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Optogenetic Approaches for Controlling Seizure Activity

Jack K. Tung [a,b], Ken Berglund [b], Robert E. Gross [a,b,*]

[a] Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA
[b] Department of Neurosurgery, Emory University, Atlanta, GA

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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 the 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.

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 people 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 also frequent. All of these factors contribute to an ineluctable downward socioeconomic spiral [4].

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 (Fig. 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

* Corresponding author. Fax: 404-778-5121.
E-mail address: rgross@emory.edu (R.E. Gross).

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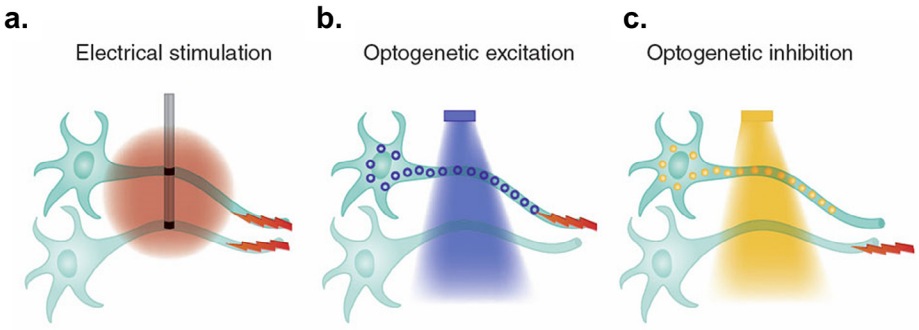

Figure 1. Optogenetic vs. electrical stimulation approaches to neuromodulation. (A) The effects of electrical stimulation are highly dependent on stimulation parameters and acts nonspecifically on cells around the stimulating electrode. The effects of optogenetic excitation (B) or inhibition (C) can be cell-type specific. Figure reprinted from *Nature Methods* 8, 26–29 (2011).

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.

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 (Fig. 1b) or inhibited (Fig. 1c) in the context of complex neural circuitry. Compared to electrical stimulation (Fig. 1a), 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].

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