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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

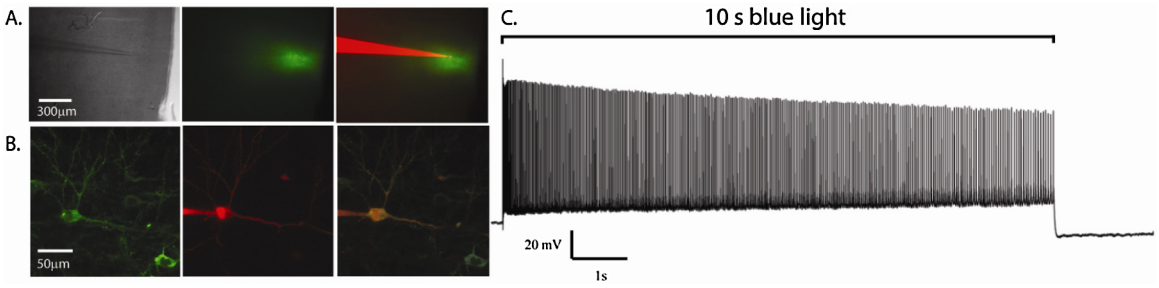


**Fig. 1.** Channelrhodopsin-mediated firing of action potentials. (A) Viral-mediated expression of channelrhodopsin tagged with a fluorescent protein (green) in a restricted part of rodent cortex. (B) 2-Photon image of a virally transduced neuron (green) patched with a pipette (pipette contained a red 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).