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Review article

Optogenetic tools for modulating and probing the epileptic network

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ABSTRACT

Epilepsy affects roughly 1% of the population worldwide. Although effective treatments with antiepileptic drugs are available, more than 20% of patients have seizures that are refractory to medical therapy and many patients experience adverse effects. Hence, there is a continued need for novel therapies for those patients. A new technique called "optogenetics" may offer a new hope for these refractory patients. Optogenetics is a technology based on the combination of optics and genetics, which can control or record neural activity with light. Following delivery of light-sensitive opsin genes such as channelrhodopsin-2 (ChR2), halorhodopsin (NpHR), and others into brain, excitation or inhibition of specific neurons in precise brain areas can be controlled by illumination at different wavelengths with very high temporal and spatial resolution. Neuromodulation with the optogenetics toolbox have already been shown to be effective at treating seizures in animal models of epilepsy. This review will outline the most recent advances in epilepsy research with optogenetic techniques and discuss how this technology can contribute to our understanding *and* treatment of epilepsy in the future.

Contents

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1. Introduction

Epilepsy affects more than 50 million people worldwide, or roughly 1% of the population. Although most patients can be adequately treated with antiepileptic drugs, more than 20% of patients continue to have seizures that are refractory to medical therapy and many additional patients experience adverse side effects (Hauser and Hesdorfer, 1990). Hence, there is a continued need for the development of novel anti-epileptic therapies. Recently, a new type of light-sensitive molecule, or "opsin", has been developed which combines light-sensitivity with the modern genetic toolbox to control and monitor brain activity on a range of spatial resolutions from individual neurons to complex neural circuits (Boyden et al., 2005). "Optogenetics" is a novel field, which uses opsins for neuromodulation at extremely high spatial and temporal resolution, which can control the activity of specific types of neurons, or populations of neurons, in preparations ranging from cultured neurons to freely moving animals. The optogenetic toolbox has already demonstrated remarkable potential in epilepsy research and epilepsy therapy (Bentley et al., 2013; Kokaia et al., 2013; Krook-Magnuson and Soltesz, 2015; Ritter et al., 2014). A second generation of optogenetic tools, including indicators as well as actuators, permits the use of light to report on, as well as control, molecular processes in specific cell sub-populations within networks of heterogeneous cell types (Knöpfel et al., 2010). Since epilepsy involves complex neurochemical changes including synaptic and non-synaptic transmission, ion channels interactions, intracellular signaling pathways and glia–neuron signaling, these new optogenetic indicators can be used to probe those changes to understand the molecular and neurochemical basis of epilepsy to develop new targets for antiepileptic therapy.

2. Opsins

The possibility of using light for controlling precise neural activity was first proposed by Francis Crick in 1999 (Crick, 1999). However, a functional gene-based light-sensitive technique was not reported until 2002 by Gero Miesenböck's laboratory (Zemelman et al., 2002). They employed Drosophila rhodopsin photoreceptors to control neural activity in cultured mammalian neurons. Georg Nagel's lab first discovered the Channelrhodopsins including Channelrhodopsin-1 (ChR1) and Channelrhodopsin-2 (ChR2) from Chlamydomonas *reinhardtii*, which functioned as light-gated cation-selective membrane channels (Nagel et al., 2002, 2003). These early genetic photostimulation techniques were only studied in a few laboratories due to technical limitations (Banghart et al., 2004; Lima and Miesenböck, 2005; Volgraf et al., 2006). The revolutionary breakthrough in optogenetics occurred in Dr. Karl Deisseroth's laboratory in 2005, where a single-component ChR2 optogenetic system was used for millisecond control of neural firing in cultured neurons (Boyden et al., 2005). Intermittently illuminating and then extinguishing a light source caused a cell to fire, or stop firing, action potentials. Soon after, a series of studies using multiple opsins extended this new technique to *in vivo* preparations (Zhang et al., 2007). Further genetic manipulations have also made brain region- and cell type-specific modulation possible (Cardin et al., 2010). Optogenetics now includes both actuators and reporters. Optogenetic actuators are proteins with a light-controllable biological function and optogenetic reporters are proteins which provide readouts of biochemical processes that occur in the context of living tissue (Alford et al., 2013).

The key element of an optogenetic actuator is the opsin. Opsins are a group of light-sensitive proteins that underlie the molecular basis of various light-sensing systems including phototaxis, circadian rhythms, eyesight, and certain types of photosynthesis. The two major classes of opsins are defined and differentiated based either on the primary protein sequence, the chromophore chemistry or the signal transduction mechanisms. Type I opsins are present in bacteria, archaebacteria, and unicellular algae including bacteriorhodopsin, bacterial sensory rhodopsin, ChR, halorhodopsin (NpHR), and proteorhodopsin (Zhang et al., 2011). Type II opsins are present in eumetazoans (animals not including sponges) and have varied function, including phototransduction, vision, circadian rhythm entrainment, papillary reflexes and photoisomerization (Sakmar, 2002; Shichida and Yamashita, 2003). Commonly used optogenetic opsins are type I microbial opsins, including light-gated cations such as ChRs and light-driven pumps such as NpHR or archaerhodopsin (Fig. 1).

ChRs are light-gated cation-selective ion channels that can be used to excite cells. ChR2, from *Chlamydomonas reinhardtii*, is the first fully genetically-encoded optogenetic tool used in neuroscience. It has been the major ChR prototype for optogenetic applications since it is expressed more highly in most host cells than ChR1 (Nagel et al., 2003). The wild-type ChR2 absorbs blue light with and action spectrum maximum at 480 nm. With blue light illumination, ChR2 induces a conformational change from all-trans to 13-cis-retinal, which opens a transmembrane protein pore to at least 6 Å depolarizing ChR2-expressed cell. Within milliseconds, the retinal relaxes back to the all-trans form, closing the pore and stopping the flow of ions.

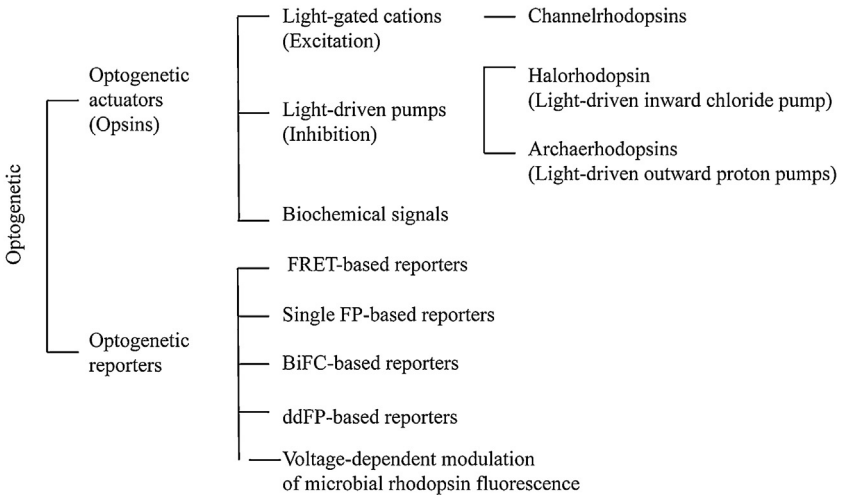

Fig. 1. Optogenetic tools. FRET: Förster (fluorescence) resonance energy transfer. FPs: fluorescence proteins. BiFC: bimolecular fluorescence complementation.