Published in final edited form as:

Adv Exp Med Biol. 2014 ; 813: 319–336. doi:10.1007/978-94-017-8914-1_26.

How might novel technologies such as optogenetics lead to better treatments in epilepsy?

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Abstract

Recent technological advances open exciting avenues for improving the understanding of mechanisms in a broad range of epilepsies. This chapter focuses on the development of optogenetics and on-demand technologies for the study of epilepsy and the control of seizures. Optogenetics is a technique which, through cell-type selective expression of light-sensitive proteins called opsins, allows temporally precise control via light delivery of specific populations of neurons. Therefore, it is now possible not only to record interictal and ictal neuronal activity, but also to test causality and identify potential new therapeutic approaches. We first discuss the benefits and caveats to using optogenetic approaches and recent advances in optogenetics related tools. We then turn to the use of optogenetics, including on-demand optogenetics in the study of epilepsies, which highlights the powerful potential of optogenetics for epilepsy research.

Keywords

On-demand; responsive; channelrhodopsin; halorhodopsin; Arch; AAV; optrode; seizure

Introduction

By enabling unprecedented possibilities for studying the cell populations and networks involved in seizure initiation, propagation, and termination, recent technological advances open exciting avenues for improving the understanding of mechanisms in a broad range of epilepsies. Through optogenetics, modulation of select cell populations is possible at specific times, providing the opportunity to not only record neuronal activity during seizures, but also to manipulate neuronal activity. In this way, it is possible to probe critical

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networks and circuits, and identify potential new therapeutic approaches. This chapter focuses on the development of optogenetics and on-demand technologies for the study of epilepsy and the control of seizures.

Optogenetics is a rapidly evolving field providing powerful tools for neuroscience (Boyden et al., 2005, Smedemark-Margulies et al., 2013), including the study of epilepsies (Bentley et al., 2013, Kokaia et al., 2013). Optogenetics is a technique in which light-sensitive proteins, called opsins, are introduced into cells. In this way, it is possible to control the activity of neuronal populations by shining light and activating the opsins. Opsins can be light-sensitive channels, pumps, G-protein-coupled receptors, or even transcriptional effectors (Konermann et al., 2013). We focus on light-sensitive channels and pumps whose activation can inhibit or excite neurons, and first discuss the benefits and caveats to using these optogenetic approaches, as well as recent advances in related tools. We then turn to the use of optogenetics in the study of epilepsies specifically.

Optogenetics: Development and technical advances

Cell-type and temporal precision are two key strengths to optogenetic approaches. Temporal precision is achieved by appropriately timed light delivery (though, of course, this can present its own challenges, as discussed below for on-demand approaches). Selective opsin expression is less straightforward, and is achievable through distinct methods. In general, expression is often achieved through the use of viral vectors, (including adeno-associated virus (AAV) or lentivirus), electroporation (Adesnik et al., 2012), or the use of transgenic animals. Inducible expression (Tanaka et al., 2012, Taniguchi et al., 2011, Zhu et al., 2009) and selective expression of opsins can be achieved for specified populations of neurons defined by their neurochemical profile (e.g., expression of parvalbumin), developmental origin (Butt et al., 2005, Corbin et al., 2011, Matta et al., 2013, Taniguchi et al., 2013), their date of birth (e.g., through the use of retroviruses which only infect actively dividing cells (Toni et al., 2008)), their location (e.g., by injecting virus in a restricted region), levels of activity at a specific time (Guenther et al., 2013), or their long-distance projections (e.g., through the use of WGA-Cre, which is retrogradely transported transynaptically (Gradinaru et al., 2010)).

Selective cell-type expression

To achieve selective expression in neurons defined by their neurochemical profile, two broad methods are used. The most straight forward approach is to place the expression of the opsin under a specific promoter (or even enhancer (Visel et al., 2013)). However, this approach has three disadvantages. First, especially when used with viruses, leaky expression is often noted (that is, expression in other cell populations). Second, long promoters do not fit in small vectors (e.g. adeno-associated viruses (AAV)). Third, in cases where the promoter is a relatively weak promoter, the expression of opsins can be insufficient to achieve strong light-induced currents and alter the activity of the neurons.

In order to overcome these drawbacks, a second method was developed: the opsin is instead placed under a strong promoter, and selectivity is achieved through the Cre/loxP system. Cre can mediate either inversion (flipping) or excision (removal) of DNA, depending on the

relative orientations of the loxP sites. For viruses, attempts at selective expression through the introduction of a floxed STOP cassette (which would be excised by Cre) can produce leaky expression (expression even in cells not expressing Cre). Additionally, attempting selective expression through a single inversion (which is then flipped by Cre to allow transcription) can produce weak expression, as Cre can continue to mediate flipping, re-inverting the sequence and inhibiting transcription. Therefore, a FLEX system ('flip-excision' (Atasoy et al., 2008, Schnutgen et al., 2003), also referred to as DIO – double-floxed inverse open reading frame (Zhang et al., 2010)) was implemented (Figure 1a). In this scenario, two sets of loxP sites are used. For one, a mutated sequence is used – lox2272. This sequence is still recognized by Cre, but is only paired with a similarly mutated sequence (Lee et al., 1998). Therefore, two distinct sets of loxP pairs can be achieved (one set carrying the mutation, and one set not). One round of Cre-mediated recombination flips the sequence, and another excises one of each type of loxP site, preventing future recombination and locking the virus in its activated state. This method has proven effective in achieving specific opsin expression, as well as sufficient levels of opsin expression (Atasoy et al., 2008). Cre can be introduced by several methods, including virus injection (note that only low levels of Cre expression are needed). WGA-Cre, mentioned above, can be used to achieve selective expression based on axonal projections (Gradinaru et al., 2010). For example, a FLEX-opsin virus can be injected into the hippocampus contralateral to WGA-Cre virus injection, to achieve opsin expression selectively in hippocampal neurons projecting contralaterally, e.g., mossy cells (Gradinaru et al., 2010). Alternatively, Cre-dependent virus can be injected into a transgenic mouse (or rat) line expressing Cre in a select population of neurons.

There is a wealth of transgenic mouse lines available, including an ever-growing resource of Cre lines (Taniguchi et al., 2011), many of which are commercially available (e.g., the Jackson laboratory Cre Repository: cre.jax.org). In addition to being useful in combination with Cre-dependent viral-based opsin expression methods, Cre lines can be crossed with lines expressing opsins in a Cre-dependent fashion (Madisen et al., 2012) (Figure 1b). For example, the Ai32 line developed at the Allen Institute expresses the excitatory opsin channelrhodopsin fused to an enhanced yellow fluorescent reporter protein (ChR2(H134R)-EYFP) from the endogenous *Gt(ROSA)26Sor* locus (a locus active in most cells) with expression enhanced with a CAG promoter (Madisen et al., 2012). Cre mediates removal of a floxed STOP cassette, and allows expression of the opsin.

An important caveat for Cre-mediated selectivity is that excision of DNA (e.g., removal of the STOP cassette) is permanent, even if Cre-expression itself is transient. This means that opsins can be expressed in cells that are not (currently) expressing Cre. Indeed, even if the cell is simply descended from a cell in which recombination has occurred, opsins will be expressed. This caveat can have significant experimental consequences. For example, following seizures, somatostatin (a neuropeptide whose expression is often used as a biochemical marker for populations of inhibitory interneurons) is transiently expressed in principal cells (Drexel et al., 2012). If selective opsin expression in somatostatin-expressing interneurons is being achieved through a Cre-dependent mechanism, selectivity of expression will be (permanently) lost following a seizure.

Another major limitation of available methods for achieving opsin-expression selectivity is the current inability to achieve selectivity in a population defined by multiple characteristics. For example, within a broad neuron population defined by a single neurochemical marker, there are several distinct cell-types. In the hippocampus alone, axo-axonic (also referred to as chandelier cells), dendritically targeting bistratified cells, and a subset of basket cells (which target the perisomatic region of postsynaptic cells) all express the calcium binding protein parvalbumin (Armstrong et al., 2012, Freund et al., 1996, Howard et al., 2005, Klausberger et al., 2008). Therefore, selective opsin expression in parvalbumin-expressing neurons still results in expression across multiple cell-types. Additionally, there are interneurons that are defined in part by expression of proteins which are also expressed by principal cells. For example, subsets of interneurons express the neuropeptide cholecystokinin (CCK) (Freund et al., 1996, Klausberger et al., 2008, Lee et al., 2011). However, as principal excitatory cells can also express CCK, selective expression in interneurons cannot be achieved through a Cre-mediated mechanism alone.

Importantly, this is a limitation of current methods which can be overcome through intersectional transgenics (Taniguchi et al., 2011). By combining the powerful Cre/loxP system with the Flp/Frt system (an analogous, but distinct, recombination system), it is possible to require expression of two markers for opsin expression. For example, Cre expression could be placed under the CCK promoter (and thus expressed in CCK-expressing cells) and Flp placed under an interneuron-specific marker. Indeed, selective expression of fluorescent proteins has already been achieved in CCK interneurons by using such an approach and a RCE-dual reporter mouse line (Taniguchi et al., 2011). However, in order for such an approach to be used for selective expression of opsins, mouse lines or viral vectors requiring both Cre and Flp for opsin expression will need to be generated. Additionally, while there is a vast resource of Cre lines, Flp-lines are markedly scarcer, and the field would certainly benefit from an increase in this resource. Note that beyond allowing access to relatively selective expression in more interneuron types (including neurogliaform and ivy cells, the numerically most dominant interneuron cell type in the hippocampus (Armstrong et al., 2012, Bezaire et al., 2013, Fuentealba et al., 2008)), intersectional transgenic approaches could also overcome the loss of selectivity for somatostatin interneurons following seizures (described above).

In order to apply optogenetics in humans, a viral-based approach will clearly have to be used. Note that viral vectors have been used in humans, including in the brain (Bartus et al., 2013, Markert et al., 2000, Murphy et al., 2013), and gene-delivery in general is being considered for a range of neurological diseases (Bartus et al., 2013, Vezzani, 2007). Beyond optogenetics, gene-delivery itself may be a new approach in epilepsy (Richichi et al., 2004, Sorensen et al., 2009, Wykes et al., 2012). Optogenetic tools to modify gene transcription may also one day be used therapeutically (Konermann et al., 2013). Note that insertional mutagenesis (and the risk for tumor generation) can be avoided by using vectors which remain extrachromosomal (e.g. recombinant AAV).

For animal studies, however, transgenic mouse methods offer several benefits over viral-based approaches. First, injection of virus is an invasive process, which is avoided through a transgenic-only approach. Second, for viral-based expression methods, the level of opsin

expression varies depending on the number of copies of viral vector in the cell. Therefore, there can be great cell-to-cell variability in the amount of opsin expression. In some cases expression can be so high that light induces toxic levels of current. Of course, high levels of expression can also be a benefit of viral-based methods, when transgenic lines do not produce strong enough photocurrents. Third, if the site of virus injection and the placement of the optical fiber delivering light to the tissue are improperly aligned, insufficient light may reach the opsin-expressing neurons. In contrast, in the transgenic lines, variability is reduced, even expression is achieved in the select cell population throughout the brain, and spatial selectivity is achieved through the location of light delivery.

In addition to the Cre-dependent opsin expressing mouse lines described above, there are several mouse lines expressing opsins directly under a specific promoter. This avoids the need for crossing strains, and leaves open the door for other Cre-based manipulations. However, a strong promoter must be used to achieve sufficiently high levels of opsin expression. The currently available lines include mice expressing the excitatory opsin channelrhodopsin under the Thy1 promoter (Arenkiel et al., 2007). Many of these mice are commercially available. Finally, there have been recent developments in achieving transgenic optogenetic rats (Tomita et al., 2009), further expanding the possibilities for using optogenetics in epilepsy research.

Direction of modulation of neuronal activity

In addition to cell-type specificity, another benefit of optogenetic approaches, over for example electrical stimulation, is the control of direction of modulation of neuronal activity (e.g., excitation versus inhibition).

Activation—Two main classes of opsins are available, allowing cell-specific activation or inhibition. Most of the optogenetic tools used for neuronal activation derive from Channelrhodopsin-2 (ChR2), a naturally-occurring, non-selective cation channel expressed by the algae *Chlamydomonas reinhardtii*. Upon exposure to blue light (470 nm absorption peak), ChR2 opens and allows passive movement of Na⁺, K⁺, Ca²⁺ and H⁺ following the electrochemical gradient (Nagel et al., 2003), depolarizing cell membranes, and if the cell is depolarized to threshold, generating action potentials. ChR2 possesses fast activation kinetics, and is able to trigger single action potentials in expressing cells following 1-2 ms light exposure, making it a particularly attractive tool for precisely timed stimulation of neuronal populations. ChR2 was the first opsin successfully expressed in mammalian neurons (Boyden et al., 2005). Since then, researchers have focused on improving its expression levels and its ON/OFF kinetics, and developed different variants with largely diverse properties. Several comprehensive reviews about Channelrhodopsin variants are available in the literature (see references (Berndt et al., 2011, Lin, 2011, Mattis et al., 2012)), and new variants are constantly being developed. Here we will focus on some important variants most often used in epilepsy research applications.

The first modification to the original ChR2 sequence, an amino acid substitution at position 134, produced a variant with improved expression levels and larger photocurrents in neurons (ChR2-H134R), but presenting slightly lower deactivation kinetics (Gradinaru et al., 2007).

The lower deactivation kinetics produces lower fidelity of light pulse to action potential generation at high light stimulation frequencies, such that cell firing may not accurately follow the stimulus (missed spikes and/or multiplet spikes per light pulse). Further research therefore then focused on improving kinetics, to allow activation of neuronal populations at higher frequencies (above 40 Hz) with better fidelity of spike generation. The first variant producing higher consistency of high frequency spike generation was developed by a chimeric combination of ChR1 (another channelrhodopsin from *C. reinhardtii*) and ChR2, and was named ChIEF (Lin et al., 2009). ChIEF displayed reduced inactivation during persistent light stimulations and improved fidelity at frequencies higher than 25 Hz. Similarly, one amino acid substitution at position 123 of the original ChR2 sequence, led to the development of ChR2(E123T), or ChETA (Gunaydin et al., 2010), a ChR2 variant displaying dramatically improved activation/deactivation kinetics, allowing consistent and reliable action potential generation at frequencies up to 200 Hz. However, photocurrents generated by ChETA were somewhat smaller than wild-type ChR2, posing a potential drawback for its successful application *in vivo*. To solve this issue, an additional modification of the amino acid sequence at position 159 resulted in the development of ChR2(ET/TC), an improved ChETA variant combining high temporal fidelity with large photocurrent generation (Berndt et al., 2011). ChR2(ET/TC) still represents to date the channelrhodopsin with the best performance in terms of spike fidelity generation and amplitude of photocurrents.

All of the ChR2 variants described above display an excitation maximum at around 470 nm, and require blue light for their activation. However, the propagation of light in tissue is directly proportional to its wavelength, with blue light presenting high scattering and low penetration compared to higher wavelengths such as red light. Additional penetration through brain tissue is achieved by avoiding wavelengths absorbed by hemoglobin. For experiments requiring coverage of large brain areas, channelrhodopsin variants with red-shifted absorption maxima are therefore preferred, as they allow activation of an increased number of neurons with lower light stimulation intensity. The first attempt towards generating red-shifted activating opsins was made by cloning VChR1, a channelrhodopsin naturally expressed by the spheroidal alga *Volvox carteri*. VChR1 presented an excitation maximum at 550 nm, but significantly lower photocurrents and expression levels in mammalian neurons when compared to ChR2 (Zhang et al., 2008). To improve VChR1 photocurrents, researchers created a chimera by substituting helices 1 and 2 of VChR1 with their analogs in ChR1, thereby developing C1V1 (Yizhar et al., 2011). Subsequent modification of glutamic acid residues at positions 122 and 162 (resulting in C1V1-T/T) further improved its photocurrents, and resulted in a channelrhodopsin variant with photocurrents comparable to ChR2(H134R) and excitation maximum at 550 nm. C1V1(T/T) also presented vastly increased light sensitivity, allowing its activation with lower light power, making it especially attractive for *in vivo* studies.

The ChR2 variants described above enable fast and precise activation of neuronal populations, but are not optimal for experiments requiring activation of specific neuronal population over longer time windows (minutes). At expression levels typically achieved in neurons, long time activation would require constant delivery of high power to the tissue, with potential and undesirable heating effects. To allow neuronal activation for longer time

periods, a separate class of activating opsins was developed, where a single brief pulse of blue light is sufficient to trigger the channel into its active state. Channelrhodopsins with these properties were named Step-Function Opsins (or SFOs), and caused depolarization of cell membranes for periods of 30-60 seconds after 10 ms blue light exposure (Berndt et al., 2009). Even slower deactivation kinetics were achieved with a Stabilized SFO (Yizhar et al., 2011), displaying dramatically improved light sensitivity and a channel deactivation time constant of about half an hour. A major advantage of SFOs is that they can be used to slightly alter the network contribution of different cell types, as the depolarization they provide following light is generally sub-threshold, and therefore does not directly activate expressing cells, but only increases cell sensitivity in responding to physiological network activity.

Suppression—The second major class of optogenetic tools available for the study of neuronal networks is constituted by opsins able to hyperpolarize the cell membrane and, if strong enough, silence action potential generation. The first opsin shown to inhibit neuronal activity was halorhodopsin (NpHR), a chloride pump driven by orange light and naturally expressed by the bacterium *Natromonas Pharaonis*. When expressed in neurons, exposure to orange light (570 nm absorption maximum) causes active pumping of chloride ions into the cell, thereby hyperpolarizing the membrane potential and inhibiting action potential generation (Zhang et al., 2007). However, expression of NpHR in neurons was not optimal, and it formed aggregates in the endoplasmic reticulum that could lead to cellular toxicity (Gradinaru et al., 2007). Further development of the NpHR sequence focused on decreasing aggregates, improving photocurrents and promoting membrane localization. Several rounds of substantial mutagenesis of the original NpHR sequence allowed researchers to develop a variant (named eNpHR3.0) displaying a three-fold increase in photocurrents and two-fold increase in membrane hyperpolarization effects, together with a significant red shift of its excitation wavelength, making eNpHR3.0 ideal for a varied range of studies involving neuronal silencing (Gradinaru et al., 2010).

Although halorhodopsin chloride pumps are able to reduce neuronal activity with high efficiency, actively pumping chloride ions into the neurons could have effects on chloride homeostasis, with potential shifts in the effect of GABAergic inhibition via chloride-permeable GABA_A receptors (i.e., shifting E_{GABA}) (Raimondo et al., 2012). E_{GABA} is already compromised in epileptic tissue (Graves, 2006). Increasing the intracellular concentration of chloride by its active pumping via NpHR activation could further exacerbate this phenomenon, and cause a shift in E_{GABA} to the point where GABA_A activation becomes depolarizing (Raimondo et al., 2012).

Together with halorhodopsins, a separate class of tools to inhibit neuronal activity was developed from naturally-occurring proton pumps derived from different strains of the bacterium *Halorubrum sodomense*. In contrast to NpHR and its variants, proton pumps hyperpolarize cell membranes by actively transporting protons to the extracellular environment, upon exposure to orange/yellow light. The most widely used proton pumps include Archaeorhodopsin-3 (also called Arch (Chow et al., 2010)) and ArchT (Han et al., 2011). Both have been shown to be able to successfully inhibit neuronal activity *in vitro* and *in vivo*, including when expressed in the brain of non-human primates (Han et al., 2011).

Recently, Arch3.0 and ArchT3.0 variants were developed, using modifications similar to those made to the original NpHR sequence, and yielded proton pumps displaying large photocurrents in neurons and increased action potential silencing effects (Mattis et al., 2012). Due to the fact that these pumps rely on active transport of protons for hyperpolarizing cell membranes (rather than chloride transport), they would not contribute to the disturbance in chloride reversal potential and GABA_A-mediated inhibition (Raimondo et al., 2012), but may have alternate effects, such as altered pH.

Channelrhodopsins and halorhodopsins or proton pumps can also be expressed simultaneously in the same cells to allow bidirectional control of the cell population of interest (Gradinaru et al., 2010, Han et al., 2009). ChR2 and most of its variants are activated by blue light, and are therefore spectrally compatible with NpHR or Arch variants, which are activated by orange/yellow light. Moreover, if particular experimental conditions require simultaneous activation of one population and silencing of another, a combination of red-shifted channelrhodopsins could be used together with NpHR or Arch. This could be used, for example, to study the effects of simultaneous pyramidal cell silencing and GABAergic interneuron activation on seizure activity.

Although the expression of opsins can be specific and directed to desired cell populations using the strategies described above, the outcome of neuronal activation and/or silencing in intact networks can be more intricate than perhaps initially expected, due to the extremely complex nature of neuronal circuits. For example, results from *in vivo* experiments using ChR2-mediated light stimulation show some cells being activated (as expected), while others are silenced, likely due to network interactions (Han et al., 2011, Han et al., 2009). Similarly, in a study using ArchT activation (expected to inhibit cells), a substantial number of neurons responded to light instead by increasing their firing rate (Han et al., 2011). As epileptic circuits often undergo considerable changes, including axon sprouting and changes in network connections, potentially unexpected network roles should also be considered when using optogenetics with epileptic tissue. Indeed, optogenetics provides a powerful means to explore these changes and their consequences on the functioning of the network in epilepsy. Provided opsin expression remains specific, optogenetics provides the ability to examine the role of specific neuronal populations in health and disease in a manner previously unachievable with techniques such as electrical stimulation.

Light delivery

In experimental conditions, light is delivered by using a variety of different systems, depending on the needs. Sources able to generate light with suitable wavelength and power include lasers and light emitting diodes (LEDs). Laser sources have the advantage of providing light with narrow wavelengths, and therefore do not require filtering. Additionally, lasers can provide high power, even when coupled to small diameter fibers which are routed through optical commutators. Lasers can be used with mechanical shutters for light on/off switching to avoid delays in reaching maximum power. However, shutters can be expensive, sensitive, and have relatively short life expectancies. A major disadvantage of lasers is their cost. LED light sources are generally more affordable and are becoming increasingly powerful. While LEDs have the disadvantage of delivering light with typical “tails” in

excitation spectrum, these can be adequately filtered to ensure proper wavelength excitation. LED sources typically reach maximum power in less than 200 microseconds even at very high frequency. Therefore, light can be switched on or off by delivering external voltage pulses (rather than via a mechanical shutter) without sacrificing light power.

For *in vitro* preparations light is typically delivered through the lens of the microscope (Krook-Magnuson et al., 2013, Tonnesen et al., 2009, Zhang et al., 2007), although other methods are also used, including optical fibers positioned in close proximity to the tissue area of interest (Ledri et al., 2012). For example, small diameter fibers (Ledri et al., 2012) or laser-scanning photostimulation (Xu et al., 2010) can be used to activate specific regions in the slice, allowing for example circuit mapping and investigation of network alterations occurring after seizures. When light is delivered through the lens of the microscope, filtered light from a mercury or xenon lamp source can also be used, similar to epifluorescence applications.

For *in vivo* situations, light is most commonly delivered through an optical fiber implanted in the region of interest and connected to the light source of choice. Sophisticated light delivery options have also been designed, including multiwaveguides capable of delivering light of different wavelengths to different locations along the guide (Zorzos et al., 2010). Additionally, the optical fiber can be combined with a recording electrode. The combination is termed an optrode, and a number of designs and protocols exist (Abaya et al., 2012, Anikeeva et al., 2012, Hung et al., 2013, Royer et al., 2010, Stark et al., 2012, Tamura et al., 2012, Wang et al., 2012, Wentz et al., 2011, Zhang et al., 2009), including a recent protocol for simple and relatively low cost optrodes designed for chronic (months long) recordings in rodents (Armstrong et al., 2013). Optical fibers can be directly implanted (Armstrong et al., 2013) or guided into the tissue by a cannula previously fixed to the animal's skull (Zhang et al., 2010). For long-term *in vivo* applications, an optical commutator is often used to reduce torque on the optical patch cord connecting the animal and the light source. There also exist wireless options for light delivery, including headborne LED devices (Wentz et al., 2011) and injectable μ -LEDs (Kim et al., 2013).

A major caveat to consider while planning *in vivo* optogenetic experiments is that brain penetrance by light is rather limited, as described above, and progressively reduces with decreasing wavelengths. Therefore, the spatial distance between the light and the cells expressing the opsin can be critical, and will determine the minimal required power for adequate activation of the transgene. The choice of opsin is also important, as some are several fold more sensitive to light than others, or have red-shifted excitation maximum allowing simultaneous activation (or inhibition) of a large number of neurons while maintaining a small diameter optical fiber (reducing tissue damage).

Optogenetics: Shedding light on epilepsy

Review of recent studies

The first attempt at using optogenetic approaches for suppressing abnormal hypersynchronized activity involved expression of eNpHR (a slightly improved NpHR protein) in pyramidal cells of the hippocampus (Tonnesen et al., 2009). The inhibitory opsin

was introduced in excitatory principal cells of organotypic hippocampal slices by using a lentivirus carrying the NpHR transgene under the control of the CaMKII alpha promoter, which is expressed in excitatory cells and absent in inhibitory interneurons. Organotypic hippocampal slices are proposed to represent an *in vitro* model of epileptic tissue, as they exhibit network reorganization, such as cell death, axonal sprouting and synaptic formation, leading to hyperexcitability (Albus et al., 2008, Bausch et al., 2000). The ability of NpHR to inhibit epileptiform activity in such “epileptic” tissue was tested by applying orange light during stimulation train induced bursting (STIB), a stimulation protocol that reliably evokes afterdischarges in the CA1 and CA3 area. Orange light application effectively suppressed STIB-induced activity, while blue light application was ineffective, indicating the specificity of the approach used (Figure 2) (Tonnesen et al., 2009).

Optogenetic approaches have also had success in inhibiting seizures *in vivo* across a range of epilepsies, including induced (acute) seizures (Sukhotinsky et al., 2013), focal cortical seizures (Wykes et al., 2012), temporal lobe seizures during the chronic (spontaneous seizures) phase of the disease (Krook-Magnuson et al., 2013), and thalamocortical epilepsy in a model of cortical stroke (Paz et al., 2013).

Using the rat pilocarpine model of acute induced seizures in awake behaving male rats, Sukhotinsky and colleagues examined the ability to inhibit seizures using optogenetic inhibition of the hippocampus (Sukhotinsky et al., 2013). The inhibitory opsin halorhodopsin (eNpHR3.0 (Gradinaru et al., 2010)) was expressed in principal excitatory cells in the hippocampus using adeno-associated virus (AAV) and a CamKII α promoter. Animals receiving light and expressing the opsin showed an increase in time to seizure onset from the time of pilocarpine injection compared to controls (time to seizure onset with opsin activation: 21 ± 1.8 min versus 15.2 ± 1.1 min in controls). Controls included animals not injected with virus and not receiving light, animals injected with virus but not receiving light, and animals receiving light but not expressing the opsin. Therefore, the activation of opsins (and inhibition of hippocampal principal excitatory cells) delayed the time to seizure onset. This study supports the notion that optogenetics can be used to inhibit seizures. Moreover, it indicates that targeted inhibition of principal cells in the hippocampus can delay the onset of pilocarpine induced seizures.

Wykes and colleagues demonstrated the successful use of an optogenetic approach to inhibit focal cortical seizures (Wykes et al., 2012). Neocortical epilepsy is frequently drug-resistant, and new therapeutic approaches are being actively sought. Focal cortical epilepsy was induced in rats by focal injection of tetanus toxin into the motor cortex. Lentivirus was co-injected with the tetanus toxin, in order to transduce excitatory pyramidal neurons in the epileptic focus with the inhibitory opsin halorhodopsin (NpHR2.0, under a CamKII α promoter). Seven to 10 days after the injection of tetanus toxin, the ability of an optogenetic approach to inhibit seizures was investigated. EEG was recorded for a 1000s baseline period, then intermittent light (20s on, 20s off) was delivered for 1000s, and then a final 1000s of post-light EEG was recorded. Compared to the periods of no-light delivery, opsin activation by light delivery attenuated recorded EEG epileptiform activity. In animals not expressing opsins, light delivery did not affect the high-frequency power of the signal, supporting the conclusion that the light-effect observed in opsin-expressing animals was due to the

activation of opsins, rather than of light delivery *per se*. Not only does this study indicate that an optogenetic approach can inhibit focal cortical epileptiform activity, but also that inhibition of a portion of excitatory cells at the focus is sufficient to do so.

These two studies support the potential for an optogenetic approach for diverse epileptic activity, and make use of the power of optogenetics to selectively target specific populations of cells. An additional major benefit of optogenetics is the temporal precision which it can provide. That is, an optogenetic approach could be employed in an on-demand or responsive fashion, such that intervention only occurred either immediately before a seizure would occur (seizure prediction) or early during a seizure onset (seizure detection). In addition to the experimental benefits of an on-demand approach, by limiting intervention to only those times when it is needed, an on-demand approach may reduce negative side effects associated with chronic treatments.

On-demand optogenetics have been used in two models of epilepsy – thalamocortical and temporal lobe epilepsy. Using a cortical stroke model of thalamocortical epilepsy, and line-length threshold crossing for automated seizure detection, Paz and colleagues demonstrated the successful inhibition of seizures (Paz et al., 2013). The inhibitory opsin halorhodopsin (eNpHR3.0 (Gradinaru et al., 2010)) was expressed under a CamKII α promoter in the ventrobasal thalamus ipsilateral to the site of induced cortical stroke. On-demand light activation of opsins interrupted seizures. In addition to illustrating the potential for on-demand optogenetics to stop seizures, these findings supported the theory that the cortical strokes produced thalamocortical seizures; that is, optogenetics can provide insight into the mechanisms of seizures, including critical brain regions and networks.

On-demand optogenetics has also been used successfully in a mouse model of chronic temporal lobe epilepsy (Krook-Magnuson et al., 2013). Seizures were detected on-line with custom-designed, tunable, multi-algorithm based detection software (Figure 3). This software, and instructions on how to use the software, is available for download through Nature Protocols (Armstrong et al., 2013). The intrahippocampal kainate mouse model used mimics unilateral hippocampal sclerosis, and displays both spontaneous electrographic-only seizures (that is, seizures with little or no overt accompanying behavior) as well as seizures that progress to overt behavioral seizures. Seizures were detected early, prior to overt behavior. Selective expression of the inhibitory opsin halorhodopsin (eNpHR3.0) was achieved by crossing mice expressing halorhodopsin in a Cre-dependent fashion with mice expressing Cre under the CamKII α promoter (Madisen et al., 2012). On-demand light delivery to the hippocampus, inhibiting excitatory cells, dramatically truncated seizures (Figure 4).

Krook-Magnuson et al. (Krook-Magnuson et al., 2013) then went on to try a second approach. Rather than inhibiting excitatory cells directly through optogenetics, the authors instead used optogenetics to excite a subpopulation of inhibitory neurons. Selective expression of the excitatory opsin channelrhodopsin (ChR2) was achieved by crossing mice expressing ChR2 in a Cre-dependent manner with mice expressing Cre selectively in parvalbumin-expressing neurons. In the hippocampus, parvalbumin-expressing interneurons represent less than 5% of the total neuronal population (Bezaire et al., 2013, Freund et al.,

1996, Woodson et al., 1989). Remarkably, seizures were significantly inhibited through this approach. Seizures were also significantly inhibited when light was delivered to the contralateral hippocampus. Finally, light delivery reduced the number of seizures progressing to overt behavioral seizures. These data indicate that focal light delivery can have a significant effect on temporal lobe seizures, that an on-demand approach can work in temporal lobe epilepsy, and that a strategy directly targeting only a small fraction of cells (that is, parvalbumin-expressing interneurons) can significantly inhibit temporal lobe seizures.

Obstacles and future potential in epilepsy research

While the results from these studies are promising, a number of hurdles need to be overcome before optogenetics, and hopefully on-demand optogenetics, can be realized in the clinical setting. These include demonstration of safe and stable opsin expression in humans, as well as a safe implantable device for on-line seizure detection and light delivery. However, on-demand optogenetics, with its cell-type, spatial, and temporal-specificity, may one day aid patients currently suffering from uncontrolled seizures and the negative side-effects of systemic treatment options. An example patient population that could benefit from the clinical realization of an on-demand optogenetic therapeutic is patients with refractory bilateral temporal lobe epilepsy for whom surgical resection is not an option.

Optogenetics additionally presents a powerful tool for expanding our understanding of mechanisms of epilepsy. While the studies discussed here have demonstrated a wide potential for optogenetics in the field of epilepsy, there is much more to be gained from fully harnessing the power of optogenetics. Through optogenetics it is possible to test hypotheses regarding critical cell-types and networks involved in the initiation, continuation, propagation, and (natural or induced) cessation of seizures. The studies described above inhibited seizures using optogenetic techniques, but it is also possible to use optogenetic approaches to study mechanisms of epilepsy through the induction (rather than inhibition) of seizures (Osawa et al., 2013). The information gained from optogenetic experiments can in turn open the door for new therapeutic approaches beyond optogenetics, including new drugs targeting key cell types or electrical stimulation targeting key brain regions.

While the field is benefiting greatly from recent technological advances, there is a continuing need for additional developments. A reliable and inexpensive long-term EEG monitoring system, with fully computerized analysis of EEG and video for automated detection and analysis of electrographic and behavioral seizures, would push the field forward dramatically. For example, this would increase the feasibility (and statistical power by allowing more animals to be monitored and analyzed) of studies with mild or moderate head injury for which only a small subset of animals go on to develop epilepsy. Recent advances in wireless devices, including those capable of delivering light (Kim et al., 2013, Wentz et al., 2011), and improvements in seizure detection (Armstrong et al., 2013, White et al., 2006) are paving the way for such future advances. These advances will additionally improve the utility of optogenetics for epilepsy research by allowing chronic on-demand light delivery to freely moving, untethered, animals.

Other technical advances: New avenues, new insights

This chapter has focused on optogenetics. Clearly, however, the field takes advantage of a large range of new technological advances, several of which are being rapidly integrated with optogenetics. For example, on-demand approaches (which as described above can be successfully integrated with optogenetics) have the potential to provide both experimental and therapeutic benefits. While electrical stimulation lacks the cell-type specificity of optogenetics, it can provide temporal precision, and thus can also be used in an on-demand fashion. Previously, on-demand electrical stimulation was found to provide superior seizure control in rats (Good et al., 2009). More recently, on-demand transcranial electrical stimulation (TES) was used to reduce spike-and-wave episodes in absence seizures in rats (Berenyi et al., 2012). There is also intense clinical interest in an on-demand therapeutic option, and clinical trials have shown promise (reviewed in reference (Wu et al., 2013)).

A step beyond early seizure detection is seizure prediction. A recent study in patients with drug-resistant partial-onset epilepsy was able to predict for a subset of patients periods of high seizure risk and periods where the chances of having a seizure were relatively low, based on an analysis of the frequency bands recorded from intracranial EEG (Cook et al., 2013). Further supporting the possibility of seizure prediction, changes in multi-unit activity are reported in human patients prior to seizure onset (Bower et al., 2012, Truccolo et al., 2011). Unfortunately, there is considerable variability in this early activity from seizure to seizure (Bower et al., 2008, Bower et al., 2012), which may limit the ability to have accurate seizure prediction. However, detecting seizures early (prior to overt behavioral manifestations) and intervening (optogenetically or otherwise) to truncate seizures to this pre-clinical stage could have a large impact on patient quality of life.

Imaging techniques are an additional example of the wide-range of expanding techniques that are being increasingly applied to the study of epilepsy, and include diffusion tensor imaging DTI, reviewed in reference (Engel et al., 2013)), magnetic resonance imaging (MRI, which can be combined with optogenetics (Lee et al., 2010)), positron emission tomography (PET), single-photon emission computed tomography (SPECT, for a review see reference (Maehara, 2007)), the new clarity brain (Chung et al., 2013), calcium imaging and voltage sensitive dyes (for recent reviews see references (Engel et al., 2013, Takano et al., 2012)). Anatomical imaging techniques of neuronal projections in intact brains allow examination of network connections between brain regions in health and disease. Appreciating long-distance network connections, and how these shape local network connections (Krook-Magnuson et al., 2012, Varga et al., 2010), will undoubtedly provide crucial information on seizure propagation mechanisms, as well as potentially mechanisms behind seizure initiation and termination. Functional imaging can reveal local as well as long-distance network dynamics, and is contributing substantially to our understanding of mechanisms in epilepsy. For example, a recent study using calcium imaging of epileptic tissue found not only variability in firing between neurons during epileptiform events, but also variability between epileptiform events, with each event comprised of different patterns of co-activated clusters of neurons (Feldt Muldoon et al., 2013).

Advances are certainly not limited to seizure detection or imaging techniques. Whole-genome sequencing, which is providing ever-expanding information on the genetics of epilepsies (reviewed in reference (Merwick et al., 2012)), is an excellent example of the driving force that new technological advances can provide to the field. Additional diverse technological advances, including uncaging of GABA (Yang et al., 2012) and devices allowing focal cooling (Rothman, 2009), are introducing unique new opportunities for studying and treating epilepsy. Advances in recording techniques are providing unprecedented information regarding the activity of neurons during epileptiform events. It is now possible to record from hundreds of units in human epileptic patients (for a discussion of the spike sorting techniques involved see reference (Einevoll et al., 2012)), providing a wealth of information on the involvement of neurons in seizures (Bower et al., 2012, Keller et al., 2010, Truccolo et al., 2011). The novel information gained from these new techniques can aid in seizure detection and prediction discussed above. Importantly, this data can also be incorporated into “big data”-driven large-scale computational models (Bezaire et al., 2013, Schneider et al., 2012). Hypotheses can then be tested *in silico*, and new hypotheses in turn generated to be tested *in vitro* or *in vivo* (for reviews of computational neuroscience in epilepsy, see references (Case et al., 2012, Soltesz et al., 2008)).

From the genetics, to the proteins, to the cell-types and networks critical in epilepsy, advances are being made and insights gained. Optogenetics, together with a vast array of novel technological developments, is expected to continue to light new avenues for studying the mechanisms of the epilepsies.

Acknowledgments

This chapter on optogenetic approaches to epilepsy highlights the fundamental veracity of Phil’s overarching conceptual framework that placed a major emphasis on the critical importance of rigorous, quantitative mechanistic understanding of epileptic neuronal circuits in order to develop new generations of temporally and spatially selective, more effective seizure control strategies. This work was supported by US National Institutes of Health grant NS74432 and the Swedish Brain Foundation.

References

- Abaya TVF, Blair S, Tathireddy P, Rieth L, Solzbacher F. A 3D glass optrode array for optical neural stimulation. *Biomed Opt Express*. 2012; 3:3087–3104. [PubMed: 23243561]
- Adesnik H, Bruns W, Taniguchi H, Huang ZJ, Scanziani M. A neural circuit for spatial summation in visual cortex. *Nature*. 2012; 490:226–31. [PubMed: 23060193]
- Albus K, Wahab A, Heinemann U. Standard antiepileptic drugs fail to block epileptiform activity in rat organotypic hippocampal slice cultures. *Br J Pharmacol*. 2008; 154:709–24. [PubMed: 18414393]
- Anikeeva P, Andalman AS, Witten I, Warden M, Goshen I, Grosenick L, Gunaydin LA, Frank LM, Deisseroth K. Optetrode: a multichannel readout for optogenetic control in freely moving mice. *Nat Neurosci*. 2012; 15:163–170. [PubMed: 22138641]
- Arenkiel BR, Peca J, Davison IG, Feliciano C, Deisseroth K, Augustine GJ, Ehlers MD, Feng G. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron*. 2007; 54:205–18. [PubMed: 17442243]
- Armstrong C, Krook-Magnuson E, Oijala M, Soltesz I. Closed-loop optogenetic intervention in mice. *Nat Protoc*. 2013; 8:1475–93. [PubMed: 23845961]
- Armstrong C, Krook-Magnuson E, Soltesz I. Neurogliaform and Ivy Cells: A Major Family of nNOS Expressing GABAergic Neurons. *Front Neural Circuits*. 2012; 6:23. [PubMed: 22623913]

- Armstrong C, Soltesz I. Basket cell dichotomy in microcircuit function. *J Physiol.* 2012; 590:683–94. [PubMed: 22199164]
- Atasoy D, Aponte Y, Su HH, Sternson SM. A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. *J Neurosci.* 2008; 28:7025–30. [PubMed: 18614669]
- Bartus RT, Baumann TL, Siffert J, Herzog CD, Alterman R, Boulis N, Turner DA, Stacy M, Lang AE, Lozano AM, Olanow CW. Safety/feasibility of targeting the substantia nigra with AAV2-neurturin in Parkinson patients. *Neurology.* 2013; 80:1698–701. [PubMed: 23576625]
- Bausch SB, McNamara JO. Synaptic connections from multiple subfields contribute to granule cell hyperexcitability in hippocampal slice cultures. *J Neurophysiol.* 2000; 84:2918–32. [PubMed: 11110821]
- Bentley JN, Chestek C, Stacey WC, Patil PG. Optogenetics in epilepsy. *Neurosurg Focus.* 2013; 34:E4. [PubMed: 23724838]
- Berenyi A, Belluscio M, Mao D, Buzsaki G. Closed-loop control of epilepsy by transcranial electrical stimulation. *Science.* 2012; 337:735–7. [PubMed: 22879515]
- Berndt A, Schoenenberger P, Mattis J, Tye KM, Deisseroth K, Hegemann P, Oertner TG. High-efficiency channelrhodopsins for fast neuronal stimulation at low light levels. *Proc Natl Acad Sci U S A.* 2011; 108:7595–600. [PubMed: 21504945]
- Berndt A, Yizhar O, Gunaydin LA, Hegemann P, Deisseroth K. Bi-stable neural state switches. *Nat Neurosci.* 2009; 12:229–34. [PubMed: 19079251]
- Bezaire MJ, Soltesz I. Quantitative assessment of CA1 local circuits: Knowledge base for interneuron-pyramidal cell connectivity. *Hippocampus.* 2013
- Bower MR, Buckmaster PS. Changes in granule cell firing rates precede locally recorded spontaneous seizures by minutes in an animal model of temporal lobe epilepsy. *J Neurophysiol.* 2008; 99:2431–42. [PubMed: 18322007]
- Bower MR, Stead M, Meyer FB, Marsh WR, Worrell GA. Spatiotemporal neuronal correlates of seizure generation in focal epilepsy. *Epilepsia.* 2012; 53:807–16. [PubMed: 22352423]
- Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci.* 2005; 8:1263–8. [PubMed: 16116447]
- Butt SJ, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G. The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron.* 2005; 48:591–604. [PubMed: 16301176]
- Case, MJ.; Morgan, RJ.; Schneider, CJ.; Soltesz, I. *Jasper's Basic Mechanisms of the Epilepsies.* 4. Bethesda, MD: Oxford University Press; 2012. Computer Modeling of Epilepsy.
- Chow BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES. High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature.* 2010; 463:98–102. [PubMed: 20054397]
- Chung K, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, Davidson TJ, Mirzabekov JJ, Zalocusky KA, Mattis J, Denisin AK, Pak S, Bernstein H, Ramakrishnan C, Grosenick L, Gradinaru V, Deisseroth K. Structural and molecular interrogation of intact biological systems. *Nature.* 2013; 497:332–7. [PubMed: 23575631]
- Cook MJ, O'Brien TJ, Berkovic SF, Murphy M, Morokoff A, Fabinyi G, D'Souza W, Yerra R, Archer J, Litewka L, Hosking S, Lightfoot P, Ruedebusch V, Sheffield WD, Snyder D, Leyde K, Himes D. Prediction of seizure likelihood with a long-term, implanted seizure advisory system in patients with drug-resistant epilepsy: a first-in-man study. *Lancet Neurol.* 2013; 12:563–71. [PubMed: 23642342]
- Corbin JG, Butt SJ. Developmental mechanisms for the generation of telencephalic interneurons. *Dev Neurobiol.* 2011; 71:710–32. [PubMed: 21485015]
- Drexel M, Kirchmair E, Wieselthaler-Holzl A, Preidt AP, Sperk G. Somatostatin and neuropeptide Y neurons undergo different plasticity in parahippocampal regions in kainic acid-induced epilepsy. *J Neuropathol Exp Neurol.* 2012; 71:312–29. [PubMed: 22437342]
- Einevoll GT, Franke F, Hagen E, Pouzat C, Harris KD. Towards reliable spike-train recordings from thousands of neurons with multielectrodes. *Curr Opin Neurobiol.* 2012; 22:11–7. [PubMed: 22023727]

- Engel J Jr, Thompson PM, Stern JM, Staba RJ, Bragin A, Mody I. Connectomics and epilepsy. *Curr Opin Neurol*. 2013; 26:186–94. [PubMed: 23406911]
- Feldt Muldoon S, Soltesz I, Cossart R. Spatially clustered neuronal assemblies comprise the microstructure of synchrony in chronically epileptic networks. *Proc Natl Acad Sci U S A*. 2013; 110:3567–72. [PubMed: 23401510]
- Freund TF, Buzsaki G. Interneurons of the hippocampus. *Hippocampus*. 1996; 6:347–470. [PubMed: 8915675]
- Fuentealba P, Begum R, Capogna M, Jinno S, Marton LF, Csicsvari J, Thomson A, Somogyi P, Klausberger T. Ivy cells: a population of nitric-oxide-producing, slow-spiking GABAergic neurons and their involvement in hippocampal network activity. *Neuron*. 2008; 57:917–29. [PubMed: 18367092]
- Good LB, Sabesan S, Marsh ST, Tsakalis K, Treiman D, Iasemidis L. Control of synchronization of brain dynamics leads to control of epileptic seizures in rodents. *Int J Neural Syst*. 2009; 19:173–96. [PubMed: 19575507]
- Gradinaru V, Thompson KR, Zhang F, Mogri M, Kay K, Schneider MB, Deisseroth K. Targeting and readout strategies for fast optical neural control in vitro and in vivo. *J Neurosci*. 2007; 27:14231–8. [PubMed: 18160630]
- Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson KR, Deisseroth K. Molecular and cellular approaches for diversifying and extending optogenetics. *Cell*. 2010; 141:154–65. [PubMed: 20303157]
- Graves TD. Ion channels and epilepsy. *QJM*. 2006; 99:201–17. [PubMed: 16495302]
- Guenther CJ, Miyamichi K, Yang HH, Heller HC, Luo L. Permanent Genetic Access to Transiently Active Neurons via TRAP: Targeted Recombination in Active Populations. *Neuron*. 2013; 78:773–84. [PubMed: 23764283]
- Gunaydin LA, Yizhar O, Berndt A, Sohal VS, Deisseroth K, Hegemann P. Ultrafast optogenetic control. *Nat Neurosci*. 2010; 13:387–92. [PubMed: 20081849]
- Han X, Chow BY, Zhou H, Klapoetke NC, Chuong A, Rajimehr R, Yang A, Baratta MV, Winkle J, Desimone R, Boyden ES. A high-light sensitivity optical neural silencer: development and application to optogenetic control of non-human primate cortex. *Front Syst Neurosci*. 2011; 5:18. [PubMed: 21811444]
- Han X, Qian X, Bernstein JG, Zhou HH, Franzesi GT, Stern P, Bronson RT, Graybiel AM, Desimone R, Boyden ES. Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain. *Neuron*. 2009; 62:191–8. [PubMed: 19409264]
- Han X, Qian X, Stern P, Chuong AS, Boyden ES. Informational lesions: optical perturbation of spike timing and neural synchrony via microbial opsin gene fusions. *Front Mol Neurosci*. 2009; 2:12. [PubMed: 19753326]
- Howard A, Tamas G, Soltesz I. Lighting the chandelier: new vistas for axo-axonic cells. *Trends Neurosci*. 2005; 28:310–6. [PubMed: 15927687]
- Hung C, Ling G, Mohanty SK, Chiao JJ. An Integrated μ LED Optrode for Optogenetic Stimulation and Electrical Recording. *Biomedical Engineering, IEEE Transactions on*. 2013; 60:225–229.
- Keller CJ, Truccolo W, Gale JT, Eskandar E, Thesen T, Carlson C, Devinsky O, Kuzniecky R, Doyle WK, Madsen JR, Schomer DL, Mehta AD, Brown EN, Hochberg LR, Ulbert I, Halgren E, Cash SS. Heterogeneous neuronal firing patterns during interictal epileptiform discharges in the human cortex. *Brain*. 2010; 133:1668–81. [PubMed: 20511283]
- Kim TI, McCall JG, Jung YH, Huang X, Siuda ER, Li Y, Song J, Song YM, Pao HA, Kim RH, Lu C, Lee SD, Song IS, Shin G, Al-Hasani R, Kim S, Tan MP, Huang Y, Omenetto FG, Rogers JA, Bruchas MR. Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. *Science*. 2013; 340:211–6. [PubMed: 23580530]
- Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science*. 2008; 321:53–7. [PubMed: 18599766]
- Kokaia M, Andersson M, Ledri M. An optogenetic approach in epilepsy. *Neuropharmacology*. 2013; 69:89–95. [PubMed: 22698957]

- Konermann S, Brigham MD, Trevino AE, Hsu PD, Heidenreich M, Cong L, Platt RJ, Scott DA, Church GM, Zhang F. Optical control of mammalian endogenous transcription and epigenetic states. *Nature*. 2013; 500:472–6. [PubMed: 23877069]
- Krook-Magnuson E, Armstrong C, Oijala M, Soltesz I. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nat Commun*. 2013; 4:1376. [PubMed: 23340416]
- Krook-Magnuson E, Varga C, Lee SH, Soltesz I. New dimensions of interneuronal specialization unmasked by principal cell heterogeneity. *Trends Neurosci*. 2012; 35:175–84. [PubMed: 22119146]
- Ledri M, Nikitidou L, Erdelyi F, Szabo G, Kirik D, Deisseroth K, Kokaia M. Altered profile of basket cell afferent synapses in hyper-excitabile dentate gyrus revealed by optogenetic and two-pathway stimulations. *Eur J Neurosci*. 2012; 36:1971–83. [PubMed: 22512307]
- Lee G, Saito I. Role of nucleotide sequences of loxP spacer region in Cre-mediated recombination. *Gene*. 1998; 216:55–65. [PubMed: 9714735]
- Lee JH, Durand R, Gradinaru V, Zhang F, Goshen I, Kim DS, Fenno LE, Ramakrishnan C, Deisseroth K. Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature*. 2010; 465:788–92. [PubMed: 20473285]
- Lee SY, Soltesz I. Cholecystokinin: a multi-functional molecular switch of neuronal circuits. *Dev Neurobiol*. 2011; 71:83–91. [PubMed: 21154912]
- Lin JY. A user's guide to channelrhodopsin variants: features, limitations and future developments. *Exp Physiol*. 2011; 96:19–25. [PubMed: 20621963]
- Lin JY, Lin MZ, Steinbach P, Tsien RY. Characterization of engineered channelrhodopsin variants with improved properties and kinetics. *Biophys J*. 2009; 96:1803–14. [PubMed: 19254539]
- Madisen L, Mao T, Koch H, Zhuo JM, Berenyi A, Fujisawa S, Hsu YW, Garcia AJ 3rd, Gu X, Zanella S, Kidney J, Gu H, Mao Y, Hooks BM, Boyden ES, Buzsaki G, Ramirez JM, Jones AR, Svoboda K, Han X, Turner EE, Zeng H. A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. *Nat Neurosci*. 2012; 15:793–802. [PubMed: 22446880]
- Maehara T. Neuroimaging of epilepsy. *Neuropathology*. 2007; 27:585–93. [PubMed: 18021381]
- Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, Palmer CA, Feigenbaum F, Tornatore C, Tufaro F, Martuza RL. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther*. 2000; 7:867–74. [PubMed: 10845725]
- Matta JA, Pelkey KA, Craig MT, Chittajallu R, Jeffries BW, McBain CJ. Developmental origin dictates interneuron AMPA and NMDA receptor subunit composition and plasticity. *Nat Neurosci*. 2013; 16:1032–41. [PubMed: 23852113]
- Mattis J, Tye KM, Ferenczi EA, Ramakrishnan C, O'Shea DJ, Prakash R, Gunaydin LA, Hyun M, Fenno LE, Gradinaru V, Yizhar O, Deisseroth K. Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nat Methods*. 2012; 9:159–72. [PubMed: 22179551]
- Merwick A, O'Brien M, Delanty N. Complex single gene disorders and epilepsy. *Epilepsia*. 2012; 53(Suppl 4):81–91. [PubMed: 22946725]
- Murphy AM, Rabkin SD. Current status of gene therapy for brain tumors. *Transl Res*. 2013; 161:339–54. [PubMed: 23246627]
- Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc Natl Acad Sci U S A*. 2003; 100:13940–5. [PubMed: 14615590]
- Osawa S, Iwasaki M, Hosaka R, Matsuzaka Y, Tomita H, Ishizuka T, Sugano E, Okumura E, Yawo H, Nakasato N, Tominaga T, Mushiaki H. Optogenetically induced seizure and the longitudinal hippocampal network dynamics. *PLoS One*. 2013; 8:e60928. [PubMed: 23593349]
- Paz JT, Davidson TJ, Frechette ES, Delord B, Parada I, Peng K, Deisseroth K, Huguenard JR. Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. *Nat Neurosci*. 2013; 16:64–70. [PubMed: 23143518]
- Raimondo JV, Kay L, Ellender TJ, Akerman CJ. Optogenetic silencing strategies differ in their effects on inhibitory synaptic transmission. *Nat Neurosci*. 2012; 15:1102–4. [PubMed: 22729174]

- Richichi C, Lin EJ, Stefanin D, Colella D, Ravizza T, Grignaschi G, Veglianesi P, Sperk G, During MJ, Vezzani A. Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. *J Neurosci*. 2004; 24:3051–9. [PubMed: 15044544]
- Rothman SM. The therapeutic potential of focal cooling for neocortical epilepsy. *Neurotherapeutics*. 2009; 6:251–7. [PubMed: 19332317]
- Royer S, Zemelman BV, Barbic M, Losonczy A, Buzsaki G, Magee JC. Multi-array silicon probes with integrated optical fibers: light-assisted perturbation and recording of local neural circuits in the behaving animal. *Eur J Neurosci*. 2010; 31:2279–91. [PubMed: 20529127]
- Schneider CJ, Bezaire M, Soltesz I. Toward a full-scale computational model of the rat dentate gyrus. *Front Neural Circuits*. 2012; 6:83. [PubMed: 23162433]
- Schnutgen F, Doerflinger N, Calleja C, Wendling O, Chambon P, Ghyselinck NB. A directional strategy for monitoring Cre-mediated recombination at the cellular level in the mouse. *Nat Biotechnol*. 2003; 21:562–5. [PubMed: 12665802]
- Smedemark-Margulies N, Trapani JG. Tools, methods, and applications for optophysiology in neuroscience. *Front Mol Neurosci*. 2013; 6:18. [PubMed: 23882179]
- Soltesz, I.; Staley, K., editors. *Computational Neuroscience in Epilepsy*. Academic Press; 2008.
- Sorensen AT, Nikitidou L, Ledri M, Lin EJ, During MJ, Kanter-Schlifke I, Kokaia M. Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. *Exp Neurol*. 2009; 215:328–33. [PubMed: 19038255]
- Stark E, Koos T, Buzsaki G. Diode probes for spatiotemporal optical control of multiple neurons in freely moving animals. *J Neurophysiol*. 2012; 108:349–63. [PubMed: 22496529]
- Sukhotinsky I, Chan AM, Ahmed OJ, Rao VR, Gradinaru V, Ramakrishnan C, Deisseroth K, Majewska AK, Cash SS. Optogenetic delay of status epilepticus onset in an in vivo rodent epilepsy model. *PLoS One*. 2013; 8:e62013. [PubMed: 23637949]
- Takano H, Coulter DA. *Imaging of Hippocampal Circuits in Epilepsy*. 2012
- Tamura K, Ohashi Y, Tsubota T, Takeuchi D, Hirabayashi T, Yaguchi M, Matsuyama M, Sekine T, Miyashita Y. A glass-coated tungsten microelectrode enclosing optical fibers for optogenetic exploration in primate deep brain structures. *Journal of Neuroscience Methods*. 2012; 211:49–57. [PubMed: 22971353]
- Tanaka KF, Matsui K, Sasaki T, Sano H, Sugio S, Fan K, Hen R, Nakai J, Yanagawa Y, Hasuwa H, Okabe M, Deisseroth K, Ikenaka K, Yamanaka A. Expanding the repertoire of optogenetically targeted cells with an enhanced gene expression system. *Cell Rep*. 2012; 2:397–406. [PubMed: 22854021]
- Taniguchi H, He M, Wu P, Kim S, Paik R, Sugino K, Kvitsiani D, Fu Y, Lu J, Lin Y, Miyoshi G, Shima Y, Fishell G, Nelson SB, Huang ZJ. A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron*. 2011; 71:995–1013. [PubMed: 21943598]
- Taniguchi H, Lu J, Huang ZJ. The spatial and temporal origin of chandelier cells in mouse neocortex. *Science*. 2013; 339:70–4. [PubMed: 23180771]
- Tomita H, Sugano E, Fukazawa Y, Isago H, Sugiyama Y, Hiroi T, Ishizuka T, Mushiaki H, Kato M, Hirabayashi M, Shigemoto R, Yawo H, Tamai M. Visual properties of transgenic rats harboring the channelrhodopsin-2 gene regulated by the thy-1.2 promoter. *PLoS One*. 2009; 4:e7679. [PubMed: 19893752]
- Toni N, Laplagne DA, Zhao C, Lombardi G, Ribak CE, Gage FH, Schinder AF. Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nat Neurosci*. 2008; 11:901–7. [PubMed: 18622400]
- Tonnesen J, Sorensen AT, Deisseroth K, Lundberg C, Kokaia M. Optogenetic control of epileptiform activity. *Proc Natl Acad Sci U S A*. 2009; 106:12162–7. [PubMed: 19581573]
- Truccolo W, Donoghue JA, Hochberg LR, Eskandar EN, Madsen JR, Anderson WS, Brown EN, Halgren E, Cash SS. Single-neuron dynamics in human focal epilepsy. *Nat Neurosci*. 2011; 14:635–41. [PubMed: 21441925]
- Varga C, Lee SY, Soltesz I. Target-selective GABAergic control of entorhinal cortex output. *Nat Neurosci*. 2010; 13:822–4. [PubMed: 20512133]

- Vezzani A. The promise of gene therapy for the treatment of epilepsy. *Expert Rev Neurother*. 2007; 7:1685–92. [PubMed: 18052764]
- Visel A, Taher L, Girgis H, May D, Golonzhka O, Hoch RV, McKinsey GL, Pattabiraman K, Silberberg SN, Blow MJ, Hansen DV, Nord AS, Akiyama JA, Holt A, Hosseini R, Phouanavong S, Plajzer-Frick I, Shoukry M, Afzal V, Kaplan T, Kriegstein AR, Rubin EM, Ovcharenko I, Pennacchio LA, Rubenstein JL. A high-resolution enhancer atlas of the developing telencephalon. *Cell*. 2013; 152:895–908. [PubMed: 23375746]
- Wang J, Wagner F, Borton DA, Zhang J, Ozden I, Burwell RD, Nurmikko AV, Wagenen RV, Diester I, Deisseroth K. Integrated device for combined optical neuromodulation and electrical recording for chronic in vivo applications. *Journal of Neural Engineering*. 2012; 9:016001. [PubMed: 22156042]
- Wentz CT, Bernstein JG, Monahan P, Guerra A, Rodriguez A, Boyden ES. A wirelessly powered and controlled device for optical neural control of freely-behaving animals. *Journal of Neural Engineering*. 2011; 8:046021. [PubMed: 21701058]
- Wentz CT, Bernstein JG, Monahan P, Guerra A, Rodriguez A, Boyden ES. A wirelessly powered and controlled device for optical neural control of freely-behaving animals. *J Neural Eng*. 2011; 8:046021. [PubMed: 21701058]
- White AM, Williams PA, Ferraro DJ, Clark S, Kadam SD, Dudek FE, Staley KJ. Efficient unsupervised algorithms for the detection of seizures in continuous EEG recordings from rats after brain injury. *J Neurosci Methods*. 2006; 152:255–66. [PubMed: 16337006]
- Woodson W, Nitecka L, Ben-Ari Y. Organization of the GABAergic system in the rat hippocampal formation: a quantitative immunocytochemical study. *J Comp Neurol*. 1989; 280:254–71. [PubMed: 2925894]
- Wu C, Sharan AD. Neurostimulation for the treatment of epilepsy: a review of current surgical interventions. *Neuromodulation*. 2013; 16:10–24. discussion 24. [PubMed: 22947069]
- Wykes RC, Heeroma JH, Mantoan L, Zheng K, Macdonald DC, Deisseroth K, Hashemi KS, Walker MC, Schorge S, Kullmann DM. Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. *Sci Transl Med*. 2012; 4:161ra152.
- Xu X, Olivas ND, Levi R, Ikrar T, Nenadic Z. High precision and fast functional mapping of cortical circuitry through a novel combination of voltage sensitive dye imaging and laser scanning photostimulation. *J Neurophysiol*. 2010; 103:2301–12. [PubMed: 20130040]
- Yang X, Rode DL, Peterka DS, Yuste R, Rothman SM. Optical control of focal epilepsy in vivo with caged gamma-aminobutyric acid. *Ann Neurol*. 2012; 71:68–75. [PubMed: 22275253]
- Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K. Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature*. 2011; 477:171–8. [PubMed: 21796121]
- Zhang F, Aravanis AM, Adamantidis A, de Lecea L, Deisseroth K. Circuit-breakers: optical technologies for probing neural signals and systems. *Nat Rev Neurosci*. 2007; 8:577–81. [PubMed: 17643087]
- Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de Lecea L, Deisseroth K. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nat Protoc*. 2010; 5:439–56. [PubMed: 20203662]
- Zhang F, Prigge M, Beyriere F, Tsunoda SP, Mattis J, Yizhar O, Hegemann P, Deisseroth K. Red-shifted optogenetic excitation: a tool for fast neural control derived from *Volvox carteri*. *Nat Neurosci*. 2008; 11:631–3. [PubMed: 18432196]
- Zhang J, Laiwalla F, Kim JA, Urabe H, Wagenen RV, Song YK, Connors BW, Zhang F, Deisseroth K, Nurmikko AV. Integrated device for optical stimulation and spatiotemporal electrical recording of neural activity in light-sensitized brain tissue. *Journal of Neural Engineering*. 2009; 6:055007. [PubMed: 19721185]
- Zhang YP, Oertner TG. Optical induction of synaptic plasticity using a light-sensitive channel. *Nat Methods*. 2007; 4:139–41. [PubMed: 17195846]
- Zhu P, Narita Y, Bundschuh ST, Fajardo O, Scharer YP, Chattopadhyaya B, Bouldoires EA, Stepien AE, Deisseroth K, Arber S, Sprengel R, Rijli FM, Friedrich RW. Optogenetic Dissection of

Neuronal Circuits in Zebrafish using Viral Gene Transfer and the Tet System. *Front Neural Circuits*. 2009; 3:21. [PubMed: 20126518]

Zorzos AN, Boyden ES, Fonstad CG. Multiwaveguide implantable probe for light delivery to sets of distributed brain targets. *Opt Lett*. 2010; 35:4133–5. [PubMed: 21165114]

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Figure 1. Strategies for selective opsin expression

(A) The FLEX system makes use of two pairs of loxP sites (triangles), including the mutated lox2272 (dark triangles). Cre mediates inversion using one set of loxP sites (for simplicity, only the inversion using lox2272 sites are illustrated), flipping the opsin sequence into the correct orientation (stage 2). Cre-mediated excision of one of each loxP sites locks the vector in an active state (stage 3). Based on figure 1 from reference (Atasoy et al., 2008). (B) Three potential ways to achieve selective opsin expression include (i) injecting a Cre-dependent virus (as in A) and a Cre-delivering virus (e.g., WGA-Cre, as further discussed in the text (Gradinaru et al., 2010)), (ii) injecting a Cre-dependent virus into a mouse expressing Cre in a subset of neurons, or (iii) crossing a mouse line expressing Cre in a subset of neurons with a mouse line expressing opsins in a Cre-dependent manner.

Figure 2. Optogenetic inhibition of epileptiform activity in vitro

NpHR expression in excitatory principal cells of organotypic hippocampal slices is efficient in inhibiting stimulation train induced bursting (STIB) when activated by orange light, in both CA3 (A) and CA1 (B) areas. Stimulation with blue light failed to alter STIB-induced bursting (B, bottom). Reproduced with permission from reference (Tonnesen et al., 2009).

Figure 3. Schematic of online seizure detection for on-demand optogenetics

EEG input (blue) recorded from the animal is amplified (Amp), digitized (A/D), and relayed to a PC running real-time seizure detection software. This software is tuned for each animal, with user-defined thresholds (green). Seizure detection algorithms utilize features of signal power (top), spikes (middle), or frequency (bottom). Once a seizure has been detected using the selected criteria, the software can activate, via a TTL signal from the digitizer to the laser, the optical output (orange) delivered to the animal. Signal power related calculations (purple, during an example seizure shown in grey), spike characteristics (e.g., amplitude, rate, regularity, and spike width, shown in red), and frequency characteristics (shown for the same seizure, with warmer colors representing higher energy) are illustrated. COMP: digital comparator. This on-line seizure detection software is available for download through reference (Armstrong et al., 2013). Figure reproduced with permission from reference (Krook-Magnuson et al., 2013).

Figure 4. Seizure control *in vivo* in mice expressing HR in principal cells in a model of temporal lobe epilepsy

(a) Crossing CamK-Cre and Cre-dependent halorhodopsin (HR) mouse lines generated mice expressing the inhibitory opsin HR in excitatory cells (Cam-HR mice). (b) Experimental timeline. (c-e) Example electrographic seizures detected (vertical green bars), activating amber light (589nm) randomly for 50% of events (light: amber line, example in d; no-light example in e). (f) Typical example distribution of post-detection seizure durations (5s bin size) during light (solid amber) and no-light internal control conditions (hashed gray). Inset: first 5s bin expanded, 1s bin size. Note that most seizures stop within 1s of light delivery. (g-i) Group CamHR data showing the percent of seizures stopping within 5s of detection (g), within 1s of detection (h), and the average post-detection seizure duration (normalized to average no-light post-detection duration for each animal) (i). Note that in one animal (shown in c-e), all seizures were stopped within 1s of light delivery. Averaged data: filled circles. Error bars represent s.e.m. Scale bars in c-e: 100 μ V, 5s. Reproduced with permission from reference (Krook-Magnuson et al., 2013).