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Applications of optogenetic and chemogenetic methods to seizure circuits: Where to go next?

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Abstract

Epilepsy is the quintessential circuit disorder, with seizure activity propagating through anatomically constrained pathways. These pathways, necessary for normal sensory, motor, and cognitive function are hijacked during seizures. Understanding the network architecture at the level of both local microcircuits and distributed macrocircuits may provide new therapeutic avenues for the treatment of epilepsy. Over the past decade, optogenetic and chemogenetic tools have enabled previously impossible levels of functional circuit mapping in neuroscience. In this review, examples of the application of optogenetics and chemogenetics to epilepsy are raised, the comparative strengths and weaknesses of these approaches are discussed for both preclinical and translational applications, and recent applications of these approaches in other areas of neuroscience are highlighted. These points are raised in an effort to highlight the potential of these methods to address additional unanswered questions in epilepsy.

Keywords

Channelrhodopsin; DREADD; CNO; Daun02

Introduction

Seizure activity propagates through anatomically constrained neural pathways, and thus an understanding of these pathways may provide insight to therapeutic interventions. A rich literature of behavioral, neuroanatomical, and neurophysiological studies have provided maps for brain circuits supporting normal functions. In parallel, these circuits have been evaluated in the context of epilepsy. Aspiration lesions, excitotoxic lesions, electrical stimulation, transient pharmacological manipulations and an array of methods for recording neural activity have provided critical insight to the network organization supporting diverse types of seizures. These studies have resulted in circuit maps that provide both preclinical and clinical insight in epilepsy, and provide a springboard for future work. However, while

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these approaches are powerful, they are limited in temporal and spatial resolution, particularly in light of recent advances in chemogenetic and optogenetic methods (Armbruster et al., 2007; Boyden et al., 2005). The introduction of these newer methodologies over the past decade has enabled cell-type specificity, real-time bidirectional control of neuronal activity, and the ability to manipulate circuits in a projection-pathway specific manner. This has put the development of high-resolution circuit maps for seizures within reach.

Both optogenetic and chemogenetic methodologies share the common feature of enabling cell-type and pathway specific activation or silencing of neurons, and have been reviewed in detail elsewhere (Bernstein and Boyden, 2011; Sjulson et al., 2016; Urban and Roth, 2015). Briefly, optogenetics accomplishes this modulation through the expression of light-sensitive ion channels or pumps; when these actuators are expressed in cells of interest, focal light delivery results in neuronal excitation (e.g., channelrhodopsin 2, ChR2) or inhibition (e.g., archaerhodopsin, ArchT, halorhodopsin, NpHR). The chemogenetic approach makes use of genetically modified G-protein coupled receptors. These receptors, commonly referred to as “designer receptors exclusively activated by designer drugs” or DREADDs, include the first-generation DREADDs, (hM4Di, hM3Dq, Gs-D) which evolved from the human muscarinic receptor and the more recently developed “Kappa opioid receptor DREADDs” (KORD) which was evolved from the kappa opioid receptor. These DREADDs no longer respond to their endogenous ligand, but instead, display high affinity for other putatively inert compounds (e.g., clozapine-n-oxide [CNO], salvinorin-B; but see below). Thus, with the DREADD approach focal or systemic delivery of the DREADD ligand results in neuronal silencing or excitation.

Here, I discuss (1) the comparative strengths of optogenetic and chemogenetic approaches in epilepsy, (2) the importance of microcircuit-macrocircuit interactions in seizure mapping, (3) ways in which optogenetics have allowed tests of *causality* within specific seizure pathways, and (4) applications of optogenetic and chemogenetic approaches in behavioral neuroscience that may be applied to epilepsy. This review is not intended to serve as a comprehensive overview of all the studies in epilepsy employing these methods, as several recent reviews have done this quite well (Choy et al., 2016; Krook-Magnuson and Soltesz, 2015; Tønnesen and Kokaia, 2017; Tung et al., 2016), but rather intends to use select examples at the level of whole-animal or macrocircuit manipulations to highlight the potential of these methods to answer questions that were previously unapproachable.

Optogenetics vs. Chemogenetics

The application of optogenetic technology to epilepsy has far outpaced the applications of chemogenetics; as of this review there were 128 references related to optogenetics and epilepsy or seizures, with the majority being primary experimental reports. The first of these reports employed optogenetics in slice (Tonnesen et al., 2009), followed by a rapid progression to *in vivo* manipulations (Krook-Magnuson et al., 2013; Paz et al., 2013; Wykes et al., 2012). These studies have utilized a variety of light-sensitive ion channels and pumps to drive or suppress activity in populations of neurons. By contrast, only thirteen reports involving a chemogenetics approach were retrieved, of which only four were primary

literature. The cumulative citations over time for the use of these two methods in epilepsy are shown in Figure 1. Both the optogenetic approach and the chemogenetic approach have achieved expression using both transgenic and viral delivery designs. While these approaches each have strengths and advantages for both research-oriented and clinical applications (outlined separately below), in situations where these two approaches have been directly compared, they have produced quite similar results (Zhu et al., 2016).

Why has the use of DREADD approaches lagged behind optogenetics in epilepsy research? A clear benefit of the optogenetic approach is its fast temporal resolution of neuronal control. Even the first generation of optogenetic constructs displayed millisecond resolution control of neuronal activity (Boyden et al., 2005), with subsequent generations of tools capable of driving neurons at even higher frequencies (Gunaydin et al., 2010; Klapoetke et al., 2014). By contrast, DREADDs do not offer spike-level temporal resolution – agonist administration results in silencing or activation of neurons for minutes to hours, with the pattern of activation outside of experimental control. If a high degree of temporal control, specific patterns of firing, or rapid switching between activated and inactivated states is required for a particular question, optogenetics is the clear “winner”. For example, closed-loop modulation of neuronal activity requires the fast temporal resolution of optogenetics (Armstrong et al., 2013; Krook-Magnuson et al., 2013; Paz et al., 2013).

While fast temporal resolution has advantages for certain applications, so does the ability to activate or silence over a longer period of time. Take, for example, the timescale of spontaneous seizure emergence following status epilepticus. Seizures gradually emerge in the days to weeks following status epilepticus (Williams et al., 2009); thus, to effectively suppress seizures after status, optogenetic methods would require continuous tethering of an animal. Although the DREADD approach would similarly require continuous treatment, recent reports have demonstrated the efficacy of long-term CNO delivery via drinking water or via osmotic minipump, thus presenting an alternative to the long term tethering for chronic optogenetic manipulations (Donato et al., 2017; Urban et al., 2016). The development of head-mounted wireless LED solutions for optogenetics may likewise provide an alternative to chronic tethering (Iwai et al., 2011; Rossi et al., 2015; Wentz et al., 2011).

Both optogenetics and chemogenetics allow for a degree of multiplexing, i.e., using two or more actuators in the same animal. Moreover, these approaches are compatible with one another so it is possible to employ both DREADD and optogenetic approaches in the same animal. Optogenetic actuators have been developed that selectively respond to blue, yellow, green, orange, and red light. These variants allow for theoretical multiplexing within a single subject, but this will depend on the precise experimental conditions, opsin pairs, and intensity of light delivery. Some opsin pairs offer minimal spectral overlap enabling excitatory multiplexing within subject. For example, Chrimson and Chronos are activated by red and blue light, respectively, and have been effectively multiplexed in vivo (Klapoetke et al., 2014), enabling within-subject targeting of multiple cell types for excitation. Similarly, Chrimson could similarly be multiplexed with ChR2 due to minimal overlap in the activation spectra. However, with any opsin pair, careful selection of the activation wavelengths and power of light delivered is required to minimize off target activation. For multiplexed

optogenetic excitation and inhibition, ChR2 can be multiplexed with eNpHR3.0 with minimal spectral overlap (Zhang et al., 2007). However, there is substantial overlap in the activation spectra for ChR2 and other inhibitory opsins (e.g., ArchT) which would limit the selectivity when used in a multiplexed setting (Mattis et al., 2012).

As with optogenetics, multiple DREADD actuators are available. Two primary families of DREADDs have been widely utilized: those based on the human muscarinic receptor (with Gi, Gs, and Gq variants) and those based on the Kappa opioid receptor (kappa opioid receptor DREADDs; Gi coupled [KORDs]) (Armbruster et al., 2007; Marchant et al., 2016). A third family - an ivermectin-gated chloride channel (GluCl) - has also been developed. However, the GluCl channel has been deployed to a much smaller degree than the other DREADD families (Frazier et al., 2013). The allatostatin receptor has also been utilized for selective silencing of mammalian cells, but is limited by the need to microinject the peptide ligand (Lechner et al., 2002). Because each of these receptor families are activated by different ligands, multiplexing is possible (Vardy et al., 2015). Thus, there are currently three DREADD-based options for neuronal silencing (hM4Di, KORD, and GluCl); each of these displays a unique pharmacology and would be thus amenable to multiplexing. The two available DREADD actuators for neuronal activation (Gs-DREADD and hM3Dq) are both activated by CNO, and would thus not be compatible for multiplexing with each other or with the hM4Di receptor. GluCl mediated neuronal inhibition is relatively straightforward, and conceptually similar to NpHR-mediated silencing - activation of the GluCl receptor with ivermectin results in chloride influx neuronal hyperpolarization, and suppression of neuronal firing (Lerchner et al., 2007). By contrast, hM4Di- or KORD-mediated silencing induces activation of G-protein coupled inward rectifying potassium channels (GIRKs). GIRK activation has been associated both with neuronal hyperpolarization and reduction in neurotransmitter release through action in presynaptic terminals (Lüscher and Slesinger, 2010).

DREADD-mediated neuronal activation (e.g., through Gq DREADDs) operates through a phospholipase C mechanism. Application of CNO in hM3Dq-expressing mice induces both an increase in presynaptic neurotransmitter release, a modest membrane depolarization, and evoked seizures (Alexander et al., 2009). Moreover, in slice, activation of Gq-coupled DREADDs induces neurotransmitter release (Buchta et al., 2017). For each of DREADD subtype, the tissue specific profile of receptor coupling and the presence of appropriate signal transduction machinery will likely modulate the efficacy of inhibition or excitation. Moreover, GPCR activation has been linked to pathways mediating cell survival (for review see: Martin et al., 2005) as well as long-term depression and long-term potentiation (for reviews see: Atwood et al., 2014; Betke et al., 2012). While DREADD methodology has generally reported to be safe, the effects of chronic modulation of these cascades is an area meriting further examination.

DREADD approaches suffer from some of the same limitations as traditional pharmacological approaches. DREADD receptors may or may not desensitize with prolonged activation – both GPCR-receptor kinase phosphorylation sites and arrestin binding sites are conserved between muscarinic receptors and DREADDs (Armbruster et al., 2007). While in cases of robust transgene expression, receptor reserve is likely to minimize

apparent desensitization, in cases where expression is less robust, desensitization may be evident (for further discussion see: Roth, 2016; Wicker and Forcelli, 2016). In addition to these pharmacodynamic concerns, DREADD activity will depend on the pharmacokinetic properties of the DREADD agonist applied.

Although CNO has been described as pharmacologically inert, recent studies have shown that it can influence amphetamine-induced locomotor activity in rats lacking DREADD receptors (MacLaren et al., 2016), and can partially substitute for clozapine in a drug-discrimination paradigm in both mouse and rat (Porter et al., 2017). These effects may be due not to the parent compound, but rather to the production of appreciable levels of clozapine or n-desmethylozapine. The pharmacokinetics of CNO have been described after systemic administration in mouse (Guettier et al., 2009), rat (Lin et al., 1996; MacLaren et al., 2016) and macaque (Raper et al., 2017), and in each case, clozapine and n-desmethylozapine have been detected after CNO administration. Clozapine displays appreciable affinity for native muscarinic receptors, histamine receptors, alpha adrenergic receptors, and serotonin receptors, and n-desmethylozapine displays affinity for many of these same receptors (PDSP Ki Database, n.d.).

Compound 21 is a novel, designer agonist for hM3Dq DREADDs which displays high selectivity for hM3Dq over serotonin and alpha adrenergic receptors, and is devoid of action at hM3 receptors. Despite these favorable properties, it displays only a 3.5-fold selectivity for hM3Dq over H1 histamine receptors (Chen et al., 2015). This degree of separation may be sufficient for many applications, but it may still present challenges if DREADD ligands are microinjected directly into the brain, as concentrations at the site of microinjection are typically much higher than those achieved systemically. The pharmacokinetic properties of Compound 21 have not been described, thus the timing of administration relative to testing, and the duration of action will need to be empirically assessed for a given experimental design. Similarly, perlapine, a hypnotic agent, displays no activity at hM3 but robust activity at hM3Dq. However, perlapine also displays nM affinity for a variety of other GPCRs (PDSP Ki Database, n.d.).

Turning toward KORD receptors, the only agonist that has been described is Salvinorin B (SalB). This compound displays a >10-fold selectivity for KORDs over native human kappa opioid receptors (hKOR), but at high concentrations maintains full agonist activity at hKOR (Marchant et al., 2016). To date, the pharmacokinetic properties of SalB have not been characterized. Appropriate dose-finding and pharmacokinetic studies, as well as the development of additional KORD agonists may further extend the utility of this class of DREADDs.

Together, these pharmacokinetic concerns underscore the importance of appropriate controls: it is essential to ensure that the dose of DREADD ligand provided is without effect on the dependent measure of interest in wild-type (i.e., non-DREADD expressing) animals. Moreover, development of DREADD reagents that avoid desensitization, and/or agonists with desirable pharmacokinetic properties may address these concerns. Even given the limits associated with systemic drug delivery, the DREADD approach still provides enhanced flexibility over classic methods of transient inactivation, e.g., intracerebral drug injection - it

is much easier to give a systemic drug injection during a seizure, than for example, to microinject a drug into the brain.

Beyond the benefits of temporal control, one of the key advantages of both the optogenetic and chemogenetic approaches is enhanced spatial control. Because both opsins and DREADDs can be expressed in a cell-type specific manner, targeting small or adjacent structures is achievable with a resolution impossible for conventional drug microinjection. Drug diffusion to adjacent regions, which has been a long-standing concern even in the best-designed microinjection experiments, may be avoided by the genetic targeting of optogenetic or chemogenetic actuators to cells of interest. These approaches have also enabled pathway-specific modulation. By delivering virus to a nucleus and applying light or DREADD agonist to a terminal field, single projection pathways may be modulated. However, this approach is not without caveats. One of the difficulties with employing optogenetic approaches to pathway-specific activation is the potential for antidromic activation of neuronal cell bodies and/or axon collaterals; this is undesirable as it limits the conclusions that can be drawn from selective modulation of a single terminal zone. When optogenetic stimulation is applied to terminal fields (Jennings et al., 2013; Sato et al., 2014), an antidromically propagating action potential can be triggered; in turn this may result in an orthodromic spike. While some of these effects can be overcome in part by microinjection of lidocaine (Stuber et al., 2011) at the site of cell bodies, this still does not preclude the possibility of antidromic activation of collaterals in a collateralized neuron. Moreover, this approach suffers from the classic drawbacks associated with drug microinjection, including diffusion of drug and in the case of lidocaine, inactivation of fibers of passage (Tehovnik and Sommer, 1997). Antidromic activation has not been reported using chemogenetic approaches, thus focal microinjection of DREADD agonist in terminal fields may avoid this confound. Given the indirect and temporally slower actions of G protein coupled signaling on ion channel conductance, the likelihood of antidromic spikes is less than that seen with optogenetics, which directly gates ion channels. However, this may depend on the complement of ion channels present in the axon terminals, and would merit experimental verification for each pathway of interest. It is worth noting that for *silencing* this confound is not an issue for either optogenetic or chemogenetic approaches. However, post-inhibitory rebound spiking has been reported after optogenetic inhibition, as has collapse of the neuronal chloride gradient with NpHR (Raimondo et al., 2012) and changes in intracellular pH with ArchT that can modulate neurotransmitter release (Mahn et al., 2016).

Most applications of optogenetics and chemogenetics to date have employed relatively focal manipulations. However, targeting multiple components of a distributed network may lead to some challenges. Light distribution in the brain is limited, and thus a relatively small volume of tissue can be activated. In some respects, the enhanced site specificity afforded by limited light distribution (i.e., the zone of activated neurons will lie underneath the fiber optic) is a strength – this allows for the discrete targeting of adjacent structures. However, because of this limited light distribution, targeting a distributed network would likely require multiple fiber optics to be placed. An alternative approach may be to employ red-shifted opsins, which offer superior penetration of brain tissue (Chuong et al., 2014).

Potential for Clinical Treatment

Looking ahead toward large-animal studies and/or clinical treatment, optogenetics offers the benefit of a self-contained system. Much akin to current deep brain stimulation approaches, optogenetics may be theoretically deployed in a self-contained manner with implanted fibers and stimulators requiring an initially invasive surgery followed by no further need for tethering or drug treatment. Importantly, proof-of-concept use of optogenetics in resected human brain tissue underscores the viability of this strategy (Andersson et al., 2016). Conversely, the chemogenetic approach, which *does not require* long term hardware implantation (just virus injection), may be seen as an advantage as it minimizes the complexity of the surgical approach. One area in which chemogenetics may offer a clear advantage over optogenetics is in *scale*. Scaling optogenetics beyond the size of the rodent brain presents a challenge: for example, the volume of the primate amygdala is almost half the total brain volume of a mouse (Chareyron et al., 2011). Given the large size of the primate amygdala, an spatially diffuse array of fibers would be required to deliver sufficient light to modulate the entire structure. The chemogenetic approach avoids this concern, because virus can be delivered to large volumes of tissue and agonist administered systemically. Both for the relatively less invasive method and for the ability to scale over larger structures, several groups have begun exploring chemogenetics in macaques (Aguilar et al., 2015; Eldridge et al., 2016; Grayson et al., 2016; Nagai et al., 2016). Similarly, several reports have demonstrated the efficacy of optogenetic stimulation (Galvan et al., 2016; Klein et al., 2016; Stauffer et al., 2016) and inhibition (Afraz et al., 2015; Cavanaugh et al., 2012) in macaques.

Seizure Circuits: Going from Black Box Diagrams to Microcircuit-Macro-circuit Interactions

Building on the background of a variety of approaches in basic neuroscience, decades of high-quality lesion and microinjection experiments have provided our current framework for understanding the pathways by which seizures propagate in the brain. These studies have identified critical regions for seizure initiation and propagation, as well as circuit nodes that can be exploited to restrain seizure activity (Bertram, 2013, 2014; Gale, 1992). The resolution of these circuit maps is currently at a “black box” level - while we have an understanding of microcircuits within nodes of the seizure circuit and rough routes of propagation between regions, tract specific mapping and microcircuit-macro-circuit interactions are now attainable.

Take, for example, amygdala kindled seizures. These seizures have a defined focal onset in the amygdala; lesion and pharmacological inactivation studies have clearly established structures such as the mediodorsal thalamus, rhinal cortex, piriform cortex, and hippocampus as nodes of propagation for these seizures (McIntyre and Gilby, 2008). However, these nodes of the limbic seizure circuit are mutually interconnected with a high degree of reciprocal and/or parallel connections (Vismer et al., 2015). Thus, microinjection approaches were not sufficient to clearly determine which regions are primarily engaged and which are secondarily engaged. Some applications of optogenetic and chemogenetic approaches have reinforced the data obtained with microinjections, laying the groundwork for more detailed projection-specific mapping. For example, we recently reported that chemogenetic inhibition of the midline thalamus is potentially anticonvulsant in the amygdala

kindling model (Wicker and Forcelli, 2016); CNO administration suppressed both behavioral and electrographic seizure responses in a dose-dependent manner. These data are consistent with prior lesion and microinjection studies, but now provide proof-of-concept for thalamic modulation by DREADDs, thus enabling pathway specific manipulation of inputs to and outputs from the thalamus, e.g., using either dual virus or CNO microinjection approaches. Some questions that are now in reach include: Is bidirectional connectivity between the midline thalamus and the amygdala (or, more broadly between any two nodes of a seizure circuit) required for seizure maintenance or propagation? Will tract-specific targeting display efficacy equal to that of less specific approaches? By contrast, will indirect and parallel pathways result in comparatively reduced efficacy with increased specificity?

The first report of therapeutic silencing of seizure activity via chemogenetics (Kätzel et al., 2014) employed hM4Di DREADDs targeted to excitatory neurons located within the seizure focus in three models of neocortical epilepsy: focal pilocarpine, focal picrotoxin, and the tetanus toxin model of *epilepsia partialis continua*. In each case, the number of electrographic seizures was significantly suppressed by CNO administration; moreover, behavioral seizures (examined in the picrotoxin model) were also suppressed. Similar suppression of electrographic seizure activity have been reported following pan-neuronal DREADD-mediated silencing in an organotypic hippocampal slice culture model (Avaliani et al., 2016). These models may be especially approachable for long term silencing, helping us determine if suppressing critical populations of neurons can disrupt epileptogenesis in slice cultures or *in vivo*.

Similarly, we have shown that optogenetic stimulation of the superior colliculus is potently anticonvulsant in a wide array of induced, acute seizure models, including absence, primarily generalized tonic-clonic, secondarily generalized and complex partial seizure types (Soper et al., 2016). Interest in the superior colliculus for seizure control dates back to the 1980s, in large part because the superior colliculus is a primary target of another region – the substantia nigra pars reticulata – that can be harnessed for seizure control (Iadarola and Gale, 1982). In fact, Gale and colleagues proposed that inhibition of the substantia nigra exerts its anticonvulsant effects by *disinhibition* of the superior colliculus (Gale et al., 1993). Yet, only with the advent of optogenetic manipulations was this hypothesis testable. Indeed, we have found that selective silencing of GABAergic terminals in the superior colliculus, originating from neurons in the substantia nigra, is sufficient to recapitulate the seizure suppressive effects of nigral inhibition and superior colliculus activation (Wicker et al., 2016). We assessed this by microinjecting virus (AAV-CAG-ArchT) into the substantia nigra and delivering light selectively to terminals in the superior colliculus. Mapping of the downstream projection targets of these regions may identify cell populations and neurochemical identities of nodes in the circuit that transduce focal activation into brain-wide changes in excitability.

Optogenetic methods have given new life to “old” targets – beyond our work in the substantia nigra and superior colliculus, several groups have targeted the cerebellum. Cerebellar deep brain stimulation was attempted for seizure control in the mid 1970s with mixed success (Cooper et al., 1976; Van Buren et al., 1978). However, selective optogenetic manipulation of subregions and cellular subtypes within the cerebellum has been

demonstrated to effectively suppress seizures in both a model of temporal lobe epilepsy (Krook-Magnuson et al., 2014) and absence epilepsy (Kros et al., 2015). The enhanced specificity provided by optogenetics may provide a route toward the revitalization of previous targets for seizure control.

Tract specific manipulation, either using optogenetics or DREADDs may offer an additional advantage: by selectively targeting the minimum subset of neurons or projections needed to suppress seizure activity, it may be possible to minimize effects on normal information processing and cognitive function. In each of the above examples, a critical question remains: what local microcircuits are engaged either by a seizure, or by an anticonvulsant manipulation and how do these microcircuits gate or permit the propagation of seizure activity within broader macrocircuits? Approaches such as the INTRSECT (intron recombinase sites enabling combinatorial targeting), which enables boolean-type logic targeting of opsins or DREADDs, may help to disentangle these questions (Fenno et al., 2014). The INTRSECT approach allows for the selection of neurons based on multiple features through the use of multiple recombinase enzymes and recognition sites (i.e., both Cre and Flp recombinases). For example, INTRSECT allows for the selection of neurons that are glutamatergic AND project to a region of interest (e.g., glutamatergic neurons in the thalamus that project to the frontal cortex). Similarly, it allows for the selection of neurons in one nucleus that project to one target region but avoid another (e.g., neurons in the thalamus that project to the frontal cortex but NOT to the amygdala).

The use of trans-synaptic viruses (e.g., glycoprotein deleted rabies viruses) may enable a stepwise deconstruction of a pathway of interest (Callaway and Luo, 2015; Wickersham et al., 2007). As the rabies glycoprotein is required for normal packaging of the virus, these rabies virus variants require the delivery of the glycoprotein in *trans* in order to replicate. Only in cells that co-express the glycoprotein and are infected by the rabies virus, will viral propagation occur. In this manner, selective delivery of the glycoprotein to a cell type of interest (e.g., Cre-dependent expression of the glycoprotein) and co-expression of the rabies virus allows for the mapping of first order neurons that project to the initially infected cells. Once the virus has infected the first order neurons, additional propagation is not possible due to the absence of the glycoprotein. This approach may be used to target the delivery of opsins, DREADDs, or other cargo of interest (Osakada et al., 2011). These strategies may be important in reconciling some recent findings in models of temporal lobe epilepsy: some have reported that optogenetic activation of GABA neurons within the hippocampus/entorhinal cortex restricts seizure propagation (Ladas et al., 2015; Lu et al., 2016; Sessolo et al., 2015) distal to the seizure initiation, but may actually exacerbate seizures focally (Sessolo et al., 2015; Yekhlief et al., 2015). Others have reported termination of local seizure activity through low frequency activation of interneurons at the seizure focus (Shiri et al., 2017). Moreover, interneurons and principal cells have been proposed to play divergent roles in the *type* of seizure onset (Shiri et al., 2016). Given the diversity of interneuron types and the complexities of local microcircuits, additional specificity (such as that achieved with INTRSECT approaches) may further unravel this complexity. For example, within the entorhinal cortex, parvalbumin interneurons display three connectivity patterns: one population targets principal cells that give rise to the perforant path, another targets those that project to extra-hippocampal sites, and a third population targets both (Armstrong et al.,

2016). Understanding the nature of these projections may help to reconcile these findings between local microcircuits and distal microcircuits.

Optogenetic Tests of Causality

Optogenetic approaches have allowed the recent test of causality in two long-held hypotheses in epilepsy: (1) that a failure of the “dentate gate” is a critical contributor to seizures within the temporal lobe and (2) that phasic firing of thalamocortical cells is necessary for absence seizure activity.

The “dentate gate” theory, first proposed in 1992 (Heinemann et al., 1992), suggests that a failure of inhibition within the dentate gyrus is a key feature of the emergence of seizures in temporal lobe epilepsy. Decades of studies have demonstrated loss of GABAergic inhibition and increased excitability within the dentate in models of epilepsy. The ability to selectively and bidirectionally manipulate activity within the dentate afforded Krook-Magnuson and colleagues the opportunity to formally test this hypothesis (Krook-Magnuson et al., 2015). By optogenetically exciting neurons within the dentate, Krook-Magnuson was able to *worsen* spontaneous seizures in a model of temporal lobe epilepsy. Moreover, consistent with electrical kindling studies (Racine et al., 1977), they reported that repeated optogenetic stimulation of the dentate resulted in progressively worsening electrographic and behavioral seizures. By contrast, they reported that “on-demand” optogenetic *silencing* of neurons within the dentate was sufficient to abort spontaneous seizures. At least for the model they employed, these optogenetic manipulations demonstrated that activity in the dentate was both sufficient for seizure induction and necessary for seizure maintenance.

A role for phasic (burst) firing of thalamocortical cells in the genesis of absence seizures has likewise been long proposed (Coulter et al., 1989a, 1989b). The canonical absence seizure loop consists of corticothalamic projection neurons, which relay in the nucleus reticularis of the thalamus. The GABAergic output of nucleus reticularis in turn hyperpolarizes thalamocortical neurons in the ventrobasal complex of the thalamus. Due to the expression of T-type calcium channels, inhibition of these thalamocortical cells results in post-inhibition rebound bursting. This burst firing presumably drives re-entrant activity in the loop through reactivation of neurons in the cortex and reticular nucleus of the thalamus. Recent work from Sorokin et al. has shown that optogenetic hyperpolarization of thalamocortical cells is sufficient to drive post-inhibition rebound bursting, and moreover, that inhibition of these cells can evoke absence-like seizures in both the stargazer mouse and the WAG/Rij models of absence seizures (Sorokin et al., 2017). The authors were able to drive thalamocortical neurons into a tonic firing mode through the use of stable step function opsin (SSFO; excitatory opsins) (Berndt et al., 2009; Yizhar et al., 2011); by doing so, they were able to robustly terminate absence seizure activity in both models (Sorokin et al., 2017). SSFOs, when activated by a pulse of light, show minimal decay (even over the course of tens of minutes) and when exposed to an alternate wavelength of light rapidly deactivate. Thus, these tools can be used to trigger prolonged depolarization with minimal light delivery. By minimizing the duration of light delivery, issues associated with tissue heating may be minimized.

These studies illustrate two applications of optogenetics to epilepsy that have helped to demonstrate causality of long-held hypotheses. However, perhaps one of the greatest tests of causality yet to be thoroughly examined in epilepsy is the notion that “seizures beget seizures”. As discussed above, both optogenetic and chemogenetic methods enable chronic manipulation of activity - will optogenetic or chemogenetic silencing of *early* seizures after status epilepticus modify disease progression?

Can we capture the engram of a seizure focus?

One of the most exciting applications of optogenetic tools to the study of learning and memory has been the quest for selective control of memory engrams. In a series of papers from the Tonegawa lab, Liu, Ramirez and colleagues pioneered an approach to optogenetically “tag” recently activated neurons, and by modulating the activity of these cells, were able to induce: fear memory recall (Liu et al., 2012); instill a “false memory” (Liu et al., 2014; Ramirez et al., 2013), switch the valence of a memory (Redondo et al., 2014), and re-activate positive memories to alleviate depressive-like behavior (Ramirez et al., 2015). The approach employed by these investigators was a tetracycline-controlled transcriptional activation strategy, with the tetracycline transactivator (tTa) driven by the c-fos immediate early gene promoter and ChR2 driven under the control of the tetracycline responsive element (TRE). In the presence of the antibiotic doxycycline, transcription is suppressed; in the absence of doxycycline, transcription is enabled in *recently activated cells*. By timing the withdrawal of doxycycline to the period immediately surrounding learning, these investigators were able to selectively express ChR2 in recently activated neurons. By resuming doxycycline treatment after learning, further expression of ChR2 was inhibited. This selective expression of ChR2 in recently activated neurons enabled the optogenetic driving of neuronal ensemble activity in subsequent training and test sessions.

This approach is similar in concept (although opposite in sign) to the “Daun02” approach that has been employed recently in drug abuse research (Cruz et al., 2013). Of the 11 papers applying this approach, 9 have been drug-abuse related, and two dyskinesia related. In this approach, Fos-lacZ animals are treated with the pro-drug Daun02; in the presence of beta galactosidase (coded for by the LacZ gene), Daun02 is converted to the cytostatic agent daunorubicin (Farquhar et al., 2002), which in turn suppresses activity of these cells. Daun02 silencing has been used to attenuate cocaine-induced locomotor sensitization in a context specific manner by targeting neurons in the nucleus accumbens (Koya et al., 2009) and to attenuate context-induced reinstatement of drug seeking through targeting of the medial prefrontal cortex (Warren et al., 2016). Some methodological concerns exist, including the degree to which neurons recover from Daun02 administration (or by contrast, are killed), and a lack of control over the duration of inactivation.

Some of these concerns may be avoided using the recently developed “Robust Activity Marking” (RAM) system (Sørensen et al., 2016). The RAM system employs a ~200 basepair synthetic promoter which displays greater activity induced induction than other activity-dependent genes (e.g., Fos), and importantly the minimal promoter size enables the packaging of all components of the system in a single AAV. By packaging both the tet transactivator and the gene of interest (e.g., ChR2, ArchT) in a single vector, the need for

transgenic animals is bypassed. This approach has been validated for both ChR2 and ArchT (thus allowing transient inhibition of cells), and is theoretically compatible with DREADD-based manipulations.

Most recently, a light- and calcium-gated transcription factor system has been developed: Fast Light- And Activity-Regulated Expression [FLARE] (Wang et al., 2017). The FLARE approach requires blue light delivery concurrent with elevated intracellular calcium to untether a membrane bound transcription factor. Upon proteolytic cleavage and release of this transcription factor (a tTa variant), transgene expression under the control of a TRE is enabled. Unlike the above systems, tetracycline administration is not needed to repress transcription, as the transcription factor is basally tethered to the cell membrane. This offers some advantages over the other systems, most notably enhanced spatiotemporal specificity. The blue light delivery has to co-occur with activity within minutes to enable transcription. However, a downside to this approach is the need to inject multiple viral constructs to achieve appropriate targeting (the light and calcium gated tTA variant, a calcium sensitive protease, and the transgene of interest under the control of a TRE).

The relevance of these approaches to epilepsy should be immediately evident given the three decades of immediate early gene mapping studies in epilepsy: seizures are a potent and robust driver of cFos expression (Morgan et al., 1987). However, these approaches will require tight control over the timing of the seizure activity and/or the timing of withdrawal of tetracycline. In models of spontaneous seizures, this is particularly daunting, as there is no experimental control over the timing of initial seizures and it remains impossible to accurately predict when the first spontaneous seizure will occur. However, electrical or chemical kindling allow for precisely timed stimulation, and may thus be a more tractable model for this purpose. The ability to optogenetically or chemogenetically manipulate neuronal ensemble activity during the process of kindling epileptogenesis will allow for the test of causality of an initial seizure focus in subsequent seizure activity. These tools may allow us to determine if modulating the initially active population of cells is sufficient to alter the trajectory of epileptogenesis, or by contrast, if we should reconsider our notion of a seizure focus as a more distributed ensemble of cells.

Conclusions

While decades of circuit mapping in epilepsy have provided us with a wide array of targets for intervention, these next-generation approaches (e.g., optogenetics and chemogenetics) have opened the door to unprecedented resolution in mapping. As summarized in Figure 2, both of these approaches have strengths and weaknesses. For preclinical research, the decision to employ DREADDs or opsins should be tailored to the needs of a specific question. If experiments require a high spatiotemporal resolution, optogenetics may be preferable. If experiments require broader spatial coverage or longer duration activation or silencing, DREADDs may be preferable. Generally, both of these approaches are amenable to activation and silencing, multiplexing, cell-type targeting and pathway specific manipulations.

Each carries with them caveats, underscoring the importance of appropriate control groups. Both approaches may also be deployed using genetic strategies to isolate populations based on multiple features (INTRSECT) and in an activity-dependent manner (tagging active cells). Together, these complementary approaches have the potential to continue to transform our understanding of both general network architecture and region- and cell-type specific roles in seizure propagation. These techniques put a full “seizure connectome” in reach.

Looking forward toward clinical and translational opportunities for these technologies, the degree to which these approaches will be deployed therapeutically is uncertain. Both tool sets offer strengths and weaknesses when scaled to the size of the human brain. The fast kinetics of optogenetics and the ability to avoid pharmacokinetic/pharmacodynamic concerns may be an advantage; conversely, as with preclinical studies, the broader spatial coverage attainable with chemogenetics may also be a strength. Even in the absence of direct translation, an enhanced understanding of these pathways may also open the door to additional electrical and pharmacological therapies that more selectively target critical populations of cells.

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Significance Statement

Seizures propagate through anatomically constrained neuronal pathways. Understanding the pathways of seizure propagation gives us insight to mechanisms of epilepsy and targets for therapeutic intervention. Approaches developed over the last decade have enabled higher resolution mapping of these circuits than was previously attainable. This review highlights optogenetic and chemogenetic approaches in epilepsy, with a focus on new directions and unanswered questions that can now be addressed using these tools.

Figure 1. Cumulative citations for optogenetic and chemogenetic methods in epilepsy over time
Data were retrieved using the Medline (PubMed) trend tool developed by Corlan (Corlan, 2004). The search term for chemogenetic methods was (chemogenetic* OR DREADD OR chemical-genetic) AND (seizure OR epilepsy). For optogenetics, the search term was (optogenetic* OR opsin) AND (seizure OR epilepsy).

Figure 2. Comparative strengths and weaknesses of the optogenetic and chemogenetic approaches in epilepsy research

The strengths and weaknesses discussed throughout this review are summarized in Venn diagram form.