. Both mechanisms, although apparently mutually exclusive, emphasize the deficiency of somatic inhibition during seizures, yet the uncertainty regarding the use of halorhodopsin/archaeorhodopsin-3 to hyperpolarize PV-containing interneurons as an efficient strategy to curtail seizures still remains unresolved.

Hitherto, the optogenetic activation of dendritic inhibition has received much less attention although it is worth investigating its potential to treat epilepsy. Unlike interneurons that contribute to peri-somatic inhibition, dendritic-targeting interneurons seem to maintain their firing during epileptiform activity (Cammarota et al., 2013; Karlocai et al., 2014), and the dendritic GABAergic drive may not switch from inhibitory to excitatory during seizures (Ellender et al., 2014). Therefore, this approach might have the ability to restrain excitation received by pyramidal neurons, thus dampening the abnormal network activity.

Exploring techniques that combine the optogenetic activation of dendritic interneurons with simultaneous inhibition of peri-somatic target interneurons or pyramidal neurons may also pay off. This could be tested by expressing an inhibitory opsin in pyramidal neurons or PV-containing interneurons and a red-shifted variant of channelrhodopsin-2 in dendritic-targeting interneurons. Illumination of the brain with yellow light should simultaneously activate and inhibit different interneurons and/or pyramidal neurons involved – resulting in greater restraint on hyper-excitable network activity. If successful this may increase the level of seizure suppression compared to a single opsin strategy.

In the long term, an optogenetic strategy that targets interneurons may be beneficial since unlike hyperpolarization of pyramidal neurons it aims to restore the excitation-inhibition balance with a minimal disturbance of the neuronal network.

A further hurdle in translating interneuron-targeted strategies to the clinic will be to develop viral vectors capable of either selectively transducing interneurons, or vectors that are large enough to carry both the insert of choice and an interneuron specific promoter. Cre-dependent recombination, as used to target interneurons in mice, has limited translational potential. Current top choice viral vectors, adeno-associated viruses and lentiviruses, do not have the size capacity to encode both an opsin and a mammalian interneuron specific promoter. A way around this may be to use non-mammalian promoters, such as those of Fugu which are smaller in size and which show some selectivity for distinct mammalian interneuron subtypes (Nathanson et al., 2009).

5. Translation into the clinic

There are a number of open questions regarding whether an optogenetic strategy can be translated from animal studies to clinical trials. Several of these are applicable to other forms of gene therapy approaches, such as the type of viral vector used to transduce neurons, and the route of delivery, and have recently been discussed (Kullmann et al., 2014).

Specifically for optogenetic approaches, a potential concern is that it is invasive – a fibre optic or light-emitting diode will have to be permanently inserted into the brain. Small, powerful, implantable light delivery devices with a long-life battery will have to be developed. As brain penetrance by light is poor, this approach may not be feasible for epilepsies with a large epileptogenic zone. Indeed, the difference in size between a rat brain and a human

brain may have particular implications in translating this approach into clinic. Multiple viral injections may be required to transduce sufficient neurons in a human brain. Also, technical advances in light delivery will be required. Local heating of the brain should, furthermore, be avoided.

Opsins are not endogenous proteins and there are uncertainties regarding how long viral-mediated expression of opsins will persist in a human brain, although long-term expression in non-human primates appears to be well-tolerated (Diester et al., 2011). However, although the brain is often thought to be protected from circulating antibodies several forms of antibody-associated autoimmune encephalitis are now recognized, and the potential for immunogenicity of non-mammalian opsins cannot be excluded.

Opsin activation may also have unexpected effects. For example, halorhodopsin activation triggers intracellular chloride accumulation. Initially, this results in membrane hyperpolarisation and an inhibition of action potential firing. However, it is possible that continued activation of the chloride pump will lead to a switch in the chloride reversal potential. This means that GABA-mediated neurotransmission could become excitatory. Although this was not found to be the case when investigated in organotypic brain preparations (Tonnesen et al., 2009), later research demonstrated that this is a possibility (Raimondo et al., 2012).

To apply optogenetics to treating epilepsy, an on-board automated detection programme that triggers the illumination of light when it detects epileptic activity (therefore preventing ictal events) would be required. This approach offers the prospect of a device very much like an automatic implantable cardiac defibrillator to stop seizures without the permanent alteration of neuronal properties. Closed-loop, on-demand systems that recognize diverse human epileptic EEG signatures are required. Implantable devices capable of real-time seizure detection and light emission in rodents are now being developed (Armstrong et al., 2013) and (Kullmann and Wykes, unpublished data). Such devices might allow the translation of promising optogenetic studies in rodent models of epilepsy into providing seizure-freedom for people living with drug-refractory epilepsy.

6. Summary

The application of optogenetics to the field of neuroscience is allowing scientists to dissect the neuronal circuits involved in a variety of behavioural responses. Optogenetic neuromodulation is being investigated as a therapy for several neurological diseases. A major advance in epilepsy therapy would be a treatment that targets only the brain regions involved in seizure generation without adverse behavioural side-effects. It is possible to target neurons within an epileptic focus to express opsins. Light-activated gene therapy can acutely suppress seizures in multiple rodent models of epilepsy without interfering with normal behaviour. Although still in the earliest stages of development, an optogenetic strategy may hold promise for treating drug-resistant epilepsies.

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