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Control of spatial memory and seizures by hippocampal mossy cells

DISSERTATION

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in Biomedical Sciences

by

Anh Bui

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2017

DEDICATION

To

my family and friends

Charles

for all their support

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Publications

Bui AD, Nguyen T, Limouse C, Kim HK, Felong S, Maroso M, Soltesz I**.** Dentate gyrus mossy cells control spatial memory and spontaneous convulsive seizures. (submitted).

Maroso M, Szabo GG, Kim HK, Alexander A, **Bui AD**, Lee SH, Lutz B, Soltesz I. 2016. Cannabinoid Control of Learning and Memory through HCN Channels. ***Neuron***. 89:1059–73.

Bui AD*, Alexander A*, Soltesz I. 2015. Seizing Control: From Current Treatments to Optogenetic Interventions in Epilepsy. ***Neuroscientist***. doi: 10.1177/1073858415619600. Epub ahead of print.

Bui A, Kim HK, Maroso M, Soltesz I. 2015. Microcircuits in Epilepsy: Heterogeneity and Hub Cells in Network Synchronization. In: ***Epilepsy: Biology of a Spectrum Disorder***, Cold Spring Harb. Perspect. Med., Eds: Noebels, J. and Holmes, G.

Krook-Magnuson E, Armstrong A, **Bui A**, Lew S, Oijala M, Soltesz I. 2015. In vivo optogenetic evaluation of the dentate gate theory in epilepsy. ***Journal of Physiology***. 593:2379–2388.

Llewellyn K, Nalbandian A, Gomez A, Wei D, Walker N, **Bui A**, Kim H, Soltesz I, Kimonis VE. 2015. Administration of CoQ10 analogue ameliorates dysfunction of the mitochondrial respiratory chain in a mouse model of Angelman syndrome. ***Neurobiology of Disease***. 76, 77-86.

Presentations

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Bui A. Optogenetic dissection of seizure networks in temporal lobe epilepsy. EpiCenter Symposium, UC Irvine, CA, March 2015. (Invited talk).

Bui A. Understanding the contribution of mossy cells to seizure propagation in temporal lobe epilepsy using closed-loop optogenetics. 4th Annual MSTP Symposium, UC Irvine, CA, Jan 2015.

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Bui A. Shining light on mossy cells and their role in temporal lobe epilepsy. 3rd Annual MSTP Symposium, UC Irvine, CA, Oct 2013.

Bui A, Krook-Magnuson E, Armstrong C, Soltesz I. Optogenetics in seizure control. 1st Annual MSTP Symposium, UC Irvine, CA, Oct 2011.

Bui A and Reimer R. What role does VGAT play in the vesicular targeting of Glutamic Acid Decarboxylase? 13th Annual MSTP Retreat, UC Irvine, CA, Oct 2009.

Bui A and Reimer R. Developing a FRET-based ATP Biosensor. Stanford University Symposium of Undergraduate Research and Public Service Abstracts. Stanford, CA, 2007.

ABSTRACT OF THE DISSERTATION

Control of spatial memory and seizures by hippocampal mossy cells

By

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Professor Ivan Soltesz

Temporal lobe epilepsy is the most common neurological disorder in adults and one of the most medically refractory. In order to develop new, more effective therapeutical approaches for TLE, we need a more complete understanding of the hippocampal structural and functional alterations underlying seizure activity and comorbid cognitive deficits. We investigated the contribution of two excitatory cell populations in the hippocampal dentate gyrus to ictal activity and spatial memory, the enigmatic hilar mossy cells and the granule cells, utilizing a combination of optogenetic, electrophysiological, and behavioral approaches in awake, behaving animals. Our main findings are: 1) decreased mossy cell activity during spontaneous, electrographic seizures permits further generalization of electrographic seizures into behavioral seizures; 2) mossy cells play a protective and anti-epileptic role in preventing seizure propagation; 3) mossy cells are necessary for encoding of spatial information, and a loss or decrease in mossy cell activity leads to memory impairments; 4) restoration of the dentate gyrus to a hyperpolarized state can robustly control seizure activity in chronic temporal lobe epilepsy, while stimulation of dentate gyrus granule cells leads to convulsive seizures. Our work has important implications for future therapeutical approaches. Our findings suggest that strategies to target the dentate gyrus

microcircuitry, for example, by limiting MC loss, directly exciting surviving MCs, or inhibiting granule cells, may provide powerful treatment options for seizure control.

INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common type of epilepsy in adults and is one of the most medically refractory. 30% to 40% of patients are pharmacoresistant (Kwan and Sander, 2004), and even those patients who have their seizures controlled with currently available anti-epileptic drugs may suffer from debilitating side effects of the medications, including sedation, cardiac arrhythmia, and vision problems. Surgery can be an option for some TLE patients whose seizure focus can be localized, but neurosurgical resection carries significant risks. Furthermore, in addition to the decreased quality of life caused by the seizures themselves, epileptic patients also have a high risk of psychological comorbidities, including depression, anxiety, and learning and memory deficits (Kwon and Park, 2014). In order to develop more effective therapeutical approaches, it is important to gain a more complete understanding of the normal brain circuitry and the pathological changes that occur during epilepsy.

One of the major regions of hippocampus that undergoes complex structural and functional alterations in temporal lobe epilepsy is the hippocampal dentate gyrus. Pathological alterations that occur to the dentate gyrus include: loss of mossy cells, granule cell dispersion, mossy fiber sprouting, and changes in the input and output to the cells (Halabisky et al., 2010; Margerison and Corsellis, 1966; Peng et al., 2013; Sanchez et al., 2012; Zhang and Buckmaster, 2009; Zhang et al., 2012, 2015). How these pathological changes affect hippocampal processing and are associated with hyperexcitability and hypersynchrony in the epileptic network have been major focuses in the field. For example, what is the consequence of mossy cell loss on the network – are mossy cells antiepileptic or proepileptic (Coulter, 2004; Ratzliff et al., 2002; Scharfman, 2016)? What role do surviving mossy cells in chronic epilepsy play in seizure

dynamics and propagation? Due to its quiescent state in the normal brain (Alme et al., 2010; Chawla et al., 2005; Tashiro et al., 2007), it has also been suggested that the dentate gyrus may act as a "gate" to the rest of the hippocampus by preventing overexcitation from spreading to the rest of the network (Heinemann et al., 1992). Therefore, would restoration of the dentate gyrus to a more hyperpolarized state stop seizures?

In addition to its key role in seizures dynamics, the hippocampus is also very important in cognitive functions such as spatial navigation and memory formation (Danielson et al., 2017; GoodSmith et al., 2017; Kheirbek et al., 2013; Leutgeb et al., 2007; Senzai and Buzsáki, 2017; Zhuo et al., 2016). Epileptic patients often present with comorbid cognitive impairments, but it is still unclear how the aberrant microcircuit changes may contribute to these deficits. The primary goal of this work is to more fully understand how the microcircuit changes observed in temporal lobe epilepsy lead to spontaneous seizures and contribute to psychological comorbidities. In the following chapters, we will discuss several approaches to address these questions, including graph theory and computational work to model the circuitry changes. We also discuss how utilization of a combination of optogenetic techniques, real-time seizure detection and intervention, and behavioral tasks can permit the direct probing of how certain microcircuit alterations contribute to cognitive deficits and to the development and maintenance of seizure activity. We will discuss implications of these findings on future treatment or prevention of epilepsy.

In Chapter 1, we discuss the importance of studying seizure dynamics at the microcircuit level to understand the neuronal connections involved in seizure activity. While previous electrophysiological work examining seizures at the macroscale suggested that seizure events are largely repetitive, it is becoming increasingly evident that these bursts of activity may not be so

repetitive after all. At the microscale level, once thought "recurrent" seizure dynamics have been found, in the same animal, to involve heterogeneous populations of neurons during sequential seizure-like events. We discuss work using two-photon imaging, network theory, and computational modeling to study and understand microcircuit changes underlying network hyperexcitability. We also explore the idea of "hub cells" as pathological niduses for seizure activity.

In Chapter 2, we look at the involvement of the hippocampal dentate gyrus in temporal lobe epilepsy, and focus on a unique population of cells called mossy cells. The partial loss of mossy cells in the dentate gyrus is a major hallmark of temporal lobe epilepsy, but the implications of this loss on seizure activity as well as on cognition is an outstanding question in the field. Implementing a novel method to selectively express light-sensitive opsins in mossy cells, we combine optogenetics and a real-time seizure detection and intervention approach to investigate how mossy cells contribute to spontaneous seizures in chronic epilepsy. This work provides the first direct in vivo evidence for the key functional role mossy cells play in controlling the spread of hyperactivity in the epileptic brain. In addition, we examine the importance of mossy cells in learning and memory, and how this might be compromised in epilepsy.

In Chapter 3, we focus on the role of the hippocampal dentate gyrus as a 'gate' to the rest of the hippocampus by protecting hippocampal circuits from overexcitation. Gate because is normally hyperpolarized. It is proposed that in epilepsy, a breakdown of this gate leads to seizure activity, but there had previously been no direct in vivo evidence for the dentate gate hypothesis. Using optogenetics to restore the dentate gate to its normal hyperpolarized state through selective optogenetic inhibition of dentate gyrus granule cells, we were able to control seizures in a mouse

model of temporal lobe epilepsy. On the other hand, we explore how an artificial breakdown of this gate through granule cell photoexcitation can induce acute seizures. Our findings emphasize how the dentate gyrus is a critical node in and suggest that targeting dentate gyrus granule cells can be a good target for seizure intervention.

In Chapter 4, we review the current treatments available for seizure control in epilepsy. We then discuss how optogenetic techniques are opening new avenues for probing and untangling the microcircuits involved in seizures. Much scientific work has laid the foundation for seizure control in various animal models of epilepsy. We explore how optogenetics itself may one day become a treatment for epileptic patients, and discuss the potential benefits and challenges of translating optogenetic techniques into the clinics.

Chapter 5 provides some concluding remarks and future directions regarding novel approaches to probe the microcircuitry underlying seizure activity, as well as a discussion on the challenges faced by scientists and clinicians in potentially harnessing optogenetic techniques as a treatment for epilepsy.

CHAPTER 1

Microcircuits in Epilepsy: Heterogeneity and Hub Cells in Network Synchronization

Abstract

Epilepsy is a complex disorder involving neurological alterations that lead to the pathological development of spontaneous, recurrent seizures. For decades, seizures were thought to be largely repetitive, and had been examined at the macrocircuit level using electrophysiological recordings. However, research mapping the dynamics of large neuronal populations has revealed that seizures are not simply recurrent bursts of hypersynchrony. Instead, it is becoming clear that seizures involve a complex interplay of different neurons and circuits. In this chapter, we will review studies examining microcircuit changes that may underlie network hyperexcitability, discussing observations from network theory, computational modeling, and optogenetics. We will delve into the idea of hub cells as pathological centers for seizure activity, and will explore optogenetics as a novel avenue to target and treat pathological circuits. Finally, we will conclude with a discussion on future directions in the field.

Introduction

Epileptic seizures are large-scale events, where synchronous neural spiking activity occurs throughout a brain region (Penfield and Jasper 1954). Traditionally, scalp and intracranial electroencephalography (EEG) have been used to record electrical activity during seizures, and have played important roles in the evaluation and management of patients with epilepsy (Mansouri et al., 2012; Smith, 2005). However, EEG has notable limitations in terms of

spatiotemporal sensitivity, due to the fact that it is essentially an average measurement of the activity of large populations of cells; it is not able to discern the firing patterns of individual neurons, or even activity at the level of microcircuits, which defines the most fundamental connections between neurons (Buzsáki et al., 2012). Therefore, while we can detect functional abnormalities in the network and pinpoint the general location of these abnormalities, the mechanisms that govern seizure activity are largely unknown (van Mierlo et al., 2014).

To begin to understand seizures as the result of aberrant circuit connections, we need to be able to monitor the activity of population of neurons with sufficient spatial resolution. Recently, more precise recordings of individual cells using techniques such as two-photon microscopy or single-unit recordings have shown that surprisingly, seizures are not as repetitive and homogeneous as originally viewed. Instead, there is complex circuit communication associated with epileptiform activity (Feldt Muldoon et al., 2013; Truccolo et al., 2011). Contributions of specific neuronal populations have been observed to be variable, and certain, well-connected networks appear to play a greater role in the initiation and progression of large-scale network events (Keller et al., 2010; Morgan and Soltesz, 2008; Sabolek et al., 2012). In addition, work from high-level network theory and data-driven computational studies have modeled how altered neural circuit connections can trigger seizure activity (Bullmore and Sporns, 2009; Dyhrfjeld-Johnsen et al., 2007; Morgan and Soltesz, 2008; Santhakumar et al., 2005). These experimental breakthroughs and theoretical and computational models have been important in advancing our knowledge of how seizures initiate and propagate through the brain. Better understanding of these circuits would open new avenues in the development of interventions that can target and treat epileptic networks.

In this chapter, we will review recent findings regarding microcircuit changes that may underlie network hyperexcitability in seizures, bringing in findings from studies using network theory, data-driven computational modeling, and optogenetic technology. In particular, we will discuss the idea of hub cells as pathological niduses for seizure activity. In addition, we will explore applications of optogenetics, as a tool to unravel the large number of microcircuit changes in epilepsy, and also as a potential novel avenue for precise targeting of pathological circuits and noninvasive treatment. Finally, we will conclude with a brief discussion on future directions and goals in the field of epilepsy research on microcircuits.

Heterogeneity in epileptiform activity: examining seizures at the microcircuit level

EEG has been successfully used in epilepsy to observe the hypersynchronous activity that occurs during seizures (van Mierlo et al., 2014). Since this hypersynchronous activity often involves large areas of the brain, it has been a major challenge to observe brainwave patterns at the level of the neurons and their individual connections. Therefore, it has been assumed that hypersynchrony is just that, large populations of neurons firing together in an abnormal manner. With the advent of technologies such as two-photon microscopy and sophisticated calcium dyes, we have begun to learn more about what occurs during seizures at a microscopic scale (Coulter et al., 2011). It has become possible to visualize the activity of hundreds of neurons during large-scale population events in order to gain insight into the brain's dynamics at the microstructure level and the alterations that occur in epilepsy (Rochefort et al., 2008).

In fact, when neuronal activity was mapped in the hippocampal granule cell layer in the mouse, there were surprisingly clusters of synchronous cells in both non-epileptic and epileptic animals, but notably, major structural and functional differences between the clusters from

epileptic versus non-epileptic animals existed (Fig. 1.1) (Feldt Muldoon et al., 2013). In particular, the clusters found in epileptic animals were much more compact – the same number of cells spanned less than half the volume of neuronal clusters from non-epileptic animals, and also fired with high correlation with large-scale synchronous network events (Fig. 1.1B and C) (Feldt Muldoon et al., 2013). In contrast, clusters from non-epileptic animals fired continuously and independently from network events (Feldt Muldoon et al., 2013). It is possible that these spatially compact clusters are densely connected and able to maintain synchronous activity more effectively than more dispersed clusters. Nevertheless, the importance of such observations highlights what can be learned about the altered microcircuitry that may underlie the broad activity normally observed in seizures.

This is not the entire story with regards to cluster recruitment, however. There is also a remarkable degree of variability in the cell populations that are recruited during seizure events, both in experimental models as well as in human patients with epilepsy. Surprisingly, in tissue from epileptic animals, the synchronous neuronal clusters did not always fire during large-scale network events (Fig. 1.1D) (Feldt Muldoon et al., 2013). Instead, the pattern of cluster recruitment was non-uniform, demonstrating large heterogeneity between macroscopically "recurrent" epileptiform events at the scale of microcircuits (Feldt Muldoon et al., 2013). This heterogeneity is not limited to laboratory models, as patients have shown non-uniform recruitment of neuronal populations during initiation and progression of epileptiform events (Keller et al., 2010; Sabolek et al., 2012; Truccolo et al., 2011). Even between sequential seizures, the fraction of active neurons was highly variable, with different populations of neurons participating in sequential events; this appeared independent of neuronal class type, as it was

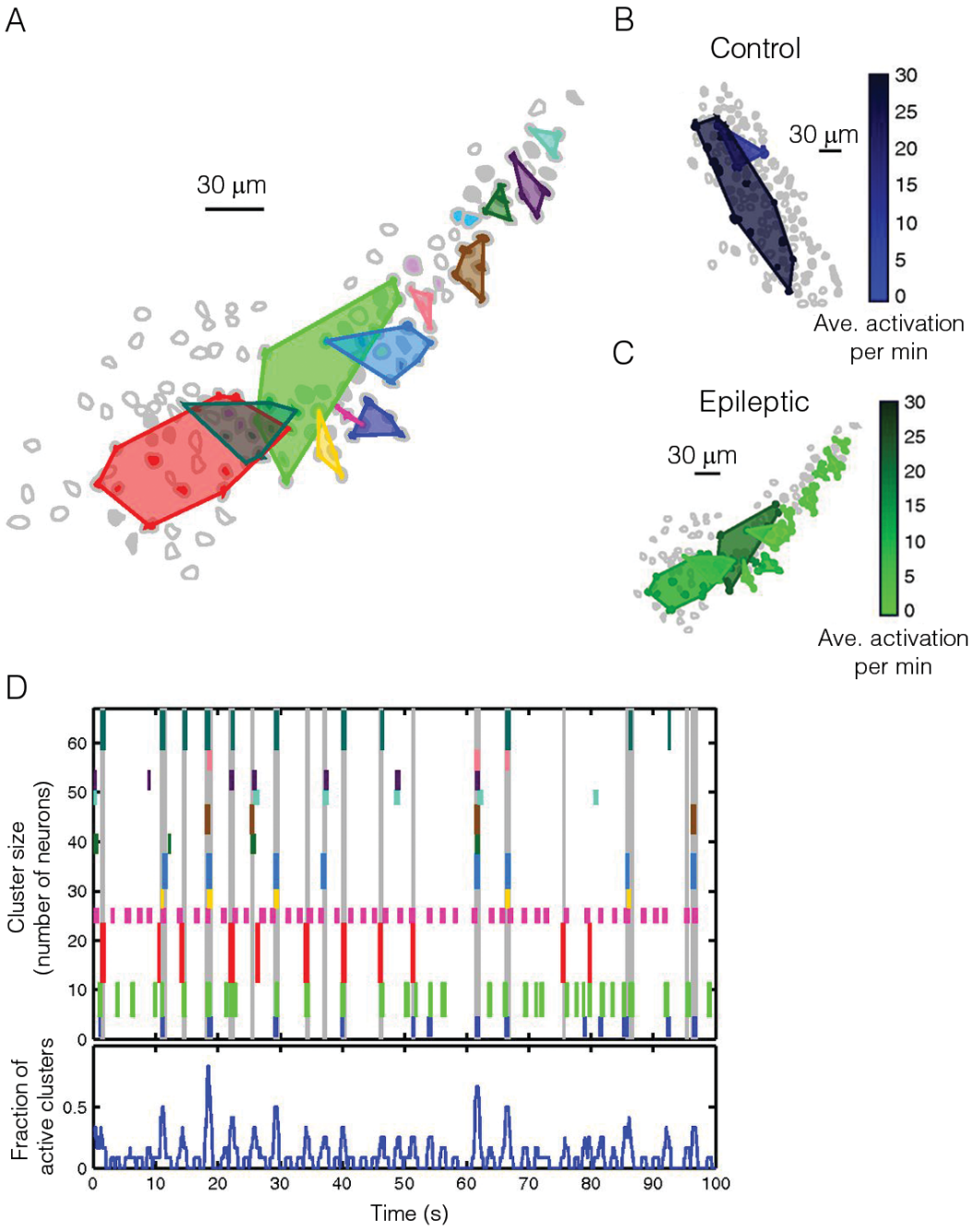

Figure 1.1. Neuronal cluster recruitment during epileptiform events. (A) Spatially localized clusters of neurons in the granule cell layer exhibiting synchronous firing patterns are demarcated by polygons. (B and C) Spatial mapping of neuronal clusters from tissue from a control (B) and an epileptic animal (C). Lighter cluster shades indicate lower frequency of activation. (D, top) Raster plot of the activity of clusters diagrammed in (A), represented by short vertical lines in corresponding colors; the height of the individual colored lines indicates the number of cells composing the cluster. Vertical gray lines extending the entire raster plot designate detected large-scale network events. (D, bottom) Fraction of active clusters over time. Adapted with permission from Feldt et al. 2013.

evident for interneurons and principal neurons (Keller et al., 2010; Truccolo et al., 2011). Additionally, even among neurons that were frequently active during seizures, the spiking patterns of those cells did not repeat between consecutive seizures (Truccolo et al., 2011), and the path of propagation during large-scale bursts of activity changed between episodes (Sabolek et al., 2012). These results highlight the existence of variable paths of epileptiform synchronization dynamics in the recruitment of pathological neuronal clusters.

While we have been able to observe the dynamics of hundreds of individual neurons, demonstrating the vast heterogeneity of large network synchronizations in epilepsy, important challenging questions remain. What cell populations are critical in the recruitment of specific microcircuits involved in the generation of epileptiform events, and how do individual neurons come together to give rise to these events? Furthermore, considering the high dimensionality and complexity of seizure events, how can we rationalize and quantify the data to better model and understand how the underlying structural connectivity of the network is related to large-scale synchronization? Importantly, being able to do so would better enable prediction of how certain disease states are the result of alterations in specific cell populations and circuits. While many of these questions have not been addressed yet by experimental methods, network theory gives us the first glimpses into how such questions may be approached.

Network models describe microcircuit brain connectivity

Network analysis: important parameters and key concepts

An emerging approach that has the potential to offer important insights into our understanding of microcircuit alterations in epilepsy is based on high-level network analysis of neuronal ensembles (Fornito et al. 2013; Sporns 2013). Drawing analogies from other disciplines

such as social sciences, information technologies, or genetics, where large networks of interactions are analyzed using mathematical and statistical tools, it is insightful to abstract and analyze neuronal assemblies as networks consisting of nodes (neurons) connected to each other via links (synaptic connections). At this level of description, the neuroanatomical and electrophysiological properties are essentially set aside and the focus is on understanding how general properties in the connectivity pattern of a network may allow or disallow certain functions, and how modifications of these properties relate to pathological conditions.

Ideas drawn from network theory have been particularly fruitful in describing which organizational principles of microcircuits are important to allow synchrony across distant populations of neurons(Hu et al., 2014)(Hu

et al., 2014)(Hu et al., 2014)(Hu et al., 2014)(Hu et al., 2014). This is especially relevant in the context of epilepsy. While long-range synchrony is a characteristic dynamic feature of healthy cortical assemblies and is important in processes such as gamma brain wave oscillations or the unification of disconnected place fields (Brun et al., 2002; Buzsáki and Draguhn, 2004; Fries, 2009), misregulation of long-range synchrony is thought to contribute to ictal activity (Cymerblit-Sabba and Schiller, 2010; Kramer et al., 2010; Mormann et al., 2003; Netoff and Schiff, 2002).

For large-scale synchronization to be possible, both in normal and pathological states, information needs to flow rapidly, locally and globally. It is important to understand these network characteristics using theoretical and computational methods to determine the most relevant and consequential network alterations in epilepsy and epileptogenesis, and to guide experimental studies. Several mathematical parameters have been used to understand how large-scale synchrony can arise from local and global connections (Telesford et al., 2011). Two fundamental variables necessary to evaluate the degree of connectivity are the clustering coefficient C and the characteristic path length L. The clustering coefficient C estimates the density of connections locally between neighboring neurons. In assessing the compactness of the network as a whole, the characteristic path length L, defined as the average number of monosynaptic connections in the shortest path connecting two cells, is an important metric to consider. Because the travel time of a signal between two spatially remote locations should not be prohibitively long to maintain synchrony across the entirety of the network, the characteristic path length L sets an upper threshold on the maximum number of synaptic relays between two distant neurons. Together, these variables quantitatively assess network structure relevant to

synchrony, and therefore allow objective comparisons to be made between normal and pathological networks (Stam, 2014).

Achieving small-worldness: high clustering and short path length, and highly connected nodes

The small-world phenomenon is commonly used to describe a network that has rich local and global connections – a high clustering coefficient C as well as a small characteristic path length L (Watts and Strogatz, 1998). One method to obtain a small-world network via random connections is through dense connectivity (Wang and Buzsáki, 1996), but an issue that arises is that a prohibitively large number of long-range connections is required for network synchronization. This problem becomes particularly relevant for large networks due to the fact that total wiring length grows linearly with the number of neurons in the network, so connection density comes at the cost of wiring efficiency (Buzsáki et al., 2004).

So how can a network exhibit the small-world property while avoiding extensive use of costly long-range connections? An elegant solution was proposed first by Watts and Strogatz (Watts and Strogatz, 1998). They considered the case of a regular network with exclusively local connections, where each node is connected only to its k closest spatial neighbors. They found that when this network is minimally modified by randomizing a small fraction of the local connections (with probability $p<0.05$, each connection from a given node to one of its neighbors was modified to target a neuron randomly selected across the entire network), the small-world property naturally emerges. It elegantly introduced the idea that a network with mostly local connections and a small number of highly connected "outlier" nodes can be sufficient to endow the network with dynamic properties reminiscent of those observed in cortical assemblies.

Experimental evidence of cortical small-world networks and "hub" cells

A particularly interesting feature of the network theoretical approach is that it does not require detailed neuroanatomical assumptions, and yet provides testable predictions that can guide and complement experimental manipulations. A brain region where the predictions of network theory (Morgan and Soltesz, 2008) have been tested is the developing hippocampus (Bonifazi et al., 2009). The developing hippocampus has been a system of choice because large-scale network synchronizations can be measured as giant depolarizing potentials (Ben-Ari, 2001), and also because the network underlying the generation of these synchronizations is confined locally to the hippocampal CA3 region in slices (Menendez de la Prida and Sanchez-Andres, 2000), permitting a more straightforward experimental strategy. As an additional benefit, the hippocampus in general is highly studied and well characterized for many areas of study, including in epilepsy. Analyzing calcium activity of the dentate gyrus, CA3 and CA1 regions in the developing hippocampus of rats and mice in *in vitro* slices, Bonifazi and colleagues drew a functional connectivity map of several hundreds of neurons. They showed the existence of essentially two populations: a large population of weakly interconnected, neighboring neurons, and a small, special population of cells with high connectivity (Bonifazi et al., 2009).

By performing simultaneous single-cell stimulations and multineuron calcium activity recordings, it was found that while perturbations applied to weakly connected neurons did not affect the large-scale dynamics of the network, stimulation of a small number of the highly connected neurons triggered network-wide synchrony (Bonifazi et al., 2009). Interestingly, when the cells were “fate mapped” via genetic markers to determine the details of their origin, it was found that a subpopulation of these distinctively influential cells were born very early during

embryonic stages and survived into adulthood (Picardo et al. 2011; Marissal et al. 2012). These super-connected cells therefore seem to play a key role in neural development and maturation, and they continue to powerfully modulate large-scale network dynamics in the adult brain (Picardo et al. 2011; Marissal et al. 2012).

These studies strongly suggest that the organization of cortical circuits is indeed governed by the same universal properties as other large-scale networks – it aims primarily to optimize the flow of information and resources used (Bullmore and Sporns, 2009; Buzsáki et al., 2004). Importantly, it also illustrates that a small population of superconnected neurons are endowed with the capability of influencing and even coordinating the dynamics of remote microcircuits. "Hub" is now a prevailing term to designate such neurons, as they share the common characteristics of high anatomical connectivity and the ability to orchestrate synchronicity of a large population of cells (Bonifazi et al. 2009; Picardo et al. 2011; Marissal et al. 2011; Cossart et al. 2014). In the context of epilepsy, hub cells may be thought of as a "microscopic epileptic focus," or a relatively small group of cells which can control and coordinate microcircuits, and recruit larger macrocircuits to lead to generalized seizure activity.

Data-driven computational models to uncover the effects of microcircuit alterations in epilepsy: small-worldness and hub neurons in the epileptic hippocampal dentate gyrus

Descriptive network models provide useful, simplified frameworks to understand the connection between a given topological organization and the dynamics of neuronal assemblies (Sporns, 2014). To also incorporate detailed, biologically relevant data including intrinsic neuronal electrophysiological properties, and to model how alterations in these properties and in the microcircuitry can affect network activity, computational modeling is becoming an increasingly important tool to tease apart the numerous changes that contribute to epilepsy and

epileptogenesis. One brain region of particular interest in the field of epilepsy has been the hippocampal dentate gyrus, which undergoes complex structural and functional transformations in chronic epilepsy and in epileptogenesis (Case and Soltesz, 2011; Santhakumar et al., 2005). "End-folium sclerosis" refers to these alterations that occur to the dentate gyrus in association with temporal lobe epilepsy, and includes two hallmark changes: hilar cell loss and mossy fiber (granule cell axon) sprouting (Sanchez et al., 2012). The link between these microcircuit changes and ictal activity has been highlighted in a series of computational studies seeking to uncover how newly sprouted mossy fiber rewiring and hilar cell loss lead to increased dentate hyperexcitability (Howard and Neu, 2007; Lytton et al., 1998; Tejada and Roque, 2014; Thomas et al., 2010).

Interestingly, results from data-driven, computational simulations indicate that the healthy hippocampal dentate gyrus has characteristics of a well-connected, small-world network (Dyhrfjeld-Johnsen et al., 2007). Additionally, the implementation of progressive levels of end-folium sclerosis (simulated in the models as mossy fiber sprouting and hilar cell loss) had the surprising effect of further increasing small-worldness of the network at sub-maximal levels of sclerosis, therefore leading to increased network hyperexcitability (Dyhrfjeld-Johnsen et al., 2007; Morgan and Soltesz, 2008; Santhakumar et al., 2005). Despite the loss of large proportions of highly connected hilar mossy cells and interneurons, which would be expected to severely reduce network connectivity globally, pathologic mossy fiber sprouting locally was able to compensate (in a network theory sense) for the hilar cell loss. In fact, mossy fiber sprouting was sufficient to spread activity coming from the cortex to the entire network of granule cells (Santhakumar et al., 2005).

However, at higher levels of sclerosis, the robust activity amplifying effects of granule cell axon sprouting could not sufficiently compensate for the loss of long-range projecting, globally connected mossy cells, leading ultimately to the transition of the hippocampal dentate gyrus to a more regular network (Dyhrfjeld-Johnsen et al., 2007). This shift is seen at 80% granule cell axon sprouting and mossy cell loss, when the dentate gyrus model network reaches a maximum level of hyperexcitability. These results not only support the functional role of network topology, but are also consistent with observations from tissue samples from patients with particularly pharmacoresistant forms of temporal lobe epilepsy, where cell counts reveal an average loss of only approximately 80% of hilar mossy cells in the hippocampus, even in the most severe cases of end-folium sclerosis (Blümcke et al., 2000; Gabriel et al., 2004).

Demonstration of the epileptic dentate gyrus as an enhanced small world network also raises the issue of the existence of putative hubs in the dentate network in epilepsy. Due to the potentially central nature of hub neurons in synchronization of the network, changes in topology that affect hubs are likely to have important consequences on the dynamics of the neuronal assembly. However, to what extent are hypersynchronous discharges attributable to aberrant connections involving newly formed or existing hub cells? Functional computational models simulating new, pathological granule cell-to-granule cell connections in several biologically plausible ways showed that while random connections between newly sprouted mossy fibers displayed no significant effect on the network (Fig. 1.2B) (Morgan and Soltesz, 2008), the incorporation of only 5% of richly connected (4-7 times more connected) granule cells was sufficient to substantially increase dentate excitability (Fig. 1.2A and C) (Morgan and Soltesz, 2008). These highly interconnected cells acted as hubs in the network that propagated and amplified activity. While experimental evidence of operational hub cells in epilepsy remains an

open question, this study emphasizes the enormous consequences of the pathological development of only a few highly connected cells in the dentate gyrus network (Morgan and Soltesz, 2008).

Optogenetic targeting of specific circuits in epilepsy

Applications of optogenetics in closed-loop seizure detection and control

Considering the complexity of epilepsy, more complete understanding of the circuits involved would greatly aid the development of newer treatments that have increased specificity, minimal invasiveness, and few side effects, while maintaining the ability to treat a wide range of epilepsies. With the emergence of a powerful technique called optogenetics, it has become possible to better elucidate the microcircuits involved in epilepsy by allowing unprecedented cell-specific, spatial and temporal control of neural activity (Aravanis et al., 2007; Boyden, 2011; Deisseroth, 2011). By targeting expression of ion-conducting opsins, which can either depolarize or hyperpolarize neurons when stimulated by light, previously unattainable goals such as the modulation of the activity of only one cell population in a small area of the brain is now possible (Deisseroth, 2011). In a very short time, optogenetics has led to tremendous progress in understanding the complex neural circuitry and the precise functional consequences of circuit alterations. Furthermore, the repertoire of optogenetic tools is ever expanding to include opsins

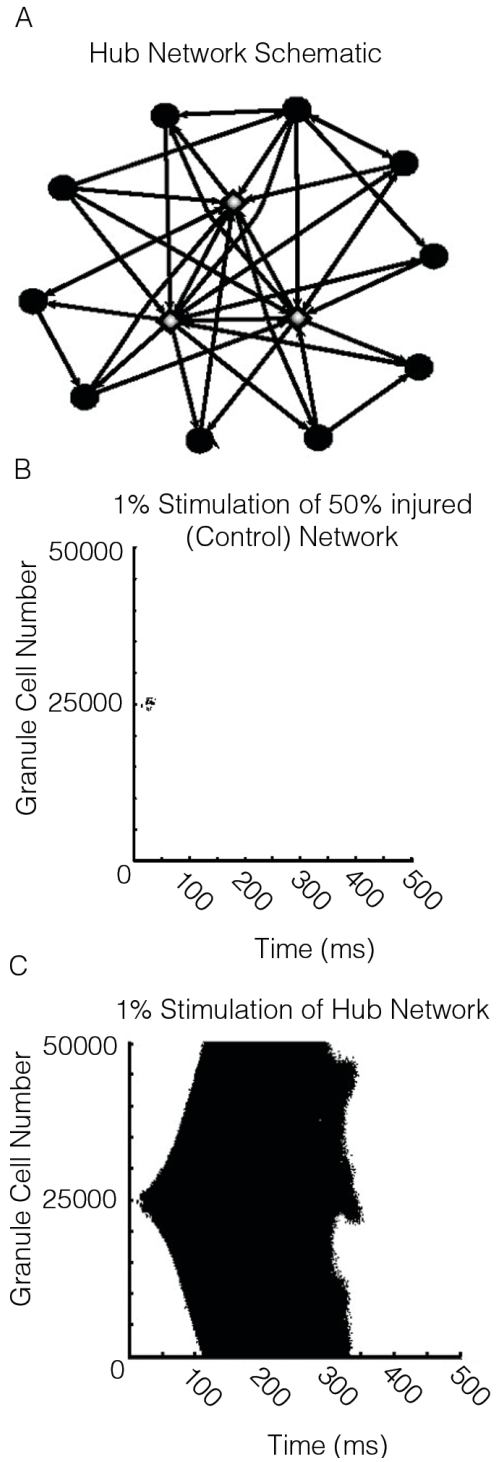

Figure 1.2. Granule cell hubs greatly increase dentate network excitability. (A) Schematic of a hub network. Granule cell hubs (gray diamonds) are four- to seven-fold more highly connected compared with average granule cells (black circles) (B and C) Raster plots of granule cell activity in a moderately injured dentate network (50% hilar cell loss, 50% of maximal mossy fiber sprouting) following perforant path stimulation to 1% of the cell population, where (B) new granule cell-to-granule cell connections are made randomly or (C) 5% of the granule cells serve as hubs. Adapted with permission from Morgan and Soltesz 2008.

with altered ion specificity and increased effectiveness (Berndt et al., 2014; Chuong et al., 2014), and this technology is being used to research possible treatments for a multitude of neurological disorders, including epilepsy, Parkinson's disease, and Alzheimer's disease (Gradinaru et al. 2009).

By using optogenetics to control the activity of specific circuits, important discoveries have been made regarding the circuits involved in epileptiform activity in *in vitro* and *in vivo* seizure or epilepsy models (Tønnesen et al. 2009; Kokaia 2011; Wykes et al. 2012; Krook-Magnuson et al. 2013, 2014; Armstrong et al. 2013; Paz et al. 2013; Sukhotinsky et al. 2013; Rossignol et al. 2013; Ledri et al. 2014; Berglind et al. 2014). Soon after early experiments demonstrating optogenetic control of seizure-like events in hippocampal culture preparations (Tønnesen et al., 2009), several *in vivo* studies showed that optogenetics can be used to manipulate specific neuronal circuits within rodent models of epilepsy to control seizures (Wykes et al. 2012; Krook-Magnuson et al. 2013, 2014; Armstrong et al. 2013; Paz et al. 2013). Their success in stopping or delaying seizure progression by using optogenetics to inhibit a small percentage of principal cells indicated the potential for optogenetics as a promising therapeutic in epilepsy.

A key aspect of using optogenetics in seizure suppression is the temporal control which allows the manipulation of neural activity only during seizure events. This requires use of closed-loop seizure detection systems, where seizures are recorded in real-time and light is administered in response to seizures. Closed-loop seizure detection systems have been used to respond to seizures in real-time with electrical stimulation, but such techniques cannot specifically target microcircuits and instead affect many cell types as well as neighboring areas of the brain (Osorio et al. 2005; Nelson et al. 2011; Liang et al. 2011; Berényi et al. 2012). With

optogenetics, however, recent studies revealed that closed-loop optogenetic intervention can significantly stop seizures in rodent models of epilepsy by targeting appropriate local or even long-range connections to the seizure focus (Paz et al. 2013; Krook-Magnuson et al. 2013, 2014). In particular, the ability to target long-range connections permits a powerful way to intervene, especially in cases where the seizure focus is in an inaccessible area of the brain. In studies of temporal lobe epilepsy, targeting hippocampal neurons contralateral to the seizure focus was as effective at suppressing seizures as intervening at the focus itself (Krook-Magnuson et al., 2013). Even more surprising, optogenetic modulation of a structure as physically distant as the cerebellum could also control seizures originating in the hippocampus (Krook-Magnuson et al., 2014), showing that perhaps there are certain "gates" or "chokepoints" that can be effective in optogenetic suppression of seizures. This phenomenon is not limited to seizures in temporal lobe epilepsy, as studies showed that seizures arising from cortical stroke could be controlled by optogenetically targeting long-range thalamic connections to the cortex (Paz et al., 2013).

Until this point, most of the work applying optogenetics to rodent models of epilepsy relied heavily on using the inhibitory chloride pump halorhodopsin to suppress the activity of principal cells (Fig. 1.3A-E) (Krook-Magnuson et al., 2013). Would targeting of GABAergic cells, a much smaller population, be equally effective at controlling seizures? Interestingly, when the excitatory cation channel channelrhodopsin was expressed only in parvalbumin-expressing interneurons and light stimulus was applied within a small area of the hippocampus, this was effective in stopping a significant number of spontaneous seizures – both electrographic and behavioral (Fig. 1.3F-I) (Krook-Magnuson et al., 2013). These findings have important implications, especially because parvalbumin-expressing neurons make up a very small percentage of the hippocampal neuronal population, so optogenetic stimulation affected less than

5% of cells exposed to light and yet was still able to effectively prevent behavioral seizures (Krook-Magnuson et al., 2013).

Future challenges and directions of optogenetic application to epilepsy

These studies show that closed-loop optogenetic intervention of seizures is a promising technique that may have applications for therapeutics in the future. As an extension of these studies, if optogenetic intervention can be achieved by targeting hub cells, which are theoretically an even smaller population of neurons that can centrally control brain activity, the "footprint" caused by altering the normal composition of neurons will be very small. For example, mossy cells in the hilus of the hippocampus are known to have extensive connections to other hippocampal neurons (1 mossy cell projects onto >30,000 granule cells) (Dyhrfjeld-Johnsen et al., 2007), and may represent an important target for seizure suppression, where modulation of just a few hub cells would be sufficient to control seizures, while minimally interfering with normal brain functions.

While the current achievements in treating seizure propagation using careful optogenetic control of neural microcircuits are remarkable, at this point, a true translation of optogenetics to epilepsy treatment will require much ground work in controlled optogenetic expression and less invasive light delivery systems. As shown in many rodent model studies, viral vectors are currently the most effective method of introducing opsins into the brain (Bentley et al., 2013; Ji and Neugebauer, 2012), but current use of viral vectors in patients is limited, although promising advances in gene therapy continue to be made in the treatment of neurological conditions such as Parkinson's disease (Bartus et al., 2014). While current methods of light delivery to activate opsins in rodent models involve directing optical fibers into the brain, new

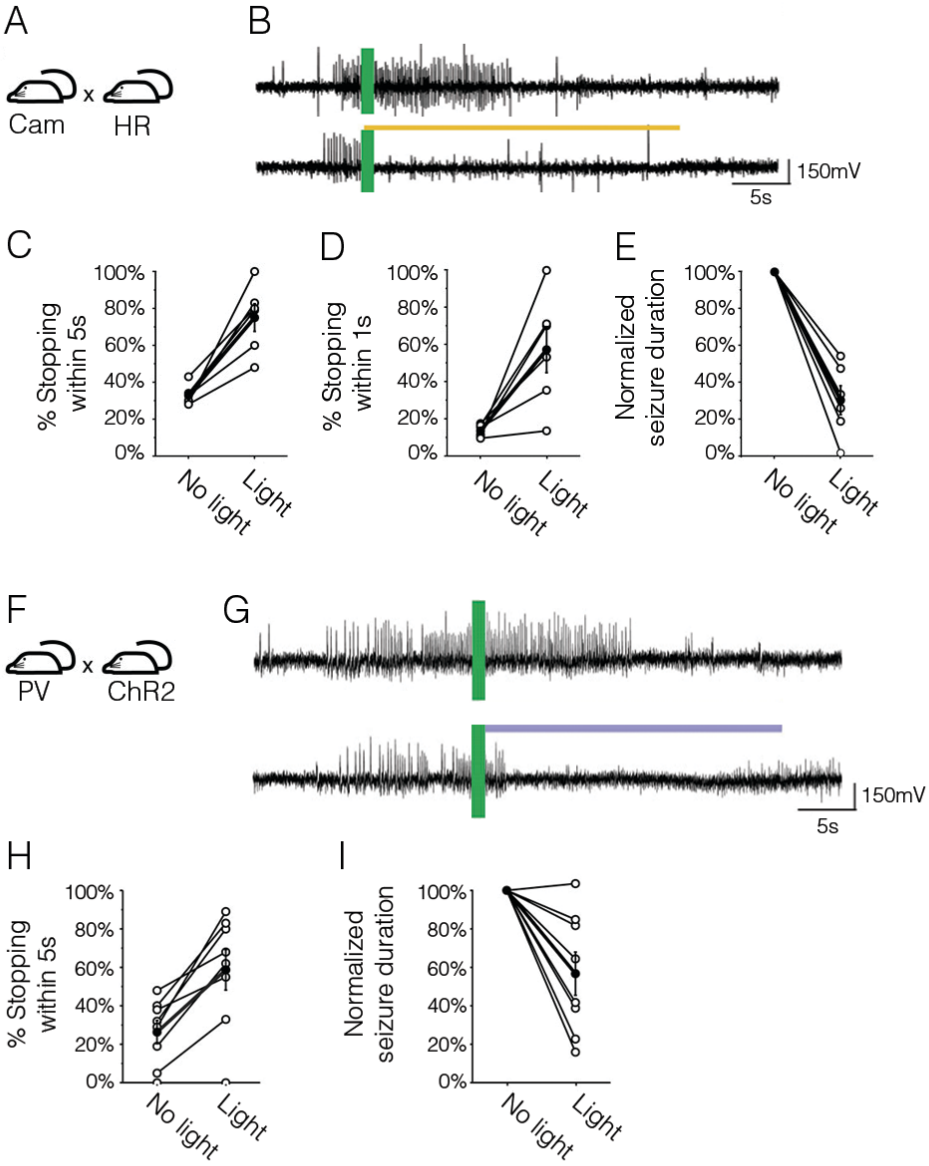

Figure 1.3. Optogenetic suppression of neural activity in the hippocampus controls seizure activity. (A) To produce animals expressing halorhodopsin (HR) within excitatory neurons, CamKII-Cre animals were crossed with those carrying Cre-dependent HR. (B) Example electrographic seizures detected (vertical green line) in Cam-HR mice, triggering delivery of no light or amber light (589nm) to the hippocampus, as indicated by the amber line. (C-D) Data from Cam-HR mice showing the percentage of seizures that stop (C) within 5 seconds of light stimulus or (D) within 1 second of light stimulus. (E) The normalized post-detection seizure duration. (F) To produce animals expressing channelrhodopsin (ChR2) within inhibitory neurons, animals expressing parvalbumin (PV)-Cre were crossed with those expressing Cre-dependent ChR2. (G) Example electrographic seizures detected in PV-ChR2 mice, triggering the delivery of no light or blue light (473nm), as indicated by the blue bar. (H-I) Data from PV-ChR2 mice showing (H) the percentage of seizures that stop within 5 seconds of light stimulus and (I) the normalized post-detection seizure duration. Scale bars represent 100mV, 5s. Error bars represent s.e.m. Adapted with permission from Krook-Magnuson et al. 2013.

opsins have been developed that can be transcranially stimulated in rodents, eliminating the need for intracranial optical fiber implants (Chuong et al., 2014). As both the field of optogenetics and the study of potential hub cells in epilepsy move forward, there is certainly a possibility that there will someday be safe and controlled methods to express opsins in specific neuronal circuit components in patients, along with safe and long-lasting methods to deliver light to these cells.

Concluding Remarks

Many advances have been made in dissecting the complex microcircuit changes in epilepsy with the use of powerful techniques that allow visualization, recording, and manipulation of specific, large populations of neurons. However, more progress needs to be made to fully understand what causes the abnormal circuit connections in epilepsy. For example, hub cells in epilepsy are still yet to be identified *in vivo* and it is still not fully understood how neural circuits are recruited during seizures.

To answer these questions, innovative methods are constantly being developed and continue to add to our growing knowledge of this serious and prevalent disorder. Super-resolution microscopy is a major advancement that allows visualization of single ion channels and receptors (Dani et al. 2010; Testa et al. 2012; Dudok et al. 2014), and has great potential to improve our understanding of how changes in the number, activity, and localization of these proteins deregulate local network activity and contribute to seizures. The development of miniaturized microscopes that can be implanted to observe cell dynamics during freely moving behavior (Ghosh et al., 2011; Wilt et al., 2009; Ziv et al., 2013) has provided opportunities to study microcircuit alterations in chronically epileptic animals. New optogenetic tools are also expanding at an astounding pace – opsins that are more sensitive or have different channel

dynamics are allowing for even finer manipulation of network activity (Chuong et al., 2014; Dhakal et al., 2014; Hochbaum et al., 2014; McIsaac et al., 2014). As experimental methods progress, computational tools for modeling neural networks likewise continue to become more complex and powerful. There have been important advances in large-scale modeling of the hippocampal networks (Case et al. 2012; Bezaire and Soltesz 2013; Schneider et al. 2012, 2014), which can give insights into cellular and circuit alterations that may lead to synchronous activity and seizures, improving and guiding experimental designs. These efforts to elucidate the microcircuitry underlying the generation and recurrence of epileptic activity will continue to open avenues in the development of increasingly specific and effective treatments for this complex neurological disorder.

Acknowledgments

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CHAPTER 2

Dentate gyrus mossy cells control spatial memory and spontaneous convulsive seizures

Abstract

Temporal lobe epilepsy (TLE) is characterized by debilitating, recurring seizures and an increased risk for cognitive deficits. Mossy cells (MCs) are a key neuronal population in the hippocampal excitatory circuit, and the partial loss of these cells is a major hallmark of TLE. However, it remains unclear whether MC loss directly contributes to seizure dynamics and cognitive deficits comorbid with epilepsy. We investigated how MCs contribute to spontaneous ictal activity and to spatial contextual memory in a mouse model of TLE with hippocampal sclerosis, utilizing a combination of optogenetic, electrophysiological, and behavioral approaches. We found that in chronically epileptic mice, real-time optogenetic modulation of MCs during the electrographic stage of spontaneous hippocampal seizures powerfully controlled the progression of activity from an electrographic to convulsive seizure. We showed that decreased MC activity is sufficient to impede encoding of spatial context, recapitulating observed cognitive deficits in chronically epileptic mice. Our results provide direct *in vivo* evidence for an important role of MCs in controlling the generalization of hyperactivity following the initiation of a spontaneous seizure and in encoding spatial memory.

Introduction

Temporal lobe epilepsy (TLE) is a serious disorder characterized by spontaneous seizures and an increased risk for cognitive impairments (e.g. learning and memory) and is the most

common form of epilepsy in adults. Antiepileptic drugs are ineffective in one-third of patients (Kwan and Sander, 2004), indicating the need for a more complete understanding of the mechanisms underlying seizure activity and comorbid cognitive deficits. Mossy cells (MCs) are a glutamatergic cell population in the hilus of the dentate gyrus (DG) in the hippocampal formation, whose partial loss is a major hallmark of TLE, both in human patients and in animal models (Blümcke et al., 2000; Houser, 1999). However, the implications of MC loss in TLE in both seizure dynamics and in cognition remain poorly understood (Scharfman, 2016). MCs are relatively few in number but have extensive connections diverging throughout the longitudinal axis of the DG (Buckmaster et al., 1996). This widespread connectivity has been proposed to enable separation of information in the DG for more efficient memory storage (Hyde and Strowbridge, 2012), but there lacks *in vivo* evidence that MCs directly participate in information encoding and how this process might be disrupted in epilepsy. Additionally, MCs drive both network excitation through their direct granule cell (GC) connections and network inhibition via their synapses onto GABAergic cells, but it remains unclear which of these projections dominate, particularly during seizure activity (Ratzliff et al., 2004; Sloviter et al., 2003). To investigate the functional role of MCs in seizure dynamics and cognition, we used a combination of electrophysiology recordings, closed-loop optogenetics, and behavioral tests. We modulated MC activity in real-time during spontaneous seizures to understand how MCs are causally involved in seizure generalization in behaving animals, and we examined the role of MCs in spatial and non-spatial learning and memory tasks.

Results

To modulate MC activity during seizures, we expressed either archaerhodopsin (ArchT), a light-sensitive inhibitory opsin, or the excitatory channelrhodopsin (ChR2) selectively in hippocampal MCs in mice. For ArchT expression, we topologically targeted MCs through their commissural projections. Trans-synaptic and retrograde trafficking of a wheat germ agglutinin (WGA)-Cre fusion protein (Gradinaru et al., 2010) by MCs with synapses in the left DG activates ArchT expression in the right DG MCs (Fig. 2.1A-C). In order to apply a second, alternative approach for MC excitation, we injected a virus for ChR2 expression into the hilus of Crlr-Cre mice (Fig. 2.1E, F) (Jinde et al., 2012). For both MC targeting strategies, opsin expression was highly specific for MCs, as confirmed by GluR2/3 costaining (Fig. 2.1H) (Ratzliff et al., 2004). Light delivery (15 s of 589 nm light) effectively suppressed current induced action potentials in ArchT-expressing MCs, and light delivery (473 nm, delivered as a 20 Hz train of 10 ms pulses for 15 s) induced firing in ChR2-expressing MCs (Fig. 2.1D, G).

We first tested how MC activity affects spontaneous electrographic, non-convulsive seizures in a model of chronic TLE with kainic acid (KA) injected unilaterally into the left hippocampus (Bragin et al., 1999). This well-established model of TLE produces robust, recurrent, spontaneous seizures (Bragin et al., 1999), reproduces key features of human unilateral hippocampal sclerosis (e.g., partial loss of MCs (Volz et al., 2011)), and shows pharmacoresistance to currently available antiepileptic drugs (Klein et al., 2015). We used a closed-loop spontaneous seizure detection and intervention method (Armstrong et al., 2013; Krook-Magnuson et al., 2014) and delivered light (15 s of 589 nm light) at the onset of seizures to inhibit ArchT-expressing MCs in chronically epileptic mice (Fig. 2.2A). Importantly, light was delivered for 50% of detected seizures in a random manner, such that light and no light epochs could be directly compared, and each animal served as its own internal control (Fig.

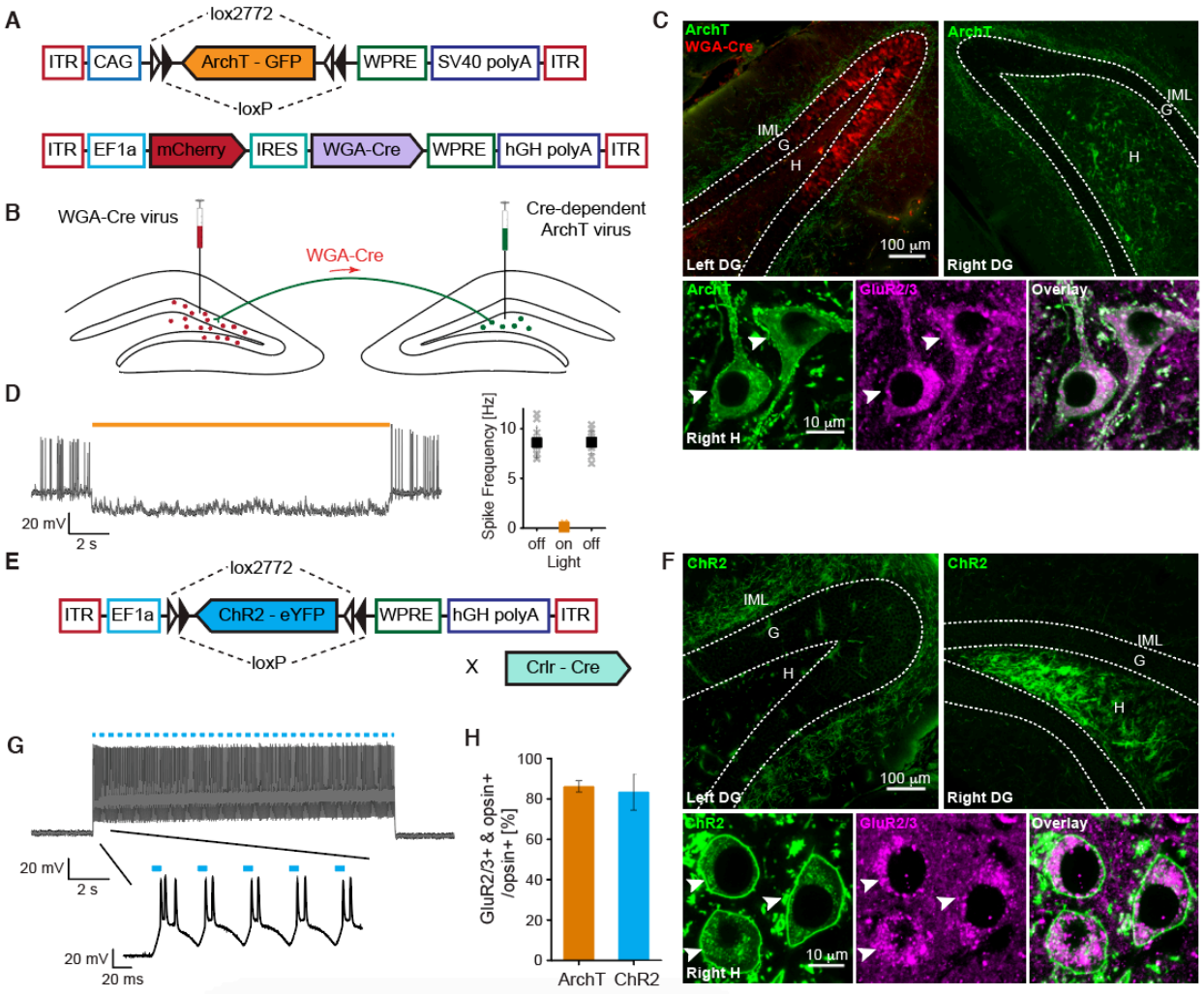

Figure 2.1. Selective optogenetic control of dentate gyrus mossy cells in the hippocampal formation.

(A) Viral vectors for Cre-dependent ArchT expression. **(B)** Topological targeting of mossy cells (MCs) with WGA-Cre. WGA-Cre fusion protein expressed in the left dentate gyrus (DG; red) is trans-synaptically and retrogradely trafficked by neurons with projections at the injection site. WGA-Cre activates ArchT expression in the right DG MCs that project to the left DG (green). **(C)** Top: confocal images of WGA-Cre and ArchT expression in the left and right DG. Bottom: high magnification images of the right hilus. ArchT-expressing MCs are identified via GFP expression and GluR2/3+ immunostaining (arrowheads). **(D)** 15 s illumination blocks current induced spiking in ArchT-expressing MCs in acute slices from an epileptic animal, quantified on the right (N = 10 recordings, n = 3 animals). **(E)** Cre-dependent ChR2 expression system. **(F)** Top: confocal images of ChR2 expression in the left and right DG. Bottom: high magnification images of the right hilus. ChR2-expressing MCs are identified via eYFP expression and GluR2/3+ immunostaining (arrowheads). **(G)** Current clamp trace showing light-induced MC spiking in acute slices from an epileptic animal during a 20 Hz train of 10 ms pulses (N = 5 recordings, n = 3 mice). **(H)** Opsin expression specificity, quantified by the proportion of ArchT+ neurons that were also GluR2/3+ (N = 103 slices, n = 3 mice) and of ChR2+ neurons

that were also GluR2/3+ (N = 24 slices, n = 5 mice). All data are presented as mean ± s.e.m. G, granule cell layer; H, hilus; IML, inner molecular layer.

2.2B). We delivered light to both DG hemispheres to target the MC somata and their axonal projections (Fig. S2.1), confirming beforehand that illumination of ArchT-expressing MC axon terminals reduces the amplitude of synaptically evoked field excitatory postsynaptic potentials (Fig. S2.2). We found that MC inhibition in response to amber light had no effect on the electrographic seizure dynamics (Fig. 2.2C). Similarly, MC excitation in ChR2-expressing mice with blue light (473 nm, delivered as a 20 Hz train of 10 ms pulses for 15 s) and light delivery in opsin-negative control mice did not alter the electrographic seizures (Fig. 2.2D, E and Fig. S2.3). These results are in contrast with the effect of inhibiting GCs, the only other excitatory cell population in the DG, where we previously showed that on-demand GC inhibition dramatically truncated electrographic seizures (Krook-Magnuson et al., 2015) (Fig. S2.4A). Therefore, unlike GCs, which can curtail electrographic seizures, MCs do not affect electrographic seizure duration.

A characteristic feature of MCs is their long, extensive projections, connecting multiple lamellae in both hippocampi (Buckmaster et al., 1996). We reasoned that this property could endow MCs with the capability to influence seizure generalization. A prediction of this model is that MC perturbation during non-generalized, electrographic only seizures may be sufficient to alter the seizure progression into more severe, widespread seizures exhibiting behavioral manifestations (e.g., rearing or tonic-clonic activity, Racine scale 4-5 (Racine, 1972)). To investigate whether MCs control seizure generalization, we focused on the seizures that began as electrographic then evolved into convulsive seizures (Fig. 2.3A, B) and quantified the proportion of such seizures that occurred following optogenetic MC inhibition. Because these seizures are much rarer than the electrographic only seizures (Bragin et al., 1999), animals were continuously recorded for ~1-6 months to accumulate a number of electrographic-to-convulsive seizures

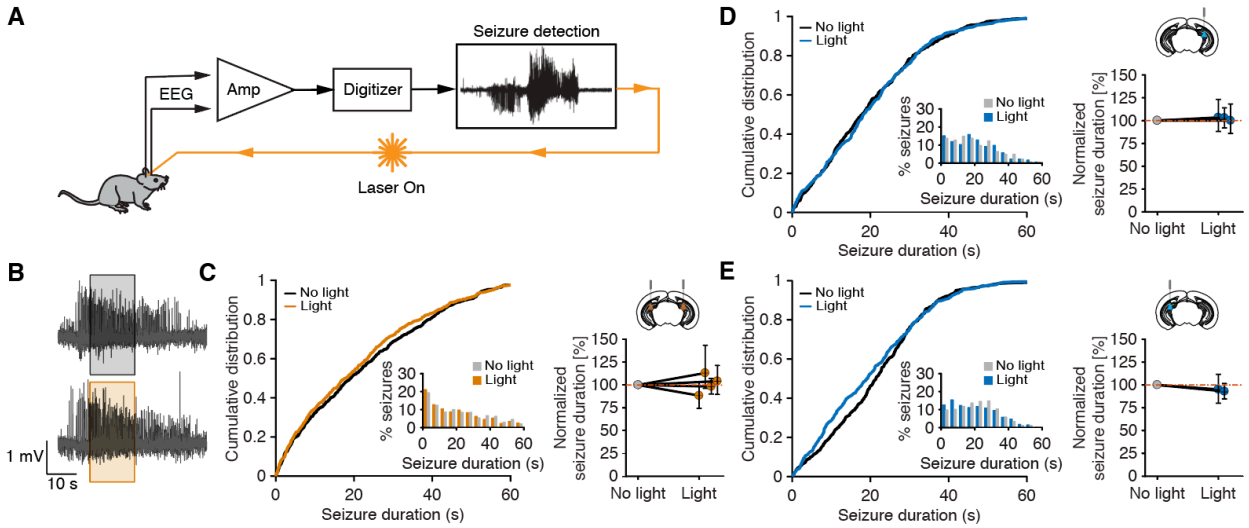

Figure 2.2. Modulation of MC activity does not alter electrographic seizure dynamics. **(A)** Closed-loop approach for i*n vivo* real-time detection and optogenetic intervention of spontaneous seizures in chronically epileptic mice. **(B)** Example electrographic seizures, where no light (gray box) or light (orange box) is delivered upon seizure detection. **(C-E)** Light delivery to mice expressing ArchT contralaterally **(C)**, ChR2 contralaterally **(D)** or ChR2 ipsilaterally **(E)** to the kainate injection site does not affect electrographic seizure duration. Left: cumulative distribution and probability density (inset) of the seizure duration following start of light or no-light delivery (all animals combined, N = 1194/903/714 seizures, n = 4/3/2 animals for ArchT/ChR2 contralateral/ChR2 ipsilateral; $P > 0.05$ Kolmogorov-Smirnov). Right: seizure duration. Data are shown as the normalized difference in seizure duration between the light and no light events and 95% CI for each individual animal ($P > 0.05$ Mann-Whitney *U* test, see Methods).

sufficient for statistical analysis (see Methods). Interestingly, we found that MC inhibition during the initial electrographic stage significantly increased the probability of seizures to generalize into overt behavioral seizures (Fig. 2.3C, D). The duration of the behavioral seizures was not significantly different between no light and light events (Fig. 2.3E), suggesting that MC inhibition does not alter ictal activity once it has reached the behavioral stage but affects solely the transition between electrographic and convulsive seizures. In agreement with this result, inhibiting MCs after the end of the behavioral seizures also did not affect the seizure frequency (Fig. S2.5). Additionally, light delivery in opsin-negative controls had no effect on the occurrence of behavioral seizures (Fig. 2.3C, D). With regard to excitatory cell populations in the DG, our experiments showed that the effect on generalized seizures was particular to MCs, as inhibition of GCs did not affect behavioral seizure occurrence (Fig. S2.4B, C). Together, these results show that decreased MC activity during spontaneous, electrographic seizures permitted further generalization of the seizures into more severe, convulsive seizures.

To test whether convulsive seizures could be prevented by increasing the activity of surviving MCs before seizure generalization, we next stimulated ChR2 expressing MCs at the onset of electrographic seizures. Since DG microcircuit alterations are different in the hemispheres ipsilateral and contralateral to the site of KA injection (e.g., degree of severity (Bragin et al., 1999; Volz et al., 2011)), we tested separately for the role of MCs contralateral and ipsilateral to the KA injection site by expressing ChR2 and delivering light either only in the right or left hemisphere, respectively. Exciting contralateral MCs prevented the electrographic seizures from generalizing into overt behavioral seizures (Fig. 2.3C, D). These results are in sharp contrast to the effect of optogenetically exciting GCs in epileptic mice, which robustly induced behavioral seizures (Krook-Magnuson et al., 2015) (Fig. S2.4B, C). Similarly, exciting

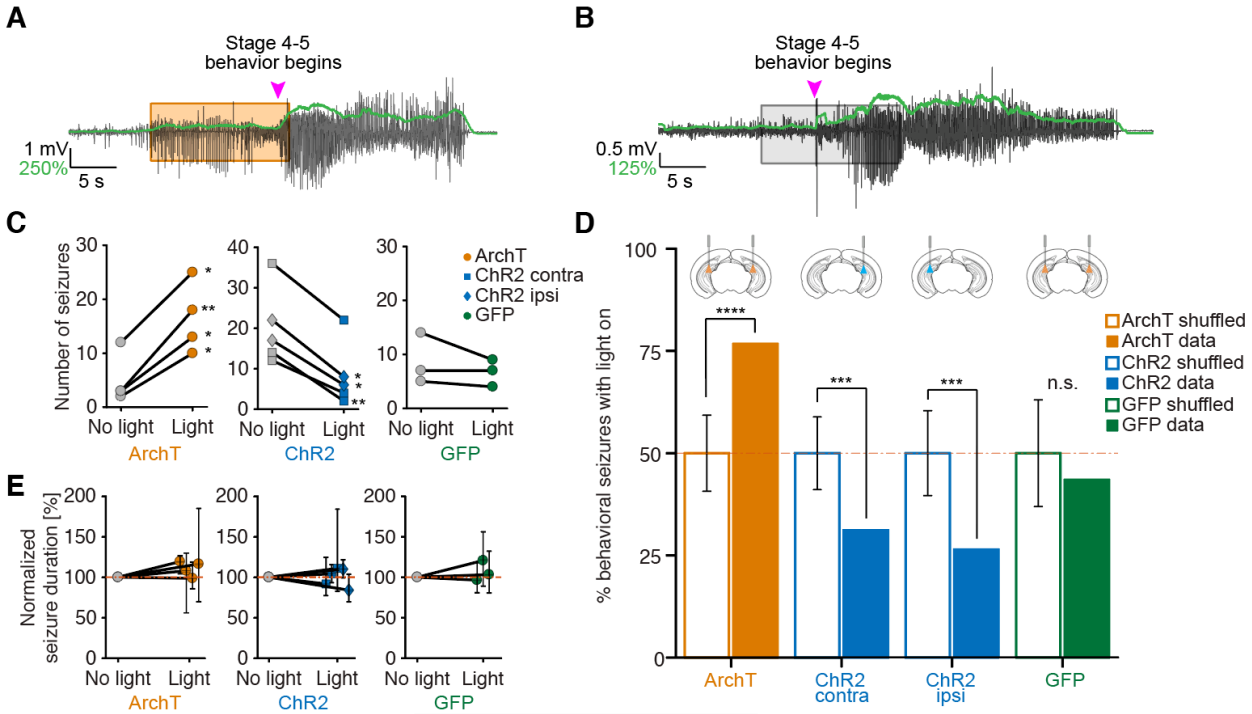

Figure 2.3. Optogenetic modulation of MCs during spontaneous seizures controls the progression of electrographic seizures into convulsive seizures.

(A, B) Representative EEG recordings of electrographic-to-convulsive seizures (black traces) and their corresponding line-length (green traces) (Sorokin et al., 2017) in **(A)** an ArchT- and **(B)** a ChR2-expressing epileptic mouse. Boxes indicate the delivery of either light (orange box) or no light (gray box) for 15 s following seizure detection. Magenta arrow indicates beginning of Racine scale 4-5 convulsive seizure. Line-length scale bars are normalized with median line-length value over the 240 s preceding the seizure. **(C)** Occurrence of convulsive seizures. Left: for each measured animal (n = 4), photoinhibition of MCs significantly increases the occurrence of convulsive seizures. Middle: photostimulation of MCs reduces the frequency of convulsive seizures (n = 5 animals). Right: light delivery to opsin-negative control mice does not affect convulsive seizure occurrence (n = 3 animals). $*P < 0.05$, $**P < 0.01$, two-tailed binomial test. **(D)** Control of seizure progression by MC modulation. Solid colored bars represent the fraction of convulsive seizures occurring after light delivery. White bars represent the expected fraction and 95% CI of seizure occurring with light, under the null hypothesis that light delivery has no effect. n.s. $P > 0.05$, $***P < 0.001$, $****P < 0.0001$, two-tailed binomial test. **(E)** Modulation of MC activity does not affect convulsive seizure duration. Data are shown as normalized difference in seizure duration between the light and no light events and 95% CI for each individual animal ($P > 0.05$ Mann-Whitney U test, see Methods). Contra, contralateral to kainate injection site; ipsi, ipsilateral to kainate injection site.

MCs on the KA injection side, where hippocampal sclerosis is more severe and where we observed a 77% MC loss (compared with 55% MC loss contralaterally, which is consistent with previous studies (Volz et al., 2011); Fig. S6) also strongly prevented the generalization of seizures (Fig. 2.3C, D). Together, our data demonstrate that despite the dramatic loss of MCs, surviving MCs play an important functional role in chronic TLE in preventing runaway excitation culminating into convulsive seizures.

Cognitive deficits are devastating co-morbidities of epilepsy, and epileptic patients present with a high risk of learning and memory disabilities (Kleen et al., 2012). However, the microcircuit alterations that lead to cognitive deficits are not well understood, and it remains unclear whether MC loss contributes to these impairments. To characterize the consequences of MC loss on cognition, we examined learning and memory processes in chronically epileptic mice and in non-epileptic mice where we optogenetically inhibited MC activity. We used two well-validated assays of spatial and object memory: the object location memory (OLM) and object recognition memory (ORM) tasks (Vogel-Ciernia et al., 2013). These two tasks rely on the innate attraction of mice for novelty, and those mice which remember the training layout will prefer the object that has been displaced or that is novel during the testing phase 24 hours later.

We found that chronically epileptic mice had significant deficits in OLM compared with non-epileptic control mice and were unable to distinguish the moved from the unmoved object, as reflected in the low discrimination index (DI, see Methods) (Fig. 2.4A). In contrast, ORM was not impaired in epileptic mice, and their DI in the ORM task was similar to that of non-epileptic control mice (Fig. 2.4B). The OLM deficits are consistent with previous studies showing that repeated generalized seizure episodes lead to decreased accuracy in spatial discrimination performance (Lin et al., 2009). The poor performance of epileptic mice was not due to

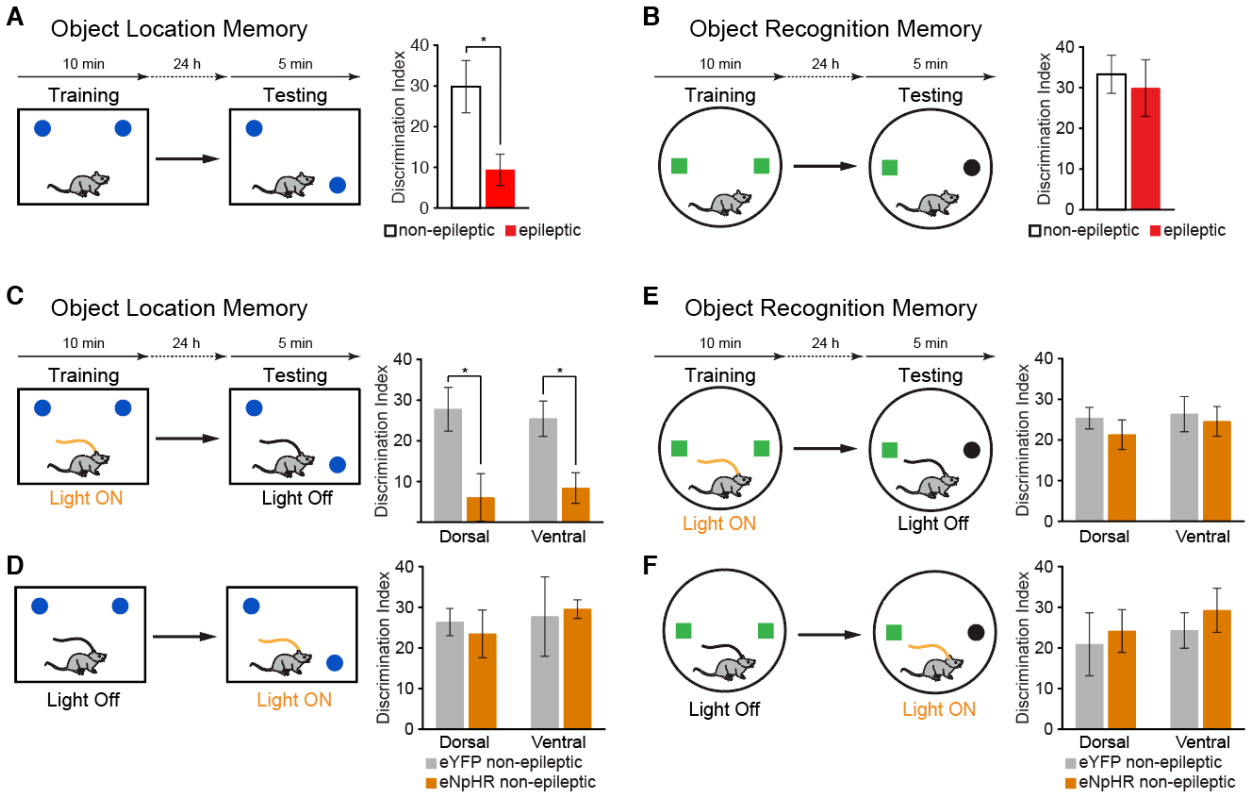

Figure 2.4. Chronically epileptic mice have impaired OLM but not ORM, and MC inhibition impairs learning, but not retrieval, of OLM.
(A) Object Location Memory (OLM) test schematic and timeline. Epileptic mice (n = 17) exhibit significantly impaired OLM, compared with control non-epileptic littermates (n = 13). **(B)** Object Recognition Memory (ORM) test schematic and timeline. Non-epileptic (n = 10) and epileptic mice (n = 14) show no significant difference in ORM. **(C)** Acute MC photoinhibition during learning interferes with OLM (dorsal: n = 9 eYFP, n = 10 eNpHR; ventral: n = 8 eYFP, n = 5 eNpHR). **(D)** MC photoinhibition during testing does not interfere with OLM (dorsal: n = 9 eYFP, n = 8 eNpHR; ventral: n = 6 eYFP, n = 7 eNpHR). **(E)** MC photoinhibition during learning of ORM does not impair task performance (dorsal: n = 10 eYFP, n = 10 eNpHR; ventral: n = 9 eYFP, n = 10 eNpHR). **(F)** MC photoinhibition during testing of ORM does not affect task performance (dorsal: n = 10 eYFP, n = 10 eNpHR; ventral: n = 7 eYFP, n = 9 eNpHR). All data are presented as mean ± s.e.m. **P* < 0.05 two-tailed Welch's *t*-test.

disinterest, as both non-epileptic and epileptic groups explored the objects at similar levels overall (Fig. S2.7), nor to increased anxiety, as assessed using the elevated plus maze test (EPM; Fig. S2.8).

To directly test whether MC loss alone is sufficient to impair spatial cognitive abilities, we turned to non-epileptic mice and mimicked MC loss in TLE by optogenetically silencing MCs during specific stages of OLM and ORM. This approach using non-epileptic animals allowed us to examine the effect of decreased MC activity on cognition independently of other network reorganizations observed in epilepsy. As inhibition with ArchT has been shown to have excitatory effects when activated for long periods (Mahn et al., 2016), we switched to halorhodopsin (eNpHR) for longer inhibition of MCs. We verified appropriate opsin response to light and expression specificity (Fig. S2.9).

When we delivered light (for patterns of stimulation, see Methods) to either the dorsal or the ventral hippocampal regions solely during the learning phase of the OLM task (Fig. S2.10), we observed a significant reduction in the performance in eNpHR mice compared with eYFP controls (Fig. 2.4C). We ruled out the possibility that this learning impairment was related to an effect of MC silencing on anxiety by assessing the exploration of the mice in the EPM (Fig. S2.8). In addition, the poor performance was not due to a lack of motivation for object exploration (Fig. S2.11). In contrast, MC activity does not appear to be necessary during retrieval of spatial memory. We found that silencing of either dorsal or ventral MCs during the testing phase of the OLM task did not impair performance, as both eNpHR and eYFP mice were able to recognize the displaced object (Fig. 2.4D). Therefore, reduced MC activity during exposure to a new spatial context precludes proper formation of the associated memory, but decreased MC firing during OLM testing does not hinder performance. Finally, we also assessed whether MCs

are involved in non-spatial ORM. Optogenetic MC inactivation during learning or testing of ORM did not interfere with ORM task performance (Fig. 2.4E, F).

Together, these results show that dorsal and ventral MCs are necessary for the encoding of the spatial context, and a loss or decrease in MC activity leads to spatial memory impairments. Furthermore, inhibiting MCs focally in healthy mice during learning reduces their OLM task performance to a level similar to that observed in epileptic mice (Fig. 2.4A, C). MC loss by itself, even partial, is therefore sufficient to degrade cognitive abilities independently of other network changes that might occur in epilepsy, such as mossy fiber sprouting or GC dispersion (Houser, 1990).

Discussion

Here, we have shown that decreased MC activity allows for the generalization of electrographic seizures into convulsive seizures and that optogenetic excitation of surviving MCs prevents the occurrence of convulsive seizures in TLE. We also found that selective MC inhibition precludes the encoding of spatial but not of object memory, recapitulating cognitive deficits observed in chronically epileptic mice. These findings indicate that MCs are key for blocking runaway hippocampal hyperexcitation and for spatial memory processes and that the MC loss often observed in TLE patients (Blümcke et al., 2000) is likely to be directly implicated in both seizures and comorbid cognitive deficits.

Earlier studies have led to conflicting models for the role of MCs on hippocampal network dynamics. Widespread chronic MC deletion led to transient disinhibition of granule cells (Jinde et al., 2012), and stimulation of commissural fibers including MC axons inhibited GCs (Buzsàki and Eidelberg, 1981; Hsu et al., 2015), indicating that MCs may exert a primarily inhibitory effect on the network. Conversely, others reported a decrease in DG activity in slices

after partial MC ablation (Ratzliff et al., 2004) or an increase in DG afterdischarges, induced by perforant path stimulation, with MC optogenetic excitation (Sugaya et al., 2016), suggesting that MCs may exert a net excitatory effect. Rather than addressing the net effect of MCs on GC and dentate activity, our study focused on elucidating how surviving MCs control the dynamics and progression of spontaneously occurring seizures in TLE. This is a critical question from a clinical perspective that had never been addressed. By leveraging closed-loop optogenetics, we established unambiguously that surviving MCs play a protective role in preventing spontaneous seizure progression.

Our learning and memory results are consistent with the emerging model that MCs have an important functional role in spatial memory. MCs have been recently shown to have multiple place fields and to exhibit stronger remapping of their place fields than GCs in response to changes in environmental clues (Danielson et al., 2017; GoodSmith et al., 2017; Senzai and Buzsáki, 2017). By controlling MC firing during memory tests, we demonstrated that MCs are critical in encoding, but not in retrieval, of spatially relevant information. As GCs have been implicated in learning contextually relevant information (Kheirbek et al., 2013; Zhuo et al., 2016), it is possible that MCs may control information encoding by regulating GC excitability in separate lamellae through their widespread, divergent projections (Hyde and Strowbridge, 2012; Jinde et al., 2012; Myers and Scharfman, 2009). Future work recording MC activity during seizures, or the activity of GCs and other hippocampal cells while simultaneously manipulating MCs, will be key to unravel how MCs control microcircuit dynamics in the DG during seizures and memory encoding. Our work suggests that strategies to limit MC loss or to directly excite surviving MCs may provide powerful treatment options for seizure control.

Materials and Methods

Animals

For mossy cell targeting, both C57BL/6J mice (Stock No: 000664, Jackson Laboratories) and mice expressing Cre in mossy cells [Crlr-Cre; C57BL/6N-Tg(Calcrl,cre)4688Nkza/J; Stock No: 023014, Jackson Laboratories] (Jinde et al., 2012) were used. Mice expressing opsins specifically in dentate gyrus granule cells were generated by crossing mice expressing Cre in GCs [B6.FVB-Tg(Pomc-cre)1Stl/J; stock 010714; Jackson Laboratories] with either floxed-STOP HR mice [Ai39; B6;129S-Gt(ROSA)26Sortm39(CAGHOP/EYFP)Hze/J; stock 014539; Jackson Laboratories] or floxed-STOP ChR mice (Ai32; Rosa-CAG-LSLChR2H134R-EYFP-deltaNeo generated by Hongkui Zeng; stock 012569; Jackson Laboratories). All mice used were more than 6 weeks old and were backcrossed by at least five generations to C57BL/6J. Male and female mice were used. Animals were housed under a 12:12 h light/dark cycle with food and water available ad libitum. For behavioral studies, animals were age matched and underwent the same surgical procedures. All behavioral testing was performed during the light portion of the cycle. All procedures were carried out in accordance with the National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine and by the Administrative Panel on Laboratory Animal Care of Stanford University.

Stereotactic injection

The mice were deeply anesthetized with isofluorane and placed into a stereotactic apparatus. For ArchT expression, C57BL/6J mice were injected with a virus encoding a Cre-recombinase wheat germ agglutinin (WGA) fusion protein (2.00 mm posterior, 1.60 mm left, 2.65 mm ventral to

bregma), and a virus encoding a Cre-dependent ArchT (2.00 mm posterior, 1.60 mm right, 2.65 mm ventral to bregma). The virus was delivered via a 2 μl syringe and a 25 gauge metal needle (Hamilton); the injection volume and flow rate were 1 μl at 0.1 μl/min. The needle was left in place after injection for 10 min before slowly being withdrawn. For ChR2 and eNpHR3.0 expression, Crlr-Cre mice were injected with the Cre-dependent opsin (2.00mm posterior, 1.60mm left and/or right, 2.65mm ventral to bregma). The virus was delivered via a 10 μl syringe and a 33 gauge metal needle (World Precision Instruments); the injection volume and flow rate were 0.2 μl at 0.1 μl/min. The needle was left in place after injection for 10 min before slowly being withdrawn. Non-opsin controls were injected similarly. Viruses were purchased from the University of North Carolina Vector Core. Mice used for behavioral tasks were also implanted with optical fibers (multimode 200 μm diameter, 0.37 NA; Thorlabs) either into the dorsal hippocampus (1.70 mm posterior, 1.35 mm left/right, 1.80 mm ventral to bregma) or the ventral hippocampus (3.30 mm posterior, 2.80 mm left/right, 3.70 mm ventral to bregma). Mice used for seizure dynamics studies were implanted as described in "Epilepsy induction and monitoring." Screws (McMaster-Carr) and dental cement (Teets Cold Curing) were used to fix the implant to the skull.

Epilepsy induction and monitoring

The procedures for epilepsy induction and monitoring are similar to those previously described (Armstrong et al., 2013; Krook-Magnuson et al., 2014). For this model, kainic acid (KA; 40-100 nL, 20 mM in saline, Tocris Bioscience) was stereotaxically injected into the left dorsal hippocampus (2.0 mm posterior, 1.25 mm left, and 1.6 mm ventral to bregma) under isoflurane anesthesia at least 3 weeks after virus injection. After two weeks, animals developed

electrographic and behavioral (Racine stage 4-5), spontaneous, recurrent seizures (Bragin et al., 1999). The mice were then stereotaxically implanted with bipolar, twisted wire, depth electrodes (1.70 mm posterior, 1.90 mm left, and 1.90 mm ventral with respect to bregma; PlasticsOne) and optical fibers (3.30 mm posterior, 2.80 mm left and/or right, and 3.70 mm ventral to bregma; 0.37 NA, 200 μm diameter multimode, Thorlabs). The location of the depth electrodes, was chosen based on previous studies showing that spontaneous seizures in the KA epilepsy model typically arise from the hippocampal formation ipsilateral to the KA injection site (Bragin et al., 1999). It is important to note, however, that the goal of our study was not to determine the spatial seizure profile or to pinpoint the site of seizure initiation. Data for the GC-HR and GC-ChR2 mice were obtained from recordings performed for a previous study (six animals (Krook-Magnuson et al., 2015)). These animals had optical fibers implanted bilaterally (2.60 mm posterior, 1.75 mm left/right, 1.40 mm ventral with respect to bregma). After recovery from the implant procedure, mice underwent 24 h video and EEG monitoring for closed-loop seizure detection and light delivery.

Closed-loop seizure detection and light delivery

Closed-loop seizure detection and light delivery were carried out as previously described (Armstrong et al., 2013; Krook-Magnuson et al., 2014). Briefly, the hippocampal EEG signal was analyzed in real-time by a PC running a custom MatLab (MathWorks) seizure detection algorithm, and seizures were detected by the software using identifiable spike features. When a seizure was detected, the software randomly decided to deliver light ($p = 0.5$) or no light ($p = 0.5$), resulting in a 50% chance of light delivery for each seizure. Light delivery consisted of a 15 s bout of 10 ms light pulses at 20 Hz for ChR2 excitation or of continuous illumination for

ArchT excitation. In both cases, light power was ~3.5-5 mW at the tip of the fiber from a fiber-coupled diode laser (Shanghai Laser & Optics Century Co.) of the appropriate wavelength to activate the expressed opsin (473 nm for ChR2, 589 nm for ArchT).

Extraction of electrographic seizure duration, and transition from electrographic to convulsive seizure

The EEG signals surrounding each trigger from the closed-loop system were analyzed offline by individuals trained to analyze EEG signals but blinded to the light condition and type of opsin expression. Post-detection electrographic seizure duration (Fig. 2, figs S3 and S4) was calculated as total duration of the electrographic seizure minus the detection dwell time (typically 1-3 s) between the beginning of the seizure and the light or no light delivery. The beginning and end of convulsive seizures were annotated based on the EEG signal and confirmed by analysis of the video recordings.

Slice electrophysiology

Whole-cell patch-clamp recordings were done at 33°C from 300 μm transverse slices from adult (>6 weeks old) mice using artificial cerebrospinal fluid containing (in mM): 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose. The patch pipette intracellular solution contained (in mM): 90 K- gluconate, 27.4 KCl, 1.8 NaCl, 1.7 $MgCl_2$, 0.05 EGTA, 10 Hepes, 2 Mg-ATP, 0.4 Na_2-GTP, 10 phosphocreatine, 8 biocytin; pH 7.2; 270–290 mOsm; pipette resistance: 3–5 MOhms. Recordings were done using a Multiclamp 700B (Molecular Devices), Digidata 1410A (Molecular Devices), a 3 kHz low pass filter and a sampling rate of 10 kHz. Light was delivered through the epifluorescence port of an Eclipse FN-1 (Nikon, Tokyo,

Japan), using a Lambda DG-4 with smart shutter and Lambda SC controller (Sutter Instruments, Novato, CA, USA) and TTL input from a Digidata 1440A (Molecular Devices). For the mossy cell terminal inactivation experiments (fig. S2), electrophysiology recordings were done as above, but in the presence of 10 μM SR 95531 hydrobromide (gabazine). Electrical stimulation of mossy cell axons in the inner molecular layer to elicit field excitatory postsynaptic potentials (fEPSPs) was performed using a platinum-iridium alloy microelectrode (FHC, cat# 30201). Stimuli consisted of 5 Hz pulses for a total of 45 s, divided into three 15 s epochs, where light was delivered continuously for 15 s during the second epoch. The recorded traces were analyzed using Clampfit 10.2 (Molecular Devices). The stimulus intensity was set such that the fEPSP amplitudes were approximately 50% of the maximum.

Object location memory and object recognition memory paradigms

Prior to training, mice were handled 2 min/day for 5 days, then habituated in the experimental apparatus 5 min/day for 6 days in the absence of objects. During training, the mice were placed in the presence of two identical objects (e.g. 100 ml beakers) for 10 min and allowed to freely explore. During testing (24 hours after training), the mice were placed again in the presence of two objects. For OLM, the objects were the same, but one object was in the same location as during training, while the other object was moved to a novel location. For ORM, one object was replaced with a novel object, but the location remained the same as during training. The determination of which object was moved or replaced was randomized and balanced. Light stimulation was delivered continuously either during the training or testing phase (bilaterally into the hippocampus, 589 nm, 5-7 mW at the tip of each fiber). Training and testing trials were video-recorded and analyzed by a researcher blind to the injection scheme. Interaction with the

object was counted as exploration when the nose of the mouse was pointing directly at and within 1 cm away from the object or when the nose was touching the object. The relative exploration times were expressed as a discrimination index (D.I. = $(t_{novel} - t_{familiar}) / (t_{novel} + t_{familiar}) \times 100$). Mice that explored the objects for less than 3 s total during either training or testing were removed from further analysis. Mice that exhibited an object preference during training (D.I. > 20) were also excluded; these criteria were pre-established (Vogel-Ciernia et al., 2013).

Elevated plus maze

The elevated plus maze consisted of two open arms (30 x 5 cm) and two enclosed arms (30 x 5 x 20 cm) which extended from a central platform (5 x 5 cm) to form a plus. The apparatus was elevated to 30 cm above the floor. Mice were placed individually in the center of the maze facing the open arm opposite to the researcher. The percentage of time spent in the open arms was scored using AnyMaze (Noldus), and movement was tracked relative to the center of the mouse body. Between subjects, the maze was cleaned with 70% ethanol. Testing with epileptic/non-epileptic mice was 5 min total. Testing with eNpHR-/eYFP-expressing mice was 15 min total, and was divided into three 5 min epochs. The mice received no light during the first epoch, light stimulation during the second epoch, and no light during the third epoch. During the light on epoch, mice received 5 min of continuous illumination bilaterally into the hippocampus (589 nm, 5-7 mW at the tip of the fiber).

Opsin expression validation, immunohistochemistry and histology

To determine specificity of opsin expression in MCs, animals were deeply anesthetized and perfused transcardially with 4% paraformaldehyde. 60 μm coronal brain sections were stained for GluR2/3 (rabbit anti-GluR2/3, 1:200, AB1506, Chemicon). Selectivity of opsin expression was quantified by comparing GFP or eYFP cells with GluR2/3-positive cells, and degree of mossy cell loss was quantified by examining GluR2/3 immunoreactivity and using the 49,6-diamidino-2-phenylindole (DAPI) stain to identify cells. Measurements were done using a confocal microscope and the optical dissector method, using every slice for the ArchT-expressing mice (Fig. 1H), and every fourth section throughout the entire anterior–posterior extent of the hippocampus for all other measurements.

Statistical analysis

Electrographic seizure durations: Differences in the distribution of post-trigger seizure duration between the light and no light triggers (Fig. 2, figs S3 and S4) were analyzed at the group level (animals pooled by type of opsin) using the Kolmogorov-Smirnov test, which is standard for comparing two empirical distributions. Outcomes of light vs. no light conditions on post-detection seizure durations were also compared within each individual animal. Since the distribution of seizure duration is visibly not Gaussian (Fig. 2C-E insets), the outcomes were compared using a two-sample Mann-Whitney *U* test, which does not require normality assumptions. This tests the null hypothesis that a randomly selected seizure from the light group is equally likely to be shorter as it is to be longer than a randomly selected seizure in the no light group, which we phrased in the text as "light has no effect on seizure duration." The effect of light on seizure duration was further quantified using a normalized Hodges Lehman (HL) estimator of the difference in median between the two conditions, which is a standard way to

analyze samples compared using a Mann-Whitney test. The normalized HL estimator was calculated as the median of the difference in seizure duration across all possible pairs of seizures from the light and no light conditions, divided by the median of the mean duration of all possible pairs of seizures from the no light group, and is approximately a relative change in median seizure duration. Data in figures 2 and 3, and supplementary figures 3 and 4 report the normalized HL estimator, along with the 95% CI computed by bootstrap (n = 10,000 bootstraps). For each animal, a random set of ~300 electrographic seizures was included in the analysis. This number allows us to detect a 3 s change in seizure duration with a 20% false negative rate (power estimated for a two-tailed t-test at 0.05 level with a standard deviation in seizure duration of ~13 s).

Convulsive seizure durations: Group level and animal level statistical analysis of the convulsive seizure duration was done in a similar manner as for the electrographic seizure analyses.

Occurrence of convulsive seizures: To compare the number of convulsive seizures occurring following either light or no light delivery in epileptic MC-ChR2, MC-ArchT, MC-GFP animals (Fig. 3), we used a binomial test with a success probability of $p = 0.5$. This tests the null hypothesis that behavioral seizures are equally likely to occur following light or no light delivery, which would be expected if light had no effect on seizure progression since each trigger independently delivered light with a 50% probability. The binomial test was also used for group data (animals pooled by type of opsin, Fig. 3D), and the test results were presented as the measured fraction of behavioral seizures occurring following light delivery, side-by-side with the expected fraction and its 95% CI under the null hypothesis (binomial distribution, $p = 0.5$).

Occurrence of convulsive seizures in opsin-expressing GC animals was analyzed in a similar manner (fig. S4). Only appropriately timed triggers for behavioral seizures (i.e., triggers during the electrographic only portion of the seizure, before the emergence of overt convulsions) were included in the analysis. Effect of post-seizure triggers (fig. S5) was quantified similarly, except that the triggers used were the first triggers occurring after the end of each convulsive seizure. Starting from the beginning of the closed-loop intervention, each animal was monitored during its entire lifespan or until the EEG signal became corrupted (e.g., due to excessive motion during seizures), amounting to up to 6 months of continuous recording per animal. For each group, at least 2-3 animals were recorded to obtain >37 seizures total, which gives a 0.2 false negative rate for the binomial test (power beta = 0.8) to detect a 20% change in seizure frequency between light and no light at the 0.05 level. Non-significance at the 0.8 power level was reported as n.s. in the text.

OLM, ORM, EPM tests: Data from OLM, ORM, and EPM were analyzed using the unpaired Welch's t-test to compare the performance of non-epileptic vs. epileptic, or eYFP vs. eNpHR mice. This test does not require equal variance between groups but assumes normality, which was verified using the Kolmogorov-Smirnov test ($p < 0.05$).

Statistical analysis was conducted using MatlabR2016 (MathWorks).

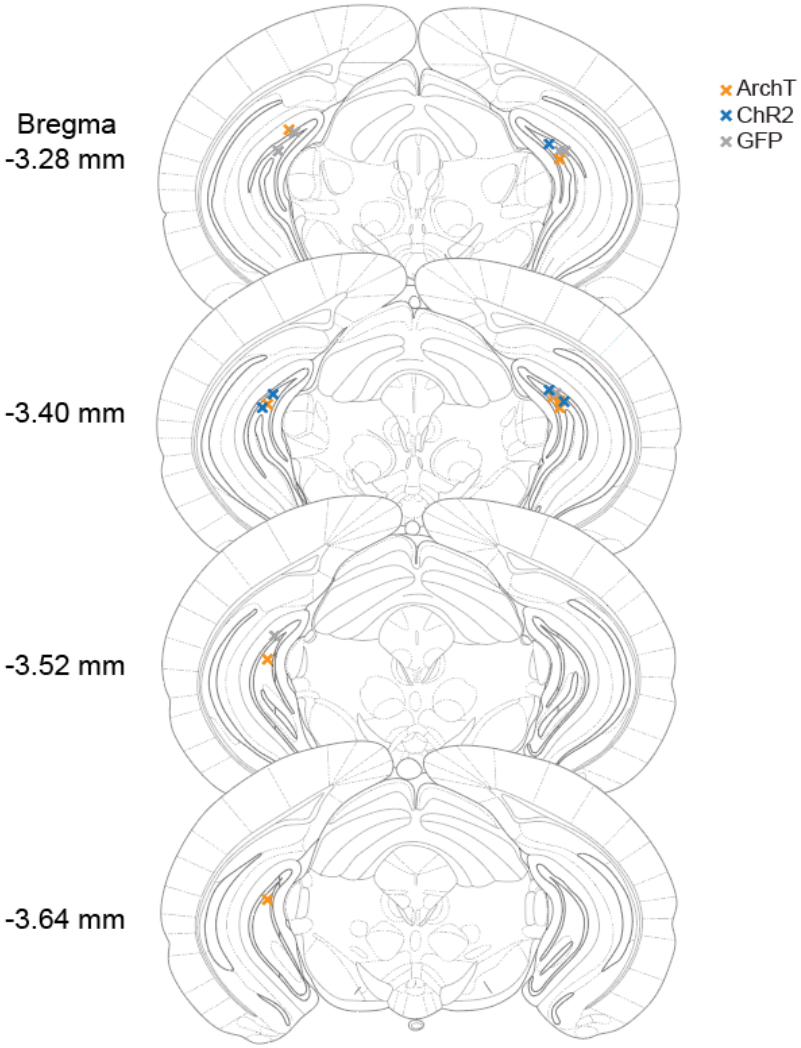

Fig. S2.1. Post-hoc verification of optical fiber locations for chronically epileptic mice with optogenetic seizure intervention. (n = 4 ArchT mice, n = 5 ChR2 mice, n = 3 GFP).

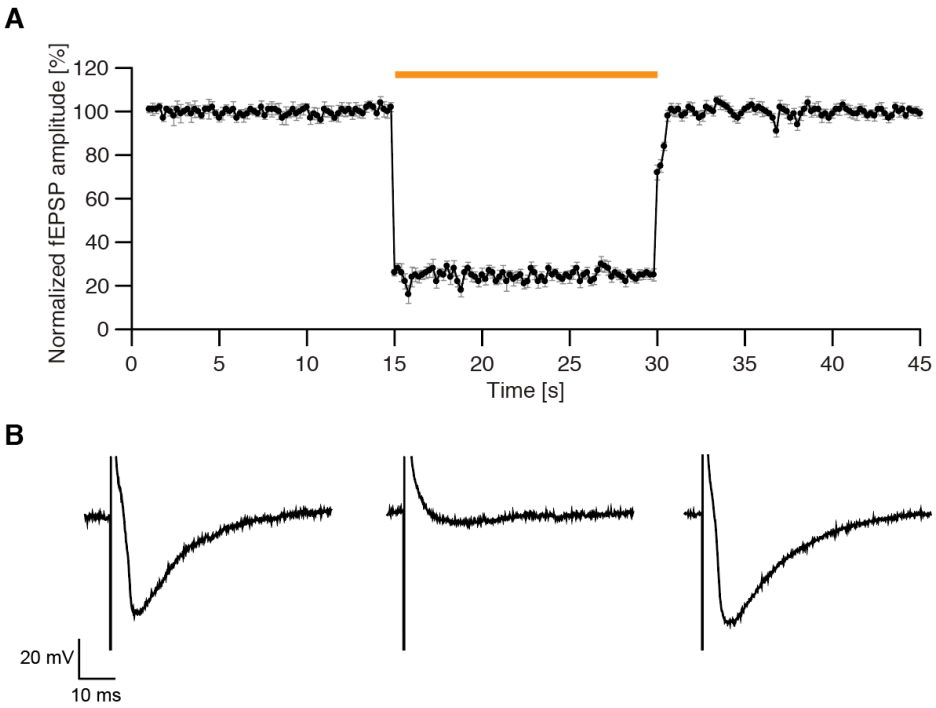

Fig. S2.2. Illumination of ArchT-expressing MC axon terminals reduces the amplitude of synaptically evoked field excitatory postsynaptic potentials (fEPSPs). (A) fEPSPs normalized to the fEPSP peak amplitude during 5 Hz electrical stimulation of MC axons in the left DG inner molecular layer in acute slices from epileptic mice. Light (590 nm; orange bar) is delivered only during the second epoch of three 15 s epochs to activate ArchT-expressing MC axons, decreasing the fEPSP amplitude. Normalized fEPSP amplitude is expressed as mean ± s.e.m. (N = 12 slices, n = 3 mice). **(B)** Representative traces of synaptically evoked fEPSP recordings from the GC layer before (left), during (middle), and after (right) light delivery.

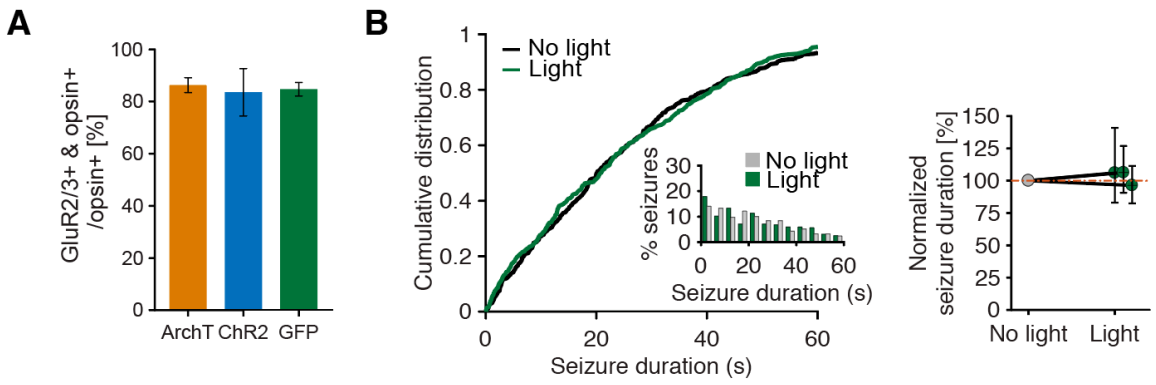

Fig. S2.3. Light delivery to opsin-negative control mice does not affect electrographic seizure duration. (A) Opsin expression specificity, quantified by the proportion of ArchT+, ChR2+ and GFP+ neurons that were also GluR2/3+ (ArchT and ChR2 replotted from Fig. 1; N = 38 slices, n = 3 mice for GFP+). **(B)** Left: cumulative distribution and probability density (inset) of the seizure duration following start of light or no light delivery (all animals combined, N = 733 seizures, n = 3 animals; $P > 0.05$ Kolmogorov-Smirnov). Right: seizure duration. Data are shown as the normalized difference in seizure duration between the light and no light events and 95% CI for each individual animal as in Fig. 2. ($P > 0.05$ for each animal, Mann-Whitney *U* test).

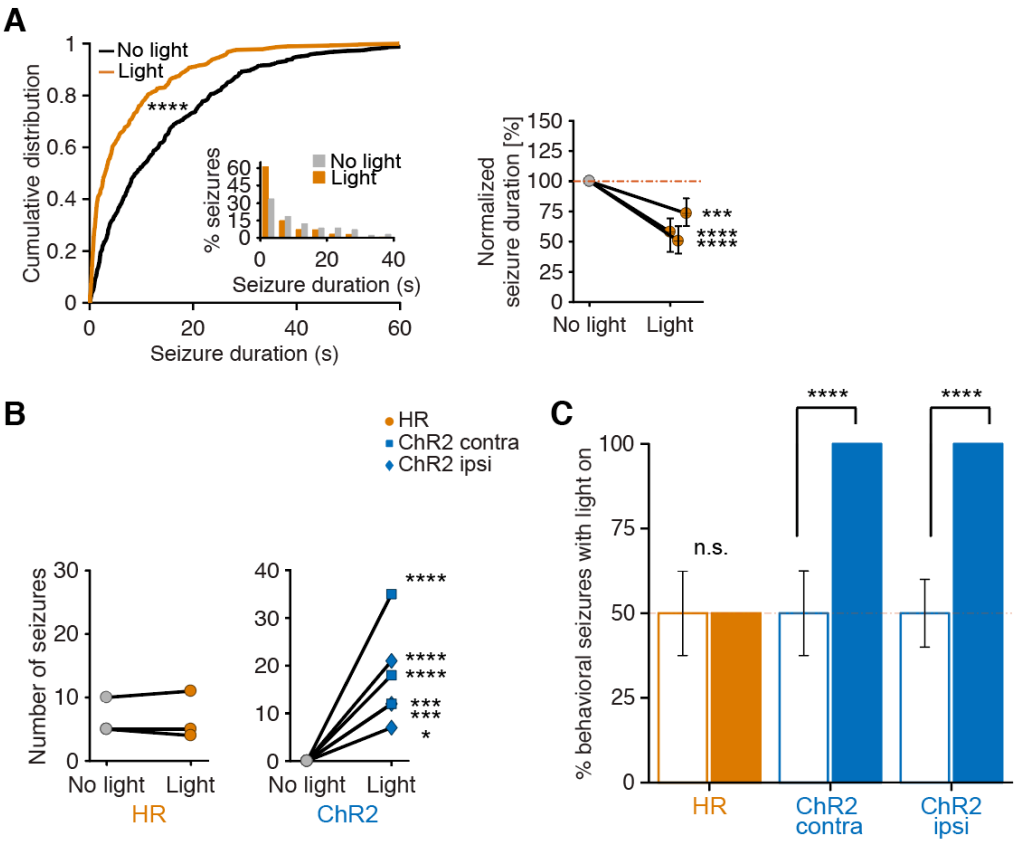

Fig. S2.4. Dentate gyrus (DG) granule cell (GC) optogenetic perturbation during spontaneous seizures in chronic TLE. (A) On-demand inhibition of DG GC significantly shortens spontaneous electrographic seizures. Left: cumulative distribution and probability density (inset) of the seizure duration following start of light or no light delivery (all animals combined, N = 841 seizures, n = 3 animals; ****P < 0.001 Kolmogorov-Smirnov). Right: seizure duration. Data are shown as normalized difference in seizure duration between the light and no light events and 95% CI for each individual animal (***P < 0.001, ****P < 0.0001, Mann-Whitney *U* test). **(B)** Left: for each measured animal (n = 3), photoinhibition of DG GCs does not affect the occurrence of behavioral seizures. Right: DG GC photostimulation significantly increases the occurrence of behavioral seizures (n = 6). *P < 0.05, ***P < 0.001, ****P < 0.0001, two-tailed binomial test. **(C)** On-demand inhibition of HR-expressing GCs has no effect on the occurrence of behavioral seizures, and photostimulation of ChR2-expressing GCs induces behavioral seizures. Solid colored bars represent the fraction of convulsive seizures occurring after light delivery. White bars represent the expected fraction 95% CI of seizure occuring with light, under the null hypothesis that light delivery has no effect. ****P < 0.0001, two-tailed binomial test. Data from **(A)** and ChR2 data from **(B & C)** are re-analyzed from data presented in Krook-Magnuson et al. 2015. HR data in **(B & C)** are new data from this study.

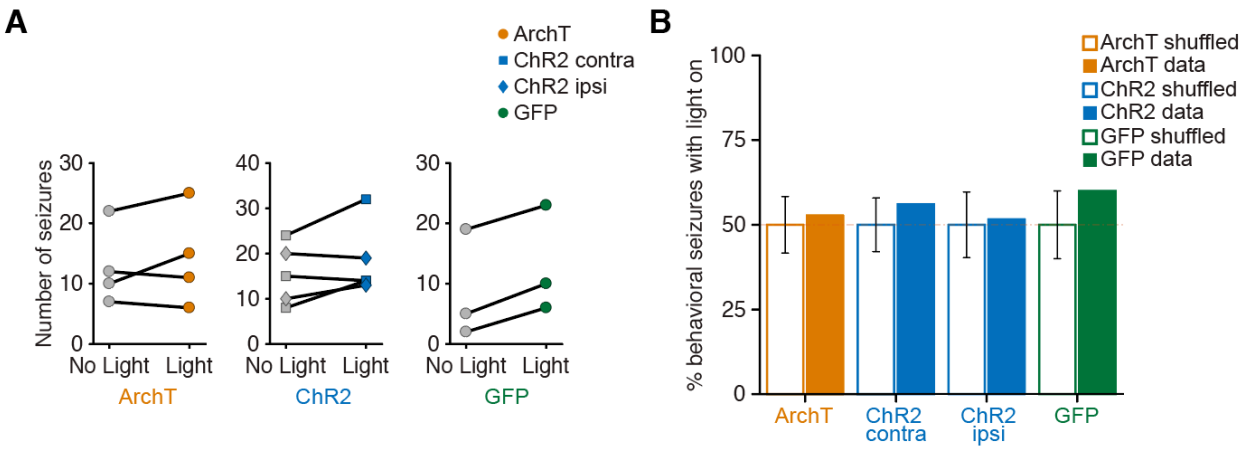

Fig. S2.5. Test of causality: light delivery following the end of the convulsive seizure does not correlate with seizure occurrence. (A) Number of convulsive seizures followed by either a light or no light trigger (post-seizure trigger). Light and no light post-seizure triggers are equally likely to occur in all ArchT- (left; n = 4 mice), ChR2- (middle; n = 5 mice) or GFP- (right; n = 3 mice) expressing mice. This is in contrast with the data shown in Fig. 3 for pre-convulsive seizure triggers, which demonstrates that the relationship between pre-convulsive seizure trigger type (light vs. no light) and convulsive seizure occurrence is causal and not due to selection bias or artifacts in light delivery. **(B)** The measured fraction of convulsive seizures followed by a light trigger (solid bars) falls in the range expected under the null hypothesis that post-seizure light delivery has no effect on the occurrence of convulsive seizure (white bars show the mean fraction and 95% CI for a binomial distribution with 50% success rate). Contra, contralateral to the kainate injection site; ipsi, ipsilateral to the kainate injection site.

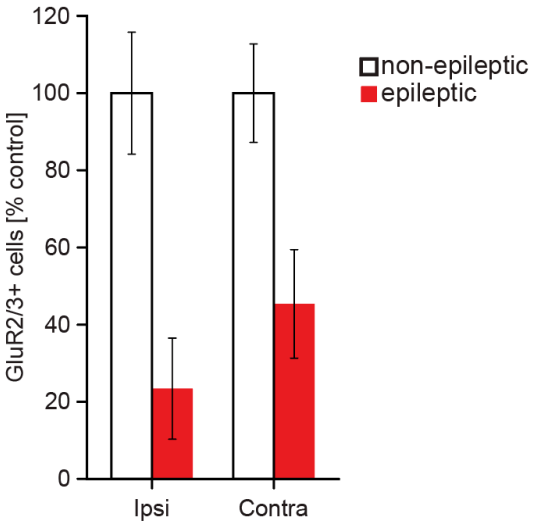

Fig S2.6. Hilar mossy cell loss in mice with chronic TLE. Number of GluR2/3+ cells in epileptic animals (N = 33 slices, n = 3 mice), compared with and normalized to non-epileptic controls (N = 35 slices, n = 3 mice). All data are presented as mean ± s.e.m. Ipsi, ipsilateral to the site of kainate injection; contra, contralateral to the site of kainate injection.

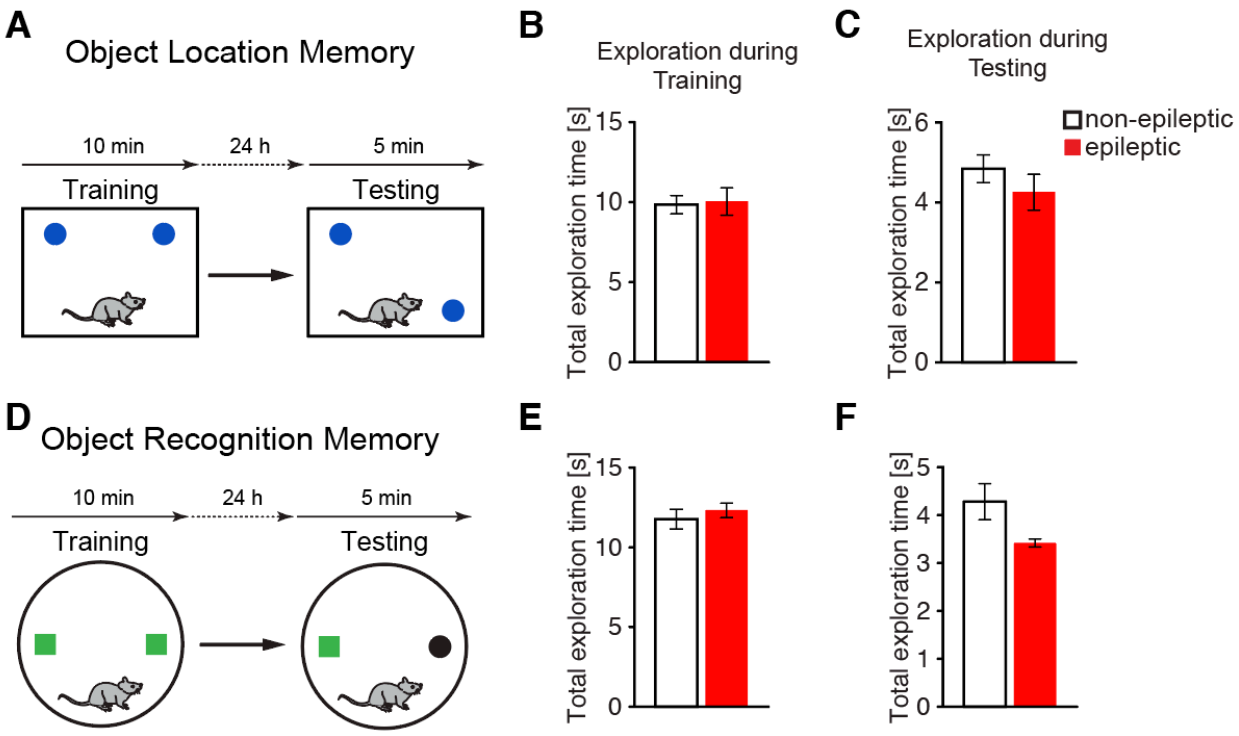

Fig. S2.7. Total exploration times for Object Location Memory and Object Recognition Memory in epileptic mice. (A-C) Epileptic mice do not have significantly different total exploration times as compared with their non-epileptic littermates during the **(B)** training or **(C)** testing phases of the OLM task. (n = 13 non-epileptic mice, n = 17 epileptic mice; P > 0.05, two-tailed Welch's t-test). **(D-F)** Epileptic mice do not have significantly different total exploration times as compared with their non-epileptic littermates during the **(E)** training or **(F)** testing phases of the ORM task (n = 10 non-epileptic mice, n = 14 epileptic mice; *P* > 0.05, two-tailed Welch's t-test).

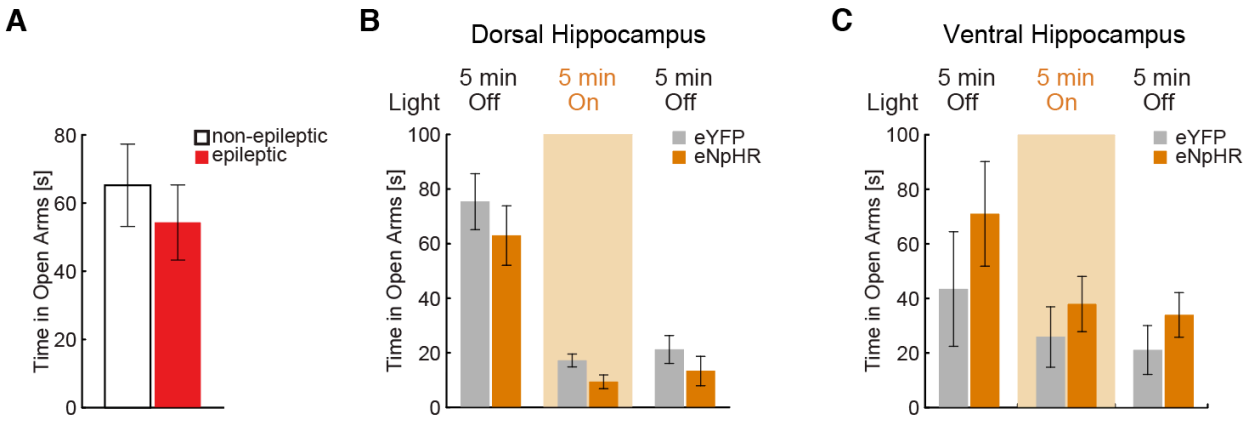

Fig. S2.8. Epileptic mice do not have altered levels of anxiety, and anxiety is not altered with mossy cell inhibition. (A) Non-epileptic (n = 12) and epileptic (n = 10) mice do not have significantly different levels of anxiety as assessed by the time spent in the open arms of the elevated plus maze (EPM). **(B)** MC photoinhibition in the dorsal hippocampus does not alter time spent in the open arms of the EPM (n = 9 eYFP, n = 8 eNpHR). **(C)** MC photoinhibition in the ventral hippocampus does not alter time spent in the open arms of the EPM (n = 10 eYFP, n = 12 eNpHR). Shaded orange boxes indicate epochs of light delivery. All data are presented as mean ± s.e.m. $P > 0.05$, two-tailed Welch's t-test.

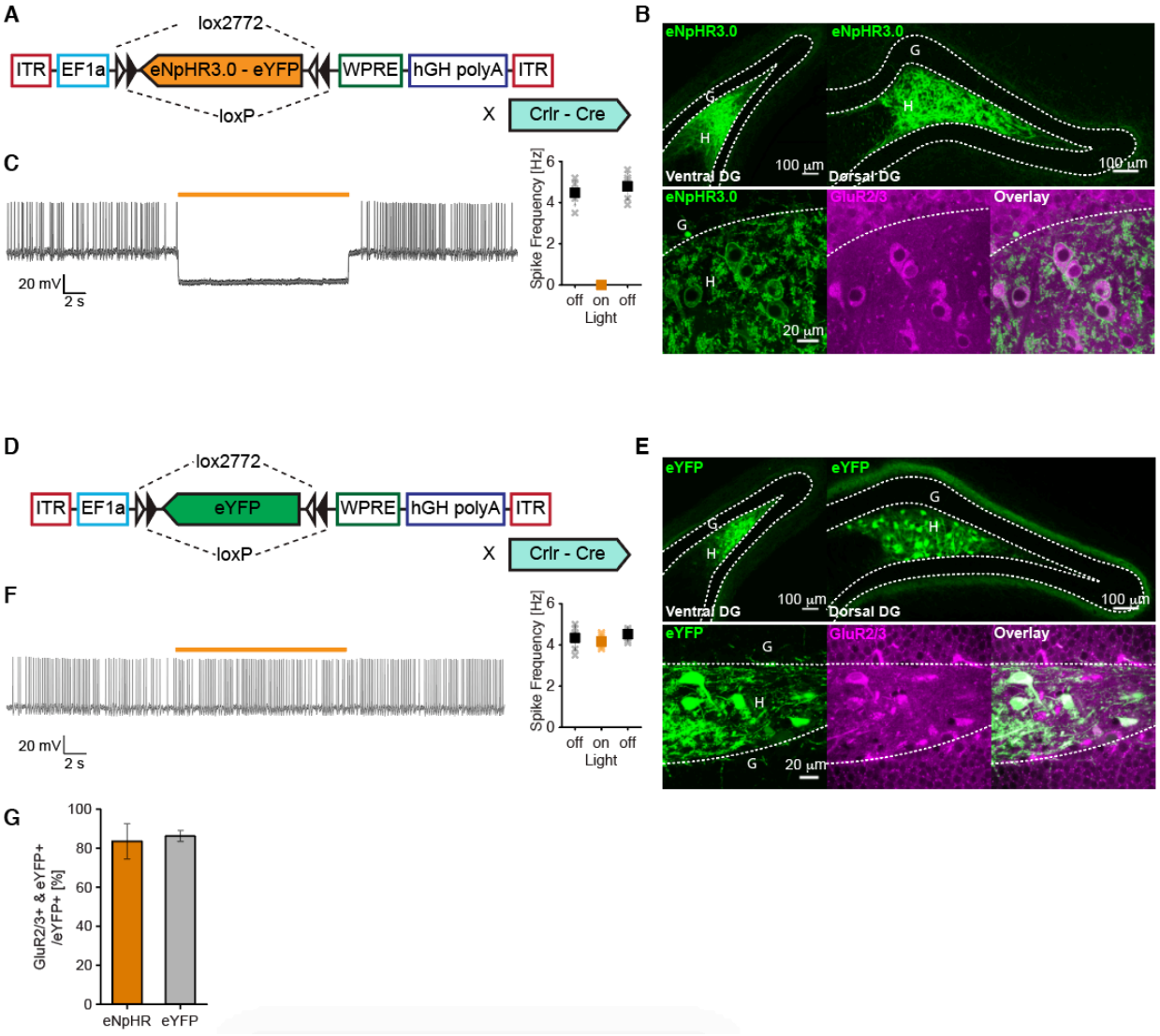

Fig. S2.9. Selective optogenetic control of hippocampal dentate gyrus mossy cells.
(A) Cre-dependent eNpHR expression system. **(B)** Top: confocal images of eNpHR expression in the ventral (left) and dorsal (right) DG. Bottom: high magnification images of the DG showing eNpHR-expressing MCs identified via eYFP expression and GluR2/3+ immunostaining. **(C)** 15 s illumination blocks current induced spiking in eNpHR-expressing MCs, quantified on the right (N = 5 recordings, n = 3 mice). **(D)** Cre-dependent eYFP expression system. **(E)** Top: confocal images of eYFP expression in the ventral (left) and dorsal (right) DG. Bottom: high magnification images of the DG showing eYFP-expressing MCs identified via eYFP expression and GluR2/3+ immunostaining. **(F)** 15 s illumination has no effect on eYFP-expressing MCs in opsin-negative control mice, quantified on the right (N = 5 recordings, n = 3 mice). **(G)** Opsin expression specificity, quantified by the proportion of eNpHR+ neurons that were also GluR2/3+ (N = 33 slices, n = 3 mice) and of eYFP+ neurons that were also GluR2/3+ (N = 16 slices, n = 3 mice). All data are presented as mean ± s.e.m. G, granule cell layer; H, hilus.

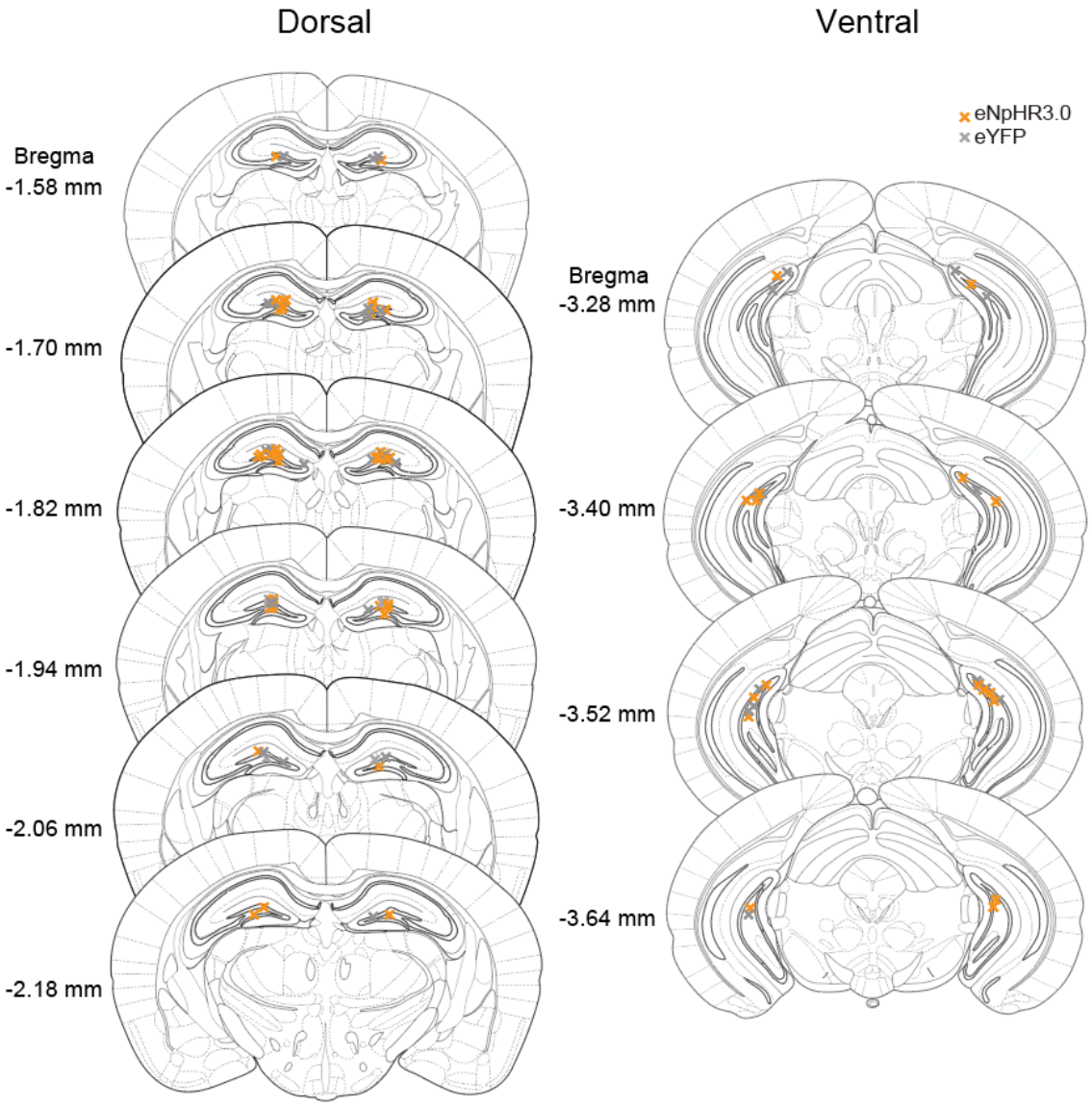

Fig. S2.10. Post-hoc verification of optical fiber locations for OLM, ORM, and EPM mice. (n = 18 eNpHR dorsal, n = 18 eYFP dorsal, n = 10 eNpHR ventral, n = 8 eYFP ventral).

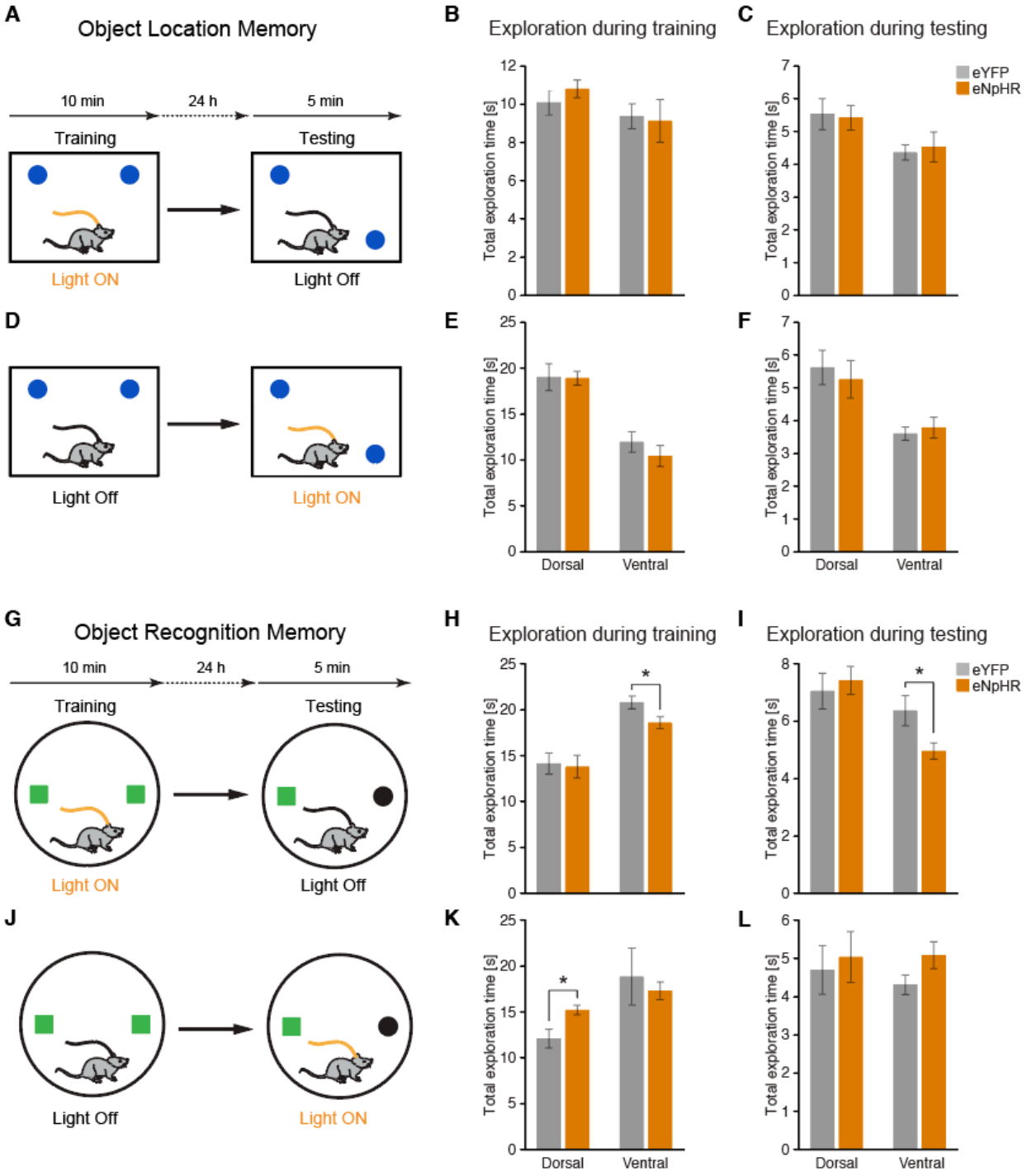

Fig. S2.11. Total exploration times for Object Location Memory and Object Recognition Memory. (A-C) MC photoinhibition during training of the OLM task does not alter total exploration time of objects during the **(B)** training or **(C)** testing phases (dorsal: n = 9 eYFP, n = 10 eNpHR; ventral: n = 8 eYFP, n = 5 eNpHR). **(D-F)** MC photoinhibition specifically during testing of OLM does not alter total exploration time of objections during the **(E)** training or **(F)** testing phases. (dorsal: n = 9 eYFP, n = 8 eNpHR; ventral: n = 6 eYFP, n = 7 eNpHR). **(G-I)** MC

photoinhibition during training of the ORM task does not alter total exploration time of objects during the **(H)** training or **(I)** testing phases (dorsal: n = 10 eYFP, n = 10 eNpHR; ventral: n = 9 eYFP, n = 10 eNpHR). **(J-L)** MC photoinhibition specifically during testing of ORM does not alter total exploration time of objects during the **(K)** training or **(L)** testing phases (dorsal: n = 10 eYFP, n = 10 eNpHR; ventral: n = 7 eYFP, n = 9 eNpHR). **P* < 0.05, two-tailed Welch's t-test.

CHAPTER 3

In vivo evaluation of the dentate gate theory in epilepsy

Abstract

The dentate gyrus is a region subject to intense study in epilepsy because of its posited role as a 'gate', acting to inhibit overexcitation in the hippocampal circuitry through its unique synaptic, cellular and network properties that result in relatively low excitability. Numerous changes predicted to produce dentate hyperexcitability are seen in epileptic patients and animal models. However, recent findings question whether changes are causative or reactive, as well as the pathophysiological relevance of the dentate in epilepsy. Critically, direct *in vivo* modulation of dentate 'gate' function during spontaneous seizure activity has not been explored. Therefore, using a mouse model of temporal lobe epilepsy with hippocampal sclerosis, a closed-loop system and selective optogenetic manipulation of granule cells during seizures, we directly tested the dentate 'gate' hypothesis *in vivo*. Consistent with the dentate gate theory, optogenetic gate restoration through granule cell hyperpolarization efficiently stopped spontaneous seizures. By contrast, optogenetic activation of granule cells exacerbated spontaneous seizures. Furthermore, activating granule cells in non-epileptic animals evoked acute seizures of increasing severity. These data indicate that the dentate gyrus is a critical node in the temporal lobe seizure network, and provide the first *in vivo* support for the dentate 'gate' hypothesis.

Introduction

Epilepsy manifests with recurrent bursts of hypersynchronous neuronal activity that can arise from a number of brain regions. The most commonly affected regions are areas where recurrent

excitatory networks pre-exist and can serve as a substrate for this type of activity, such as the temporal lobe, where recurrent excitatory connections of the hippocampus are required for normal spatial navigation, learning and memory (Eichenbaum and Cohen, 2014, Hartley *et al.*, 2014). Thus, the dentate gyrus (DG), classically considered as the first stop in the trisynaptic loop of entorhinal-hippocampal circuitry within the temporal lobe (van Strien *et al.*, 2009), has been extensively studied in both healthy and pathological circumstances. Both the microcircuitry of the DG and unique features of granule cells (GCs; the main principal cell type of the DG) make them less easily excitable than other types of principal cells: in healthy tissue, GCs not only have extensive feedforward and feedback inhibition, but also intrinsic properties, such as a particularly hyperpolarized resting membrane potential, low input resistance and a relatively high threshold for firing (Heinemann *et al.*, 1992, Lothman *et al.*, 1992, Mody *et al.*, 1992, Heinemann *et al.*, 1993, Acsady *et al.*, 1998, Coulter, 1999, Henze *et al.*, 2002, Lysetskiy *et al.*, 2005, Coulter and Carlson, 2007, Leutgeb *et al.*, 2007, Morgan *et al.*, 2007, de Almeida *et al.*, 2009, Armstrong *et al.*, 2011, Armstrong *et al.*, 2012, Yu *et al.*, 2013). Therefore, although substantial barrages of excitatory input may arrive at the DG, there is normally only sparse activation of GCs, limiting the input to downstream regions of the hippocampus, where pyramidal cells can generate action potentials more easily and where there are abundant intrinsic recurrent excitatory connections. The limited activation of GCs is important for pattern separation functions during normal brain activity (Leutgeb *et al.*, 2007, de Almeida *et al.*, 2009), but, in addition, this may help protect the vulnerable downstream hippocampal formation and prevent seizure activity. The latter idea is the basis of the dentate ‘gate’ theory of temporal lobe epilepsy (TLE), which posits that seizures occur when the gate function of the DG is disrupted

such that excess excitation emerges from or passes through the DG to downstream regions (Heinemann *et al.*, 1992, Lothman *et al.*, 1992).

The dentate gate hypothesis has been a major and frequently cited mechanistic concept in epilepsy for over 20 years (Heinemann *et al.*, 1992, Lothman *et al.*, 1992) and, further illustrating the importance of this concept, the National Institutes of Health has invested millions of dollars in projects related to the DG and epilepsy in this fiscal year alone (http://projectreporter.nih.gov). A wealth of evidence in support of the theory has been found in the numerous changes in this region in TLE that are predicted to cause hyperexcitability. These changes include, amongst many others, mossy fibre sprouting (such that GCs form aberrant recurrent synapses onto other GCs, a feature not observed in healthy tissue; (Nadler *et al.*, 1980, Zhang *et al.*, 2012)), altered intrinsic properties and receptor expression (Coulter, 1999, Stegen *et al.*, 2012), and reduced GABAergic inhibition (Acsady *et al.*, 1998, Bouilleret *et al.*, 2000, Kobayashi and Buckmaster, 2003, Coulter and Carlson, 2007). A recent study found that dysregulation in GCs of molecular pathways known to be important in epileptogenesis can result in epilepsy (Pun *et al.*, 2012), and correlations have been reported between seizure frequency and changes seen in the DG, including mossy fibre sprouting (Marucci *et al.*, 2010, Hester and Danzer, 2013).

However, other work has demonstrated that, unexpectedly, it is possible to suppress certain changes considered to lead to hyperexcitability, such as mossy fibre sprouting, without also suppressing seizures, questioning the causality of the morphological changes observed in the dentate in TLE (Buckmaster and Lew, 2011). Additionally, calcium imaging of hippocampal slices suggests only an initial, transient breakdown in the dentate gate prior to the emergence of spontaneous seizures in a rat model of TLE, with the temporoammonic pathway to the CA1

region instead showing the greatest dysregulation at a time when the first spontaneous seizures appear (Wozny *et al.*, 2005, Ang *et al.*, 2006, Pathak *et al.*, 2007, Coulter *et al.*, 2011). These data raise the possibility that there is a primary alternate pathway by which seizures spread that bypasses the DG altogether. If this were the case, therapeutic interventions targeting the DG in an attempt to restore the dentate gate in TLE would be ineffective at controlling seizures. Importantly, direct *in vivo* evidence supporting the dentate gate hypothesis in TLE has been conspicuously lacking.

For the dentate gate hypothesis to be demonstrated *in vivo*, inhibition of GCs during spontaneous temporal lobe seizures to restore gate function should prevent overexcitation of the rest of the hippocampal formation and effectively stop seizure activity. If, however, an alternate pathway bypassing the DG is the critical pathway involved in TLE, targeting GCs would be ineffective at inhibiting ongoing seizure activity. Similarly, overexcitation of GCs to mimic a breakdown of the gate might be expected to cause seizures. Optogenetics is a powerful tool that can be harnessed to study epilepsy (Krook-Magnuson *et al.*, 2014a) and has been successfully applied in animal models of focal cortical epilepsy (Wykes *et al.*, 2012), cortical stroke-induced thalamocortical epilepsy (Paz *et al.*, 2011) and TLE (Krook-Magnuson *et al.*, 2013), as well as to inhibit acute seizures *in vivo* (Sukhotinsky *et al.*, 2013, Berglind *et al.*, 2014, Chiang *et al.*, 2014) and epileptiform activity *in vitro* (Tonnesen *et al.*, 2009, Berglind *et al.*, 2014, Ledri *et al.*, 2014). Importantly, by allowing direct and selective manipulation of GCs *in vivo*, on-demand optogenetic techniques (Armstrong *et al.*, 2013) provide a straightforward way of directly testing the dentate gate hypothesis.

M**ethods**

Ethical approval

All procedures were approved by the UC Irvine Animal Care and Use Committee and were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* of the National Research Council.

Animals

The molecular Cre-lox system permitted the selective introduction of the inhibitory halorhodopsin (HR) or the excitatory channelrhodopsin (ChR2) into specific cell populations. Mice were generated by crossing Cre lines expressing Cre either in DG GCs selectively (B6.FVB-Tg(Pomc-cre)1Stl/J; stock 010714 from Jackson labs (McHugh *et al.*, 2007)) or in principal cells broadly (including CA1 pyramidal cells: CamK-Cre; B6.Cg-Tg(Camk2a-cre)T29-1Stl/J; stock 005359 from Jackson labs (Tsien *et al.*, 1996)) with either floxed-STOP HR mice (Ai39; B6;129S-Gt(ROSA)26Sortm39(CAGHOP/EYFP)Hze/J; generated by Hongkui Zeng, available from Jackson labs, stock 014539 (Madisen *et al.*, 2012)), or floxed-STOP ChR mice (Ai32; Rosa-CAG-LSLChR2H134R-EYFP-deltaNeo generated by Hongkui Zeng, obtained from the Allen Institute and now available from Jackson labs, stock 012569 (Madisen *et al.*, 2012)). Ai39 and Ai32 lines were maintained by crossing with C57BL/6J mice (Jackson labs stock number 000664). For visualization, the GC-Cre line was also crossed with a line expressing the red fluorescent protein tdTomato in a Cre-dependent fashion (B6;129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J; stock 007905 from Jackson labs (Madisen *et al.*, 2012)). While Cre is transiently expressed in developing GCs in the B6.FVB-Tg(Pomc-cre)1Stl/J line, recombination leads to permanent opsin or tdTomato expression in GCs with this approach. Images of expression were acquired using either a Zeiss Axioskop 2 plus and post-

acquisition coloring, or the confocal facility of the Optical Biology Shared Resource center at the University of California, Irvine. GC-ChR2, GC-HR, and GC-Tomato mice demonstrated occasional scattered cortical cells with expression. The location of these varied between animals and was not within the path of light. However, in a subset of GC-ChR2, GC-HR, and GC-Tom animals, we found wide-spread non-specific expression (potentially due to transient Cre expression during early development of the animal). Therefore, the expression in all GC mice was examined post-hoc, and animals with such non-selective expression were not included in the analysis. This resulted in the exclusion of 10 animals. Negative littermates were used for opsin-negative controls. Animals were housed with a 12hr light/dark cycle and with ad libitum access to food and water. Male and female mice were used. At the end of experiments, animals were deeply anaesthetized with isoflurane, followed by rapid decapitation.

Epilepsy induction and monitoring

The procedures employed are similar to those described in (Krook-Magnuson *et al.*, 2013) and a detailed protocol is provided in Armstrong *et al*. (2013). We used the unilateral intrahippocampal kainate model of TLE (Cavalheiro *et al.*, 1982), which best mimics unilateral hippocampal sclerosis. Kainic acid (KA; 50–100 nL, 20 mM in saline; Tocris Bioscience, St Louis, MO, USA) was stereotaxically injected directly into the left dorsal hippocampal formation (posterior 2.0 mm, lateral 1.25 mm, ventral 1.6 mm with respect to bregma) of mice under isoflurane anaesthesia on or after postnatal day 46. After at least 2 weeks, allowing for the emergence of spontaneous recurrent seizures, optical fibres and twisted wire bipolar electrodes were implanted into the dorsal hippocampal formation (posterior 2.6 mm, lateral 1.75 mm, ventral 1.4 mm with respect to bregma). Light was delivered ipsilaterally or contralaterally to the site of previous kainate injection, as noted in the Results. Based on previous estimates of the volume of light

delivery (Krook-Magnuson *et al.*, 2013) and volume of the GC layer (Peirce *et al*. 2003), in healthy tissue, this fibre location would result in less than 5% of GCs being illuminated. Seizures were detected and recorded from electrodes located in the hippocampal formation ipsilateral to previous kainate injection; direct information about the source or spatial profile of seizures was not obtained in our experiments. The selection of our electrode placement was based on previous studies indicating that, in this model of epilepsy, spontaneous seizures typically arise ipsilateral, and slightly posterior, to the site of previous kainate injection (Cavalheiro *et al.*, 1982, Bragin *et al.*, 1999, Haussler *et al.*, 2012), such that the light stimulation was directed at seizure activity in an area of the DG where gate function was expected to be most compromised. The extent of hippocampal sclerosis is variable between animals; an example of the extent of sclerosis is provided by (Krook-Magnuson *et al.*, 2013), who illustrate GC dispersion and CA1 cell loss; note that the optical fibre was not placed in the area of maximal hippocampal sclerosis but rather where sclerotic and non-sclerotic tissue tended to interface. In separate experiments, animals not previously injected with kainate (non-epileptic, kainate-naïve animals), were similarly implanted with optical fibres and bipolar depth electrodes. Data for some of the Cam-HR mice used for comparison with the effects seen in GC-HR mice were reported previously (in six animals). These animals had optrodes targeted to above the CA1 region (ventral 1.25 mm with respect to bregma), with light reaching an estimated depth of at least 0.55 mm from the tip of the fibre, and thus also reaching the DG (Krook-Magnuson *et al.*, 2013). In two additional Cam-HR animals, the placement of the optical fibre was lowered to match that of GC-HR animals. The results obtained at this location were not substantially different, and the results from all Cam-HR animals were combined. After recovery, 24 h video and EEG monitoring for seizures and subsequent closed-loop seizure detection and/or light delivery were initiated. On average, KA-

injected animals were implanted 15.9 ± 2.3 weeks after KA injection and the effect of light on seizures was examined 19.9 ± 2.6 weeks after KA injection. There was no correlation between seizure duration reduction and time subsequent to kainate injection ($P = 0.34$, Spearman test).

Closed-loop seizure detection and light delivery

Closed-loop seizure detection and light delivery was performed in a manner similar to that described by (Krook-Magnuson *et al.*, 2013) and in more detail by (Armstrong *et al.*, 2013). For experiments using on-demand light delivery, the hippocampal EEG signal was analysed in real-time by a PC running a custom MATLAB (http://www.mathworks.com) seizure detection algorithm. A version of this software is available for download from (Armstrong *et al.*, 2013). Once the presence of spontaneous recurrent seizures in individual animals was established, an experimenter identified features of the early ictal electrographic signal to be used in triggering the real-time closed-loop seizure detection software. The recording software has been modified slightly and interfaces with a custom-built trigger control box for a more accurate timing of light pulses. A fibre-coupled diode laser (Shanghai Laser & Optics Century Co., Ltd, Shanghai, China) of an appropriate wavelength to activate the opsin expressed (473 nm for ChR2, 589 nm for HR) was used. Steady state power measured *post hoc* from the tip of the optical fibres was 9.3 ± 0.6 mW. When a seizure was detected, it was flagged for later review and, for a preset percentage of events (in a random sequence), light delivery (30 s of 50 ms on, 100 ms off for 473 nm; 30 s of 2000 ms on, 50 ms off for 589 nm) was immediately triggered. This allowed for each animal to serve as its own control, in addition to opsin-negative controls. In non-epileptic animals, triggering was instead performed in a scheduled manner, with triggers occurring every 15 min and light being delivered for 50% of triggers.

Slice electrophysiology

Whole-cell patch-clamp recordings were made at 36°C from coronal slices using artificial cerebrospinal fluid (aCSF) containing (in mM) 2.5 KCl, 10 glucose, 126 NaCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 CaCl, 26 $NaHCO_3$. The intracellular solution contained (in mM): 90 potassium gluconate, 27.4 KCl, 1.8 NaCl, 1.7 $MgCl_2$, 0.05 EGTA, 10 Hepes, 2 Mg-ATP, 0.4 Na_2-GTP, 10 phosphocreatine, 8 biocytin; pH 7.2; 270–290 mosmol l^{-1}; pipette resistance: 3–4.5 MΩ. Recordings were made a using a Multiclamp 700B, Digidata 1322A (Axon Instruments, Foster City, CA, USA), a 4–10 kHz low pass filter and a sampling rate of 10–50 kHz. Light was delivered through the epifluorescence port of a Eclipse FN-1 (Nikon, Tokyo, Japan), using a Lambda DG-4 with smart shutter and Lambda SC controller (Sutter Instruments, Novato, CA, USA) and TTL input from a Digidata 1322A (Axon Instruments). Where noted in the text, 1 μM of TTX was added to the aCSF.

Scoring of behavioural seizures

The extended Racine scale of Pinel and Rovner (1978), which captures also more severe behavioral seizures, was modified to include additional phenotypes observed (Racine, 1971, Pinel and Rovner, 1978, Luttjohann *et al.*, 2009): stage 1: a change in behavioural state (sudden behavioural arrest or sudden motion); stage 2: head nodding; stage 3: forelimb clonus; stage 4: rearing, or clonus when on belly, or strong hindlimb clonus (bucking); stage 5: falling, or clonus when on side; stage 6: multiple sequences of rearing and falling, or brief jumps; stage 7: violent jumping; and stage 8: class seven, followed by a period of tonus lasting longer than 5 s.

Statistical analysis

Electrographic seizure durations after the time of the trigger and the time to next seizure were analysed offline by reviewers who were blinded to the light condition and genotype of the animal, and behavioural seizures were confirmed by video and EEG analysis. Post-detection

seizure durations for light and no light conditions in GC-HR animals were compared in each animal using a two-sample Kolmogorov–Smirnov test and a two-tailed Mann–Whitney test (93 ± 5 seizure events per animal were analysed to assess the effect of ipsilateral light delivery; 102 ± 2 seizure events per animal were analysed to assess the effect of contralateral light delivery). Group level statistics for duration reduction and time to next seizure used a Wilcoxon signed ranks test (light *vs*. no light) and a Mann–Whitney test (opsin-expressing *vs*. opsin-negatives; GC-HR *vs*. Cam-HR). Comparisons for the frequency of events progressing to behavioural seizures in epileptic GC-ChR2 animals with and without light delivery was made using chi-squared tests (137 ± 44 events per animal were examined to assess the effect of ipsilateral light delivery; 75 ± 22 events per animal for contralateral light delivery). In kainate-naïve animals, a correlation between the number of light deliveries (for light deliveries one to eleven) and seizure duration or behavioural seizure score was tested using Spearman's correlation coefficient. Note that this is a non-parametric test and does not assume a linear relationship. Values presented are the mean ± SEM. $P < 0.05$ was considered statistically significant. Statistical analysis was conducted using Excel 2007 and 2013 (Microsoft, Redmond, WA, USA), OriginPro 8 and 9 (OriginLab Corp., Northampton, MA, USA) and Google documents (https://docs.google.com).

Results

On-demand inhibition of dentate GCs

To gain selective optogenetic inhibition of GCs, we generated chronically epileptic mice expressing the inhibitory opsin HR in DG GCs (GC-HR) (Fig. 3.1) utilizing a model of TLE with unilateral hippocampal sclerosis as described in the Methods. GC-HR mice were injected with

KA in the left dorsal hippocampus, and the effect of inhibition of GCs on spontaneous seizures was examined during the chronic phase of the disorder. Expression of HR selectively in GCs remained after chronic, spontaneous seizures emerged (specificity of expression was confirmed *post hoc* for all animals included in the present study). Light delivery produced robust inhibitory currents, hyperpolarizing GCs (Fig. 3.1*D* and and *E)*.

During closed-loop spontaneous seizure detection and intervention *in vivo*, amber light was immediately delivered upon seizure detection above the DG (reaching a depth of ~0.55 mm from the tip of the optical fibre; (Krook-Magnuson *et al.*, 2013), and thus reaching both blades of the DG) for 50% of detected events in a random fashion, allowing each animal to serve as its own internal control. As noted above, seizures in this model typically arise ipsilateral to the site of prior kainate injection (Bragin *et al.*, 1999), and thus the region of the hippocampal formation with the greatest probablility of having a compromised dentate gate was the region both recorded from and targeted with light. Supporting the dentate gate hypothesis, we found that ipsilateral on-demand light delivery in GC-HR mice dramatically truncated seizures (75 ± 7% stopping within 5 s of light delivery; post-detection seizure duration, light *vs*. no light, $P < 0.05$ Wilcoxon test; six animals) (Fig. 3.1F-H). On average, there was a 66 ± 4% duration reduction ($P < 0.01$, opsin-positive *vs*. opsin-negative controls, Mann–Whitney). There was no effect on time to next seizure, indicating a lack of any rebound effect ($P = 0.2$). These data illustrate that selective inhibition of dentate GCs is able to inhibit temporal lobe seizure activity arising from regions of the hippocampus where there is probably most deficient DG function.

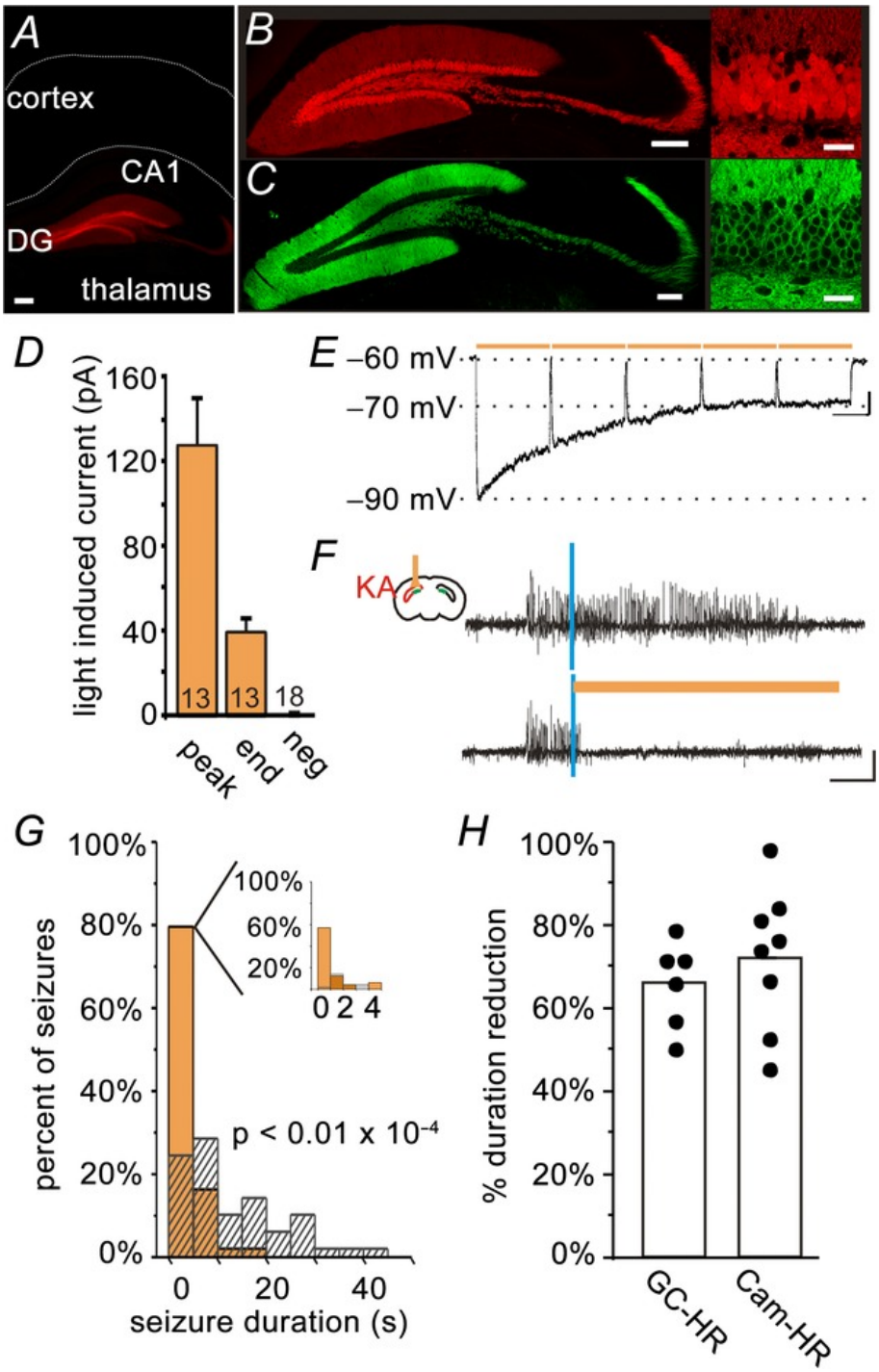

Figure 3.1. On-demand restoration of the dentate gate inhibits spontaneous temporal lobe seizures. Crossing a mouse line expressing Cre in DG GCs (visualized in *A* and *B*, by crossing with a tdTomato reporter line; red in the online version) with a mouse line expressing the inhibitory opsin HR in a Cre-dependent fashion, produced mice with HR expressed selectively in DG GCs (GC-HR). Selectivity of opsin expression was maintained in epileptic animals (*C*, yellow fluorescent protein tagged HR; green in the online version). The edge of the slice and the border between CA1 and the alveus are drawn in *A* for reference. *D* and *E*, whole-cell patch-

clamp recordings from epileptic brain slices revealed robust light-induced inhibition of GCs of opsin-expressing but not opsin-negative animals. (Peak, peak-induced currents; End, current measured at the end of 10 s of pulsed light delivery; Neg, opsin-negative controls. The number of cells recorded is indicated in each bar. *D*, summary voltage clamp data. *E*, example current clamp recording). *F–G*, *in vivo* online detection of spontaneous seizures allowed on-demand light delivery, which rapidly truncated seizures when delivered to the hippocampus ipsilateral to prior KA injection (example animal: vertical blue lines indicate seizure detection; amber bars indicate light delivery; hashed bars indicate events not receiving light; *G*, inset: expansion of the first 5 s after light delivery; 100 seizures; colour is shown in the online version). *H*, the inhibition of seizure duration achieved with selective inhibition of GCs is comparable to that achieved with broader inhibition of excitatory cells including CA1 pyramidal cells (Cam-HR; shown for reference in *H*; each dot represents one animal). Scale bars: *A*, 200 μm; *B* and *C*, left 200 μm, right 30 μm; *E*, 5 mV, 1 s; *F*, 0.2 mV, 5 s.

We previously demonstrated significant seizure control with on-demand light delivery to the hippocampal formation in mice broadly expressing HR in excitatory cells, including CA1 pyramidal cells (Cam-HR mice; (Krook-Magnuson *et al.*, 2013). We therefore also directly compared the seizure control obtained with this broader inhibition with the seizure control obtained with selective inhibition of GCs. Selectively inhibiting GCs produced comparable seizure control (Cam-HR 72 ± 6% duration reduction; eight animals; GC-HR 66 ± 4% duration reduction, as discussed above; Cam-HR *vs*. GC-HR: $P = 0.33$, Mann–Whitney) (Fig. 3.1H), indicating that, by identifying a key component of the network, improved intervention specificity can be achieved without sacrificing efficacy, and that the DG is a necessary participant in ongoing seizure activity.

We additionally tested the efficacy of light delivered contralateral to the site of previous kainate injection and recording electrode in GC-HR mice and found that it did not affect seizure duration (light *vs*. no light, GC-HR: $P = 1.0$, Wilcoxon). Therefore, inhibition of GCs ipsilaterally (and not contralaterally) inhibits seizures. Given that seizures in this model typically arise ipsilateral and in relatively close proximity to the site of prior kainate injection (Bragin *et al.*, 1999, Haussler *et al.*, 2012), and that GCs do not have contralateral projections, in the context of the dentate gate hypothesis, these findings suggest that inhibiting GCs can prevent seizure activity only where changes indicative of dentate gate breakdown occur.

Direction of modulation is a critical factor

Recent work examining cerebellar directed intervention for TLE unexpectedly found that the direction of modulation of cerebellar neurons was not a critical factor in achieving a reduction in seizure duration, with optogenetic excitation or inhibition of cerebellar neurons inhibiting seizures (Krook-Magnuson *et al.*, 2014b). We thus investigated whether the direction of

modulation is a critical factor in achieving seizure inhibition when targeting DG GCs in the hippocampal formation, or whether a disruption of on-going activity through on-demand optogenetic activation rather than inhibition of GCs could also inhibit seizures. To be able to selectively excite GCs, we generated mice expressing the excitatory opsin ChR2 selectively in GCs (GC-ChR2) and tested the effect of on-demand optogenetic intervention in these animals. Light delivery produced strong direct excitation of GCs (Fig. 3.2*A*and *B*; light-induced action potentials in 6 of 6 GCs recorded in current clamp; on average, light induced 820 ± 150 pA in 7 GCs recorded in voltage clamp), which remained in the presence of TTX (Fig. 3.2*B*, inset; 90 ± 5% of induced current remained in TTX; 3 GCs), as well as indirect excitation of downstream CA3 pyramidal cells, which was blocked in the presence of TTX (Fig. 3.2C; average peak current in regular aCSF: 270 ± 110 pA; abolished by TTX; 3 CA3 pyramidal cells).

Rather than truncating spontaneous seizures, *in vivo* on-demand light delivery to chronically epileptic GC-ChR2 mice increased the probability of electrographic seizures becoming large behavioural seizures (seizures were 90 times more probable to become a large behavioural seizure with light delivery; light *vs*. no light, $P < 0.001$, χ^2; behaviour included rearing with forelimb clonus, falling and violent jumping; five animals; Fig. 3.2*D*. Light delivery to the contralateral hippocampus also induced behavioural seizures (Fig. 3.2E light *vs*. no light, $P < 0.001$, χ^2; four animals). These data indicate that increased excitatory drive to GCs selectively is sufficient to push the network to behavioural seizures in epileptic animals, and that this occurs not only when excitatory drive is increased in areas of expected dentate gate compromise, but also in the contralateral hippocampus where dentate gate restoration had no effect on ongoing seizure activity.

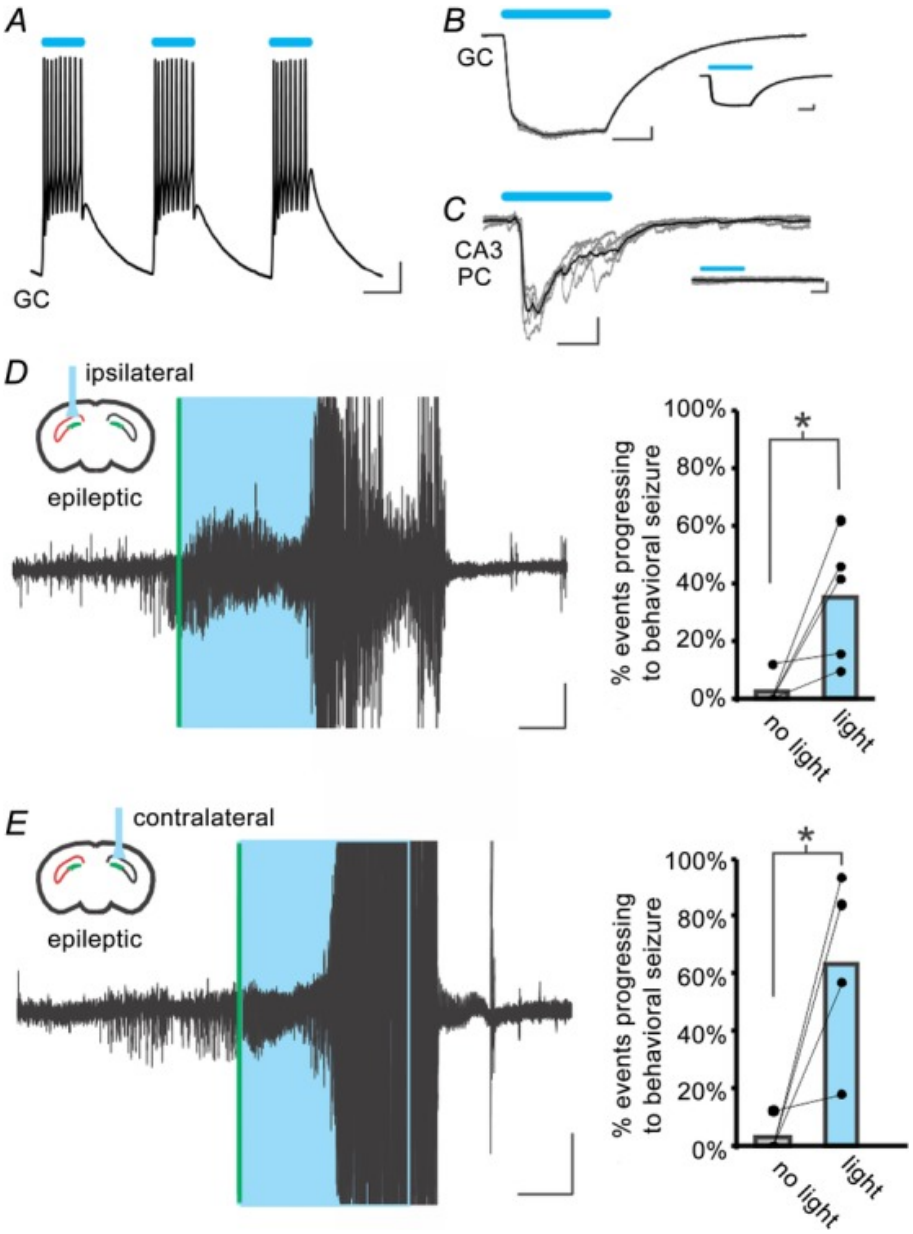

Figure 3.2. Excitation of GCs worsens spontaneous seizures. *A*, light-induced excitation of a GC in a slice from an epileptic GC-ChR2 animal. *B*, robust light-induced currents in a GC, which remain in the presence of TTX (inset). Grey traces: individual sweeps; black traces: average. *C*, optogenetic activation of GCs produces postsynaptic currents in a downstream CA3 pyramidal cell (PC), which are eliminated by the application of TTX (inset). *D* and *E*, on-demand light delivery to the dentate gyrus in mice expressing the excitatory opsin ChR2 in GCs (GC-ChR2 mice) ipsilateral (*D*) or contralateral (*E*) to previous kainate injection causes electrographic seizures to progress to large behavioural seizures. Right: each dot pair represents one animal. *$P < 0.001$ (chi-squared). Scale bars: *A*, 10 mV, 50 ms; *B* and *C*, 100 pA, 20 ms; *D* and *E*, 0.1 mV, 10 s. Boxes (coloured blue in the online version) denote light delivery. Vertical lines (coloured green in the online version) indicate online seizure detection. Large amplitude signals include a movement artefact and have been truncated.

Seizure induction in kainate-naïve animals

Given the finding that activation of GCs worsened, rather than inhibited, seizures in epileptic animals, and that this occurred even with contralateral activation (distant from the site of dentate gate injury, in a location where inhibition had no effect on seizure activity), we reasoned that overexcitation of GCs, effectively selectively collapsing or bypassing the dentate gate in the targeted region, may be sufficient to induce seizures in kainate-naïve non-epileptic animals. To achieve this, we implanted optrodes in non-epileptic (kainate-naïve) GC-ChR2 animals and repetitively stimulated DG GCs unilaterally for 30 s with at least 15 min between stimulation periods. This protocol of 30 s of pulsed light delivery was chosen to mimic the previous on-demand experiments performed in epileptic animals and determine whether the DG is a sufficiently powerful region to generate seizures in kainate-naïve animals. As such, the parameters do not represent an attempt to mimic the overexcitation of GCs probably occurring spontaneously in epileptic animals during endogenously occurring seizure activity.

Light delivery to the DG in kainate-naïve GC-ChR2 animals was capable of inducing seizures (Fig.(Fig.3)3) (in total, 30 of 47 light deliveries produced behavioural seizures in three animals). The duration and severity of seizure activity in response to light increased with repeated light delivery, resembling a kindling effect seen with electrical stimulation (Racine *et al*. 1973; Pinel & Rovner, 1978), and seizure duration outlasted light delivery (Spearman's correlation coefficient for seizure duration: 0.50; $P < 0.01$; Spearman's correlation coefficient for behavioural seizure score: 0.69; $P < 0.001$; Fig 3.3). Light never produced a seizure in opsin-negative kainate-naïve controls (367 light deliveries in four animals). These findings indicate that the selective excitation of GCs, mimicking a breakdown of the dentate gate, is sufficient to induce seizures even in non-epileptic animals.

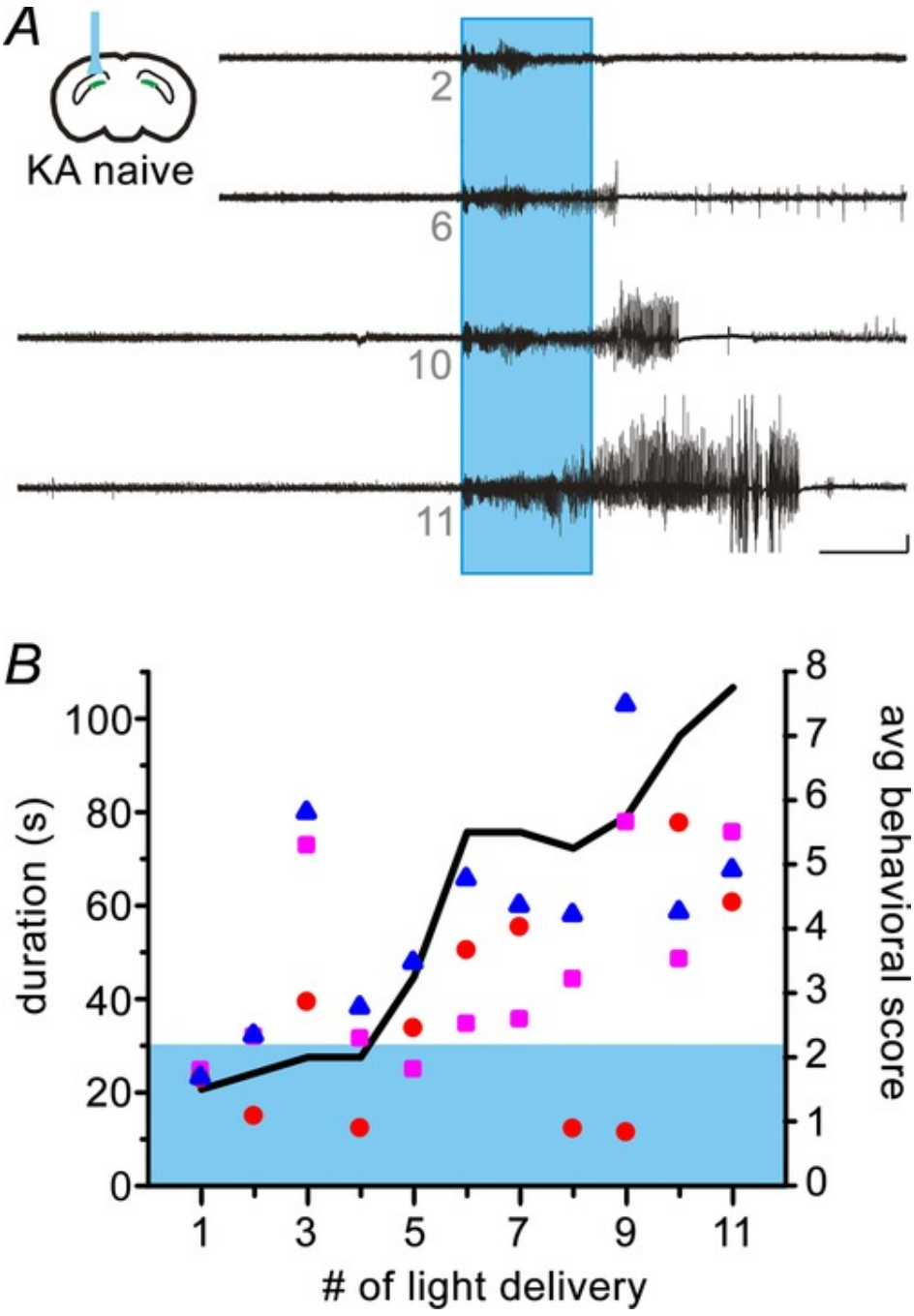

Figure 3.3. Excitation of GCs is sufficient to induce seizures in kainate-naïve animals. *A*, example responses to repeated light delivery in a kainate-naïve GC-ChR2 animal. Small grey numbers under traces indicate the number of light delivery. Boxes (coloured blue in the online version) denote 30 s of pulsed light delivery. Large amplitude signals include a movement artefact and have been truncated. Scale bar: 0.1 mV, 20 s. *B*, seizure duration and severity increases with repeated light delivery. Each symbol represents the seizure duration from a given animal; shading (coloured blue in the online version) indicates the duration of light delivery (left axis). The black line indicates the average behavioural seizure score (right axis). Seizure scoring was based on the extended Racine scale of Pinel & Rover (1978), as detailed in the Methods.

Discussion

Using optogenetics to selectively manipulate GCs in the DG, we demonstrate that (i) on-demand selective inhibition of GCs ipsilateral to kainate injection effectively stops spontaneous temporal lobe seizures; (ii) the direction of modulation is a critical factor, such that excitation of GCs worsens, rather than inhibits, seizures; and (iii) selective optogenetic activation of GCs can induce acute seizures in kainate-naïve, non-epileptic animals. These findings provide direct support for the dentate gate hypothesis because inhibition of the DG in areas of hippocampal sclerosis, and thus of predicted dentate gate breakdown, inhibits spontaneous temporal lobe seizures being propagated through the region. In addition, the DG is a sufficiently powerful node in the circuit such that driving GCs can generate seizures even in healthy, non-sclerotic tissue.

Our findings suggest that, although blocking some of the changes in the dentate associated with TLE may not be sufficient in isolation to stop the generation of seizures (Buckmaster and Lew, 2011), and while alternative pathways may exist (Wozny *et al.*, 2005, Coulter *et al.*, 2011), the DG is a critical component of the seizure circuitry. The numerous changes seen in hippocampal sclerosis that lead to DG hyperexcitability are indeed probably pathogenic, and the DG can be an effective target for the inhibition of seizures. The fact that selective inhibition of GCs produced a robust inhibition of seizures comparable to the broad inhibition of excitatory neurons in the hippocampal formation (including CA1 pyramidal cells) illustrates that, by identifying a key component in the network, increased specificity of intervention can be achieved without greatly sacrificing efficacy.

In considering these findings, it is important to emphasize that, although dysfunction of the dentate clearly allows the propagation of seizure activity through the hippocampal formation in temporal lobe seizures, our findings have limited spatial implications about the propagation

patterns, and certainly do not imply that upstream regions such as the entorhinal cortex are not involved in seizure initiation, nor do they indicate a unique or privileged role of the dentate in stopping seizure activity. Modulating any essential node within the seizure circuitry, or even modulating other brain regions that have strong influence on any of the essential nodes, may effectively inhibit seizures as well. Indeed, modulating the activity of certain extrahippocampal regions is known to modulate temporal lobe seizures (Fisher, 2013, Bertram, 2014, Krook-Magnuson *et al.*, 2014b), and changes seen in other brain regions, including in the CA1 and the temporoammonic pathway, may also contribute to seizure propagation. Alternative potential sites of intervention do not detract from the significance of the dentate gate hypothesis, nor from the finding that the DG is a critical node in the network and an effective target for seizure inhibition. With the recent approval of medical devices in human patients that are capable of delivering closed-loop electrical interventions to specific brain regions in response to seizure activity (Heck *et al.*, 2014), understanding seizure circuitry becomes even more beneficial because the findings may be more readily translated for clinical use. Future work examining other nodes or modulatory regions will provide increasing insight into the mechanisms and underlying architecture of seizures and epilepsy, and will also identify new intervention strategies.

Traditional medications, which have broad effects in numerous brain areas, can have major negative side effects, and restricted intervention strategies may reduce any negative side effects. Although our understanding of the exact changes that critically contribute to the breakdown of the dentate gate is still incomplete, the findings obtained from numerous studies of this region in epilepsy hint at a number of unique changes that may make excellent targets for interventions. The results of the present study clearly demonstrate that the DG is an essential node in TLE with hippocampal sclerosis, and indicate that an intervention approach selectively

targeting GCs or otherwise improving dentate gate function may be an effective strategy for inhibiting seizures and also reducing side-effects.

Acknowledgments

We thank Cecilia Lozoya, Rose Zhu, Judit Vargane, Chris Krook-Magnuson and Dhrumil Vyas for providing technical support. This work was made possible, in part, through access to the confocal facility of the Optical Biology Shared Resource of the Cancer Centre Support Grant (CA-62203) at the University of California, Irvine.

CHAPTER 4

Seizing Control: From Current Treatments to Optogenetic Interventions in Epilepsy

Abstract

The unpredictability and severity of seizures contribute to the debilitating nature of epilepsy. These factors also render the condition particularly challenging to treat, as an ideal treatment would need to detect and halt the pathological bursts of hyperactivity without disrupting normal brain activity. Optogenetic techniques offer promising tools to study and perhaps eventually treat this episodic disorder by controlling specific brain circuits in epileptic animals. Here we briefly review the current treatment options for patients with epilepsy. We then describe the many ways optogenetics has allowed us to untangle the microcircuits involved in seizure activity, and how it has, in some cases, changed our perception of previous theories of seizure generation. Control of seizures with light is no longer a dream, and has been achieved in numerous different animal models of epilepsy. Beyond its application as a seizure suppressor, we highlight another facet of optogenetics in epilepsy, namely the ability to create "on-demand" seizures, as a tool to systematically probe the dynamics of networks during seizure initiation and propagation. Finally, we look into the future to discuss the possibilities and challenges of translating optogenetic techniques to clinical use.

Introduction

Epilepsy: Epidemiology and current treatments

Epilepsy is a widespread neurological disorder, affecting 50 million people worldwide (World Health Organization (WHO), 2015). The overall prevalence is approximately 0.5% in developed countries and 1% or higher in developing countries (Banerjee et al., 2009; Hirtz et al., 2007; Picot et al., 2008; World Health Organization (WHO), 2015). Overall, 30-40% of patients are pharmacoresistant (Kwan and Sander, 2004). Unfortunately, this high percentage of non-responding patients persists despite the advent of a large number of new antiepileptic drugs (AEDs) since the 1990s. Currently, there are over 20 clinically utilized AEDs (Duncan et al., 2006; Zaccara and Perucca, 2014). Patients with chronic refractory epilepsy have at least a two-fold increase in mortality when compared to non-epileptic age-matched controls (Mohanraj et al., 2006). This is partially due to the risk of sudden death associated with epilepsy (SUDEP), which rises from 1 per 1000 patient-years in all patients with epilepsy to 6 per 1000 patient-years in patients with refractory epilepsy (Laxer et al., 2014; Thurman et al., 2014). Additionally, the cause of death is directly epilepsy-related in one-third of patients (Brodie et al., 1997). Epileptic patients also suffer have a high risk of psychological comorbidities, with nearly one-third of patients suffering from depression and/or anxiety (Kwon and Park, 2014). Finally, persistent seizures drastically decrease the patient's quality of life, limiting their independence and preventing them from driving or holding many types of employment (Taylor et al., 2011).

The first-line treatment for epilepsy is AEDs, which can offer excellent seizure control for many patients. However, they only treat the symptoms, i.e. the seizures, but not the disease, i.e. epilepsy, (Duncan et al., 2006). Additional drawbacks are that drugs are not effective for all patients or seizure types, they are not specific to the seizure focus, they must be taken at all times (not just during a seizure), and they can have serious negative side effects. Another issue is that except for a few drugs directed at specific types of seizures, such as ethosuximide for absence

seizures, the selection of an AED is not tailored to the patient's seizure semiology (Duncan et al., 2006). Furthermore, once patients have intractable epilepsy, they have a very low chance (less than 4% per year) of achieving seizure remission with the addition of new drugs (Choi et al., 2008). Drug-drug interactions are common among AEDs and can cause problems with affecting with the efficacy of drugs, such as other AEDs, oral contraceptives, antibiotics, analgesics, antidepressants, antihypertensives, and chemotherapeutic drugs (Zaccara and Perucca, 2014). Much research has been done to investigate why there is such a significant population of drug-resistant patients. Hypotheses of pharmacoresistance include a failure of the drug to reach its target, an alteration of the drug's target, and the possibility that the drug may not be acting on the true pathogenic target (Kwan et al., 2011).

Surgical treatments are the second-line therapy for patients with drug-resistant epilepsy (Figure 4.1). Well-established surgical treatments include temporal lobectomy, which leads to an excellent chance of seizure freedom. A randomized controlled trial of temporal lobectomy versus medical management for patients with medically refractory temporal lobe epilepsy demonstrated that 58% of surgical patients were completely seizure-free after one year, compared to only 8% of pharmacologically treated patients (Wiebe et al., 2001). Focused resections of other lesions such as cortical dysplasia, tubers in tuberous sclerosis, and low grade tumors can also be very effective in treating epilepsy. Overall, surgical resection of epileptic lesions leads to very good results, with about 70% of patients being seizure free after surgery with an MRI-identified lesion (Téllez-Zenteno et al., 2010). Patients without a clear lesion can go through so-called Phase I and Phase II monitoring. Phase I consists of inpatient video-EEG. If the electrographic and clinical features of the seizures localize to one side of the brain, the patient may be eligible for phase II

monitoring, where either subdural or depth electrodes are surgically implanted. The patient then undergoes further video-electrocorticography (ECoG analysis), and if a resectable seizure focus

Figure 1

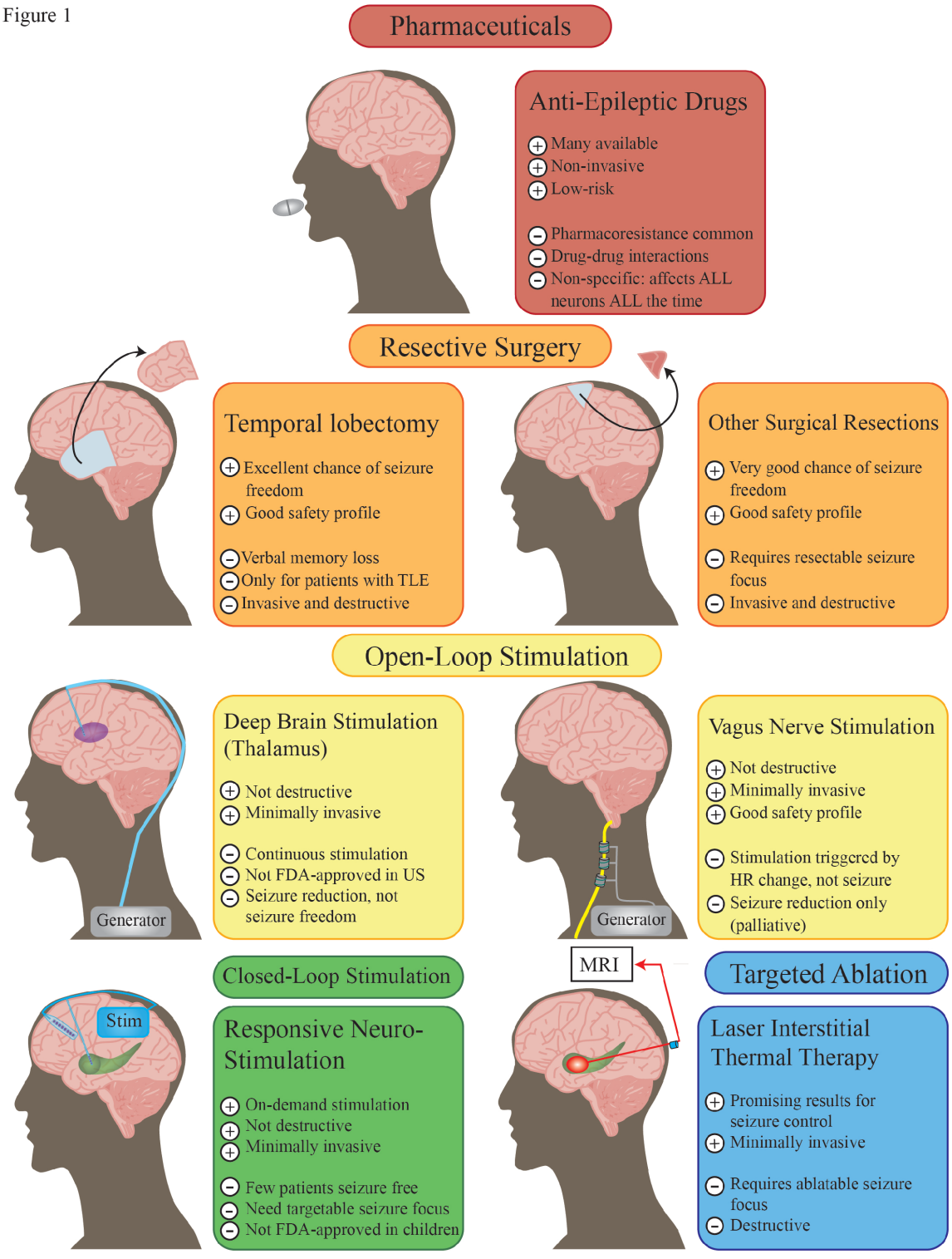

Figure 4.1. Currently used treatments for epilepsy in human patients. Medical and surgical treatments for epilepsy are shown with schematic diagrams. Major pros (+) and cons (–) of each type of treatment are noted.

is identified, this is then surgically removed. A recent study showed that after 10 years of follow-up, 38% of such patients remained seizure free (Noe et al., 2013). Although long-term seizure freedom of 38% of patients is much higher than can be achieved with medical management, this number demonstrates that there is still a need for more efficacious treatments.

Surgical implantation of electrodes for stimulation offers another type of treatment for refractory epilepsy. Deep brain stimulation (DBS), which involves the delivery of electrical impulses through electrodes surgically implanted into subcortical regions of the brain, is well-known as a highly effective treatment for Parkinson's Disease. DBS is also currently approved for treatment of epilepsy in Europe and Canada, and there is evidence that DBS has particularly beneficial outcomes for patients with temporal lobe epilepsy (Beudel and Brown, 2015; Miocinovic et al., 2013; Nune et al., 2015). A randomized controlled trial of DBS in the anterior nucleus of the thalamus demonstrated significant seizure reduction in patients with refractory epilepsy (Fisher et al., 2010). Vagal nerve stimulation is another surgical option for patients with nonlesional epilepsy or an unresectable seizure focus, and this technique offers about 50% seizure reduction. With this stimulation, small electrodes are wrapped around the left vagus nerve in the patient's neck, and these electrodes stimulate the vagus nerve in response to substantial increases in the patient's heart rate, which serves as a proxy for seizure activity (Nune et al., 2015). Drawbacks to VNS include that an unknown mechanism of action, open-loop rather than closed-loop control, and lack of curative potential (Orosz et al., 2014).

Over the past ten years, several new neurosurgical technologies have emerged with applications for epilepsy. One very promising, newly-approved treatment is responsive

neurostimulation (RNS). This is a closed-loop system in which electrodes are implanted either deep into the brain or onto the surface of the brain. The attached device is then programmed to detect seizures, and delivers electrical stimulation to the implanted electrode(s) upon detection of a seizure. The device has a built-in seizure detection algorithm, which is customizable to the patient's own seizures. The initial clinical trial showed promising results in terms of seizure control (Heck et al., 2014), and follow-up studies are promising in terms of improved cognition (Loring et al., 2015). This new and promising device represents a first step towards the goal of "no seizures, no side-effects." Laser interstitial thermal therapy (LITT) is another new surgical option for refractory epilepsy. With this technique, a laser fiber is stereotactically implanted into the patient's pre-identified seizure focus, and then the tissue is heated to 90°C, using real-time thermal MRI imaging to verify the tissue temperature and the zone of the lesion (Medvid et al., 2015). This technique has been successfully used for the treatment of many types of lesional epilepsy, including hypothalamic hamartoma, mesial temporal sclerosis, focal cortical dysplasia, cortical tubers, insular lesions, and Rasmussen's encephalitis (Curry et al., 2012; Esquenazi et al., 2014; Hawasli et al., 2013; Lewis et al., 2015; Willie et al., 2014). LITT ablation of the hippocampus has also been used for mesial temporal sclerosis. A small study of this technique suggests that postoperative language function is better preserved with LITT than with temporal lobectomy (Drane et al., 2015). Overall, although surgical techniques for epilepsy can offer excellent outcomes in select patients, drawbacks include: patient hesitation to undergo surgery, the need for an identified and accessible seizure focus, the fact that many of these techniques are destructive, the risk of surgical complications, and the fact that many patients are not candidates for these procedures. Therefore, given the fact that current surgical and medical techniques

cannot adequately treat a substantial population of epilepsy patients, new treatments must be developed.

Optogenetics: theory and current scientific uses

Optogenetics has greatly evolved over the past decades since the first opsin (light-sensitive protein) was discovered in 1971 in *Halobacterium salinarum*, a single-celled archaeon which uses the opsin to pump protons out into the extracellular environment, thus creating a gradient that drives ATP synthesis (Oesterhelt and Stoeckenius, 1971, 1973). The field of optogenetics was brought into the limelight in 2005 when the light-gated cation channel channelrhodopsin-2 (ChR2), from the unicellular green alga *Chlamydomonas reinhardtii,* was successfully expressed in cultured rat hippocampal neurons. With the expression of this protein, it became possible to activate transfected neurons on a millisecond timescale with simple photostimulation (Boyden et al., 2005). Since these early breakthroughs, the field of optogenetics has exploded, and now encompasses a wide array of light-gated excitatory channels, inhibitory pumps and channels, and G-protein coupled receptors (Figure 4.2). Two additional opsins which are especially relevant to epilepsy are the chloride pump halorhodopsin (NpHR), identified in the archaeon *Natronomonas pharaonis*, and the proton pump archaerhodopsin-3 (Arch) from the bacterium *Halorubrum sodomense* (Chow et al., 2010; Yizhar et al., 2011). The harnessing of natural opsins and engineering of novel opsins with desired kinetic properties, has led to unprecedented spatial, and temporal control of neuronal activity.

However, the real power of optogenetics lies in the ability to express these opsins selectively and specifically in spatially or genetically targeted populations of neurons. This allows the activation or silencing of nearly any imaginable definable neuronal population. There

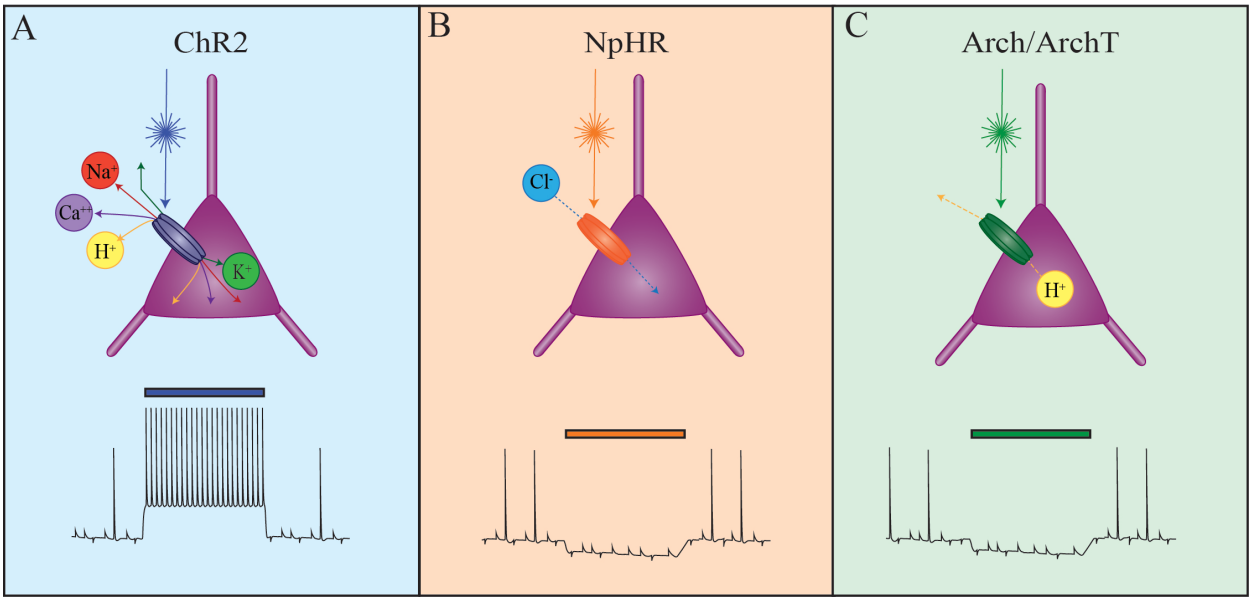

Figure 4.2. Optogenetic tools for light-mediated control of neuronal activity with high temporal precision. (A) Following light activation, the cation channel of channelrhodopsin-2 (ChR2) opens to allow passive movement of Na+, Ca2+, K+, and H+ ions down their respective electrochemical gradients (top) causing neurons to depolarize and fire action potentials (bottom, blue bar signifies time of light activation). (B) Halorhodopsin (NpHR) pumps Cl– into the cell (top) following light stimulation. At standard chloride concentrations, this results in hyperpolarization and a decrease in neuronal firing (bottom, orange bar signifies time of light activation). (C) When activated, archaerhodopsin (Arch/ArchT) pumps H+ out into the extracellular environment (top), thus suppressing cell firing and causing hyperpolarization (bottom, green bar signifies time of light activation). Note that Arch/ArchT may thus be particularly useful in epilepsy as it is not dependent on the reversal potential for chloride.

is a large, and ever expanding, repertoire of viral vectors and transgenic animals available for opsin targeting. One common method is to package an opsin into a viral vector (such as lentivirus or adeno-associated virus) that includes a fluorescent protein and a cell-type specific promoter. The virus is then injected into a target area, and only the neurons with cell bodies near the injection site will take up the virus and express the opsin. Not all promoters are amenable to viral delivery, however, and some situations call for expression of the opsin in a larger area than accessible by viral injection. Therefore, transgenic mouse lines are also available. A common strategy is to use a Cre recombinase-loxP targeting strategy. In this situation, one set of mice expresses Cre under a specific promoter (for example, in parvalbumin (PV)-expressing neurons). These are crossed to another set of mice which express an opsin in a Cre-dependent manner. The double mutants will express the opsin only under the control of the specific promoter. Further details of optogenetic targeting techniques as well as the different opsins have been reviewed in detail elsewhere (Krook-Magnuson and Soltesz, 2015; Paz and Huguenard, 2015; Yizhar et al., 2011).

Using optogenetics to control epileptiform events and to dissect critical microcircuits in epilepsy

One of the key advantages of optogenetic techniques in studying neurological disorders is that they are uniquely suited to dissect neuronal circuits. With optogenetic tools, researchers can selectively switch on or off specific elements of a circuit, one at a time, and thereby probe the effect of each element on total circuit function. In the context of epilepsy, this has been useful in examining the role of specific microcircuits in seizure dynamics, by permitting precise perturbation of neuronal subpopulations and investigating how it alters epileptiform activity.

The first study to demonstrate that epileptiform events could be controlled with optogenetic manipulation *in vitro* was published in 2009. NpHR was expressed in principal cells of hippocampal neurons, in organotypic slice culture. Optical silencing of either CA1 or CA3 pyramidal led to the suppression of stimulation-induced bursting in these slices (Tønnesen et al., 2009). Shortly after, it was shown that epileptiform activity could also be controlled in awake, behaving animals. More recently, in the intrahippocampal kainic acid model of temporal lobe epilepsy, spontaneous seizures were suppressed optogenetically, by inhibiting hippocampal principal cells as well as by exciting a subset of interneurons (Krook-Magnuson et al., 2014). Optogenetic inhibition of thalamocortical cells was able to suppress stroke-induced cortical seizures (Paz et al., 2013), and suppression of the activity of principal cells in a model of tetanus toxin-induced neocortical seizures could also abort electrographic seizures (Wykes et al., 2012).

These studies have been essential in demonstrating the potential for translational applications of optogenetics in seizure control. They have also set a basis for a wave of studies that have used similar experimental schemes to probe the contribution of various microcircuits in seizure activity and to test previously posited hypotheses in new ways. One such hypothesis, the dentate gate theory, proposes that the dentate gyrus normally acts as a "gate" to the rest of the hippocampus by shutting down incoming hyperexcitability before it can travel to the rest of the hippocampus. In epilepsy, however, it has been hypothesized that this gate breaks down and the normally quiescent granule cells are overexcited, thus allowing the spread of hyperexcitation throughout the hippocampus (Heinemann et al., 1992; Lothman et al., 1992). An experimental restoration of the dentate gate *in vivo* in epileptic mice was created by silencing selectively-labeled granule cells with NpHR. Consistent with the hypothesis, on-demand gate restoration was able to stop seizures (Krook-Magnuson et al., 2015). As additional proof, mimicking a

breakdown of the dentate gate via ChR2-induced excitation of granule cells, both in epileptic and in control animals, was able to exacerbate and induce seizures.

Understanding the contribution of interneuronal subpopulations in epilepsy has also been a major research focus. An often-posed hypothesis is that, in epilepsy, interneurons are no longer able to shut down out-of-control excitation because GABA can become excitatory during seizures. Supporting this theory, selective optogenetic activation of PV-positive interneurons led to depolarizing—i.e. excitatory—GABAergic events in CA3 pyramidal cells during low-magnesium-induced ictal events, due to a brief collapse in the Cl^- reversal potential. This contribution of PV cells to network excitability during ictal events was further validated by the fact that optical silencing of these cells leads to decreased afterdischarges during the ictal event (Ellender et al., 2014).

In addition to being a powerful approach for characterizing the role of various neuronal populations in seizure activity, optogenetic studies can also reveal detailed mechanistic information about circuit function and dysfunction, such as changes in synaptic transmission. In that respect, in a novel model of absence seizures, created by selectively deleting P/Q-type calcium channels in rhombic lip-derived neurons, optogenetic activation of cerebellar granule cells (GC) showed that the GC to Purkinje cell (PC) synaptic transmission was substantially reduced, implicating alterations to the GC to PC circuit in this type of seizure (Maejima et al., 2013). In another model of absence seizures triggered by deletion of the GluA4 AMPA receptor, the thalamocortical circuit's normal oscillatory action is perturbed, leading to pathologic seizures rather than physiologic spindles. Specific optogenetic labeling of different synapses within the circuit was utilized not only to show pathologic problems in the thalamocortical circuit during

seizures, but also to help map out the synaptic pathway involved in normal oscillations (Paz et al., 2011).

Finally, it is becoming clear that results obtained from optogenetic studies can have profound implications for future epilepsy treatments. For example, a set of recent studies showed that in temporal lobe epilepsy, seizures could be effectively stopped by optogenetically manipulating neurons in areas physically distant from the ictal focus. Targeting PV interneurons contralateral to the site of seizure initiation significantly curtailed seizure activity (Krook-Magnuson et al., 2014), and intervening at a site even as remote as the cerebellum could effectively stop seizures originating in the hippocampus (Krook-Magnuson et al., 2014). The cerebellum proved to be a powerful seizure "choke-point" in absence epilepsy as well (Kros et al., 2015), and in the case of cortical seizures, long-range thalamic projections to the cortex could be targeted to interrupt both electrographic and generalized seizures (Paz et al., 2013). These results highlight the feasibility of "remote seizure control," which could be a useful clinical strategy in cases where the seizure focus is unknown, diffuse, or not surgically approachable. Additionally, optogenetic control of seizures is not limited to chronic epilepsy but has been shown to be effective in postponing status epilepticus following the injection of a convulsant. By optically inactivating halorhodopsin expressing pyramidal cells, the onset of acute electrographic and behavioral seizures induced by injection of lithium-pilocarpine into the hippocampus of living rats, could be successfully delayed (Sukhotinsky et al., 2013).

Closed-loop optogenetic intervention

The studies demonstrating the success of optogenetics in the control of seizures *in vitro* and *in vivo* have greatly advanced the field, and another important leap towards the translational

usefulness of optogenetic technology would be a fully automated system. This type of system would offer advantages over most current treatments of epilepsy. Namely, an on-demand automated system (referred to as "closed-loop") would allow treatment to occur only during seizure initiation and at the location of a seizure focus, thus providing temporal and spatial specificity. Closed-loop optogenetic control involves a real-time readout of the neural activity recorded from electroencephalographic, electrocorticographic or electromyographic signals. Once a seizure is detected, the system immediately responds with the delivery of light to excite or inhibit the opsin-expressing cells and abort the seizure (Figure 4.3). In neurological disorders like epilepsy where abnormal activity occurs sporadically, such activity-guided intervention is extremely attractive as the optogenetic stimulation can be restricted to those precise moments when seizures occur, thus avoiding unnecessary perturbations during normal brain activity.

Various seizure detection systems for use with closed-loop optogenetics have been developed for studying rodent models of temporal lobe epilepsy and thalamocortical epilepsy (Armstrong et al., 2013; Krook-Magnuson et al., 2014; Paz et al., 2013). Since seizures in temporal lobe epilepsy are notoriously heterogeneous in terms of their electrophysiological signatures, it has been crucial to implement detection systems that are highly flexible and tunable. Typically, multiple characteristics of the recorded electrical signal, such as spike rate, amplitude, and frequency band power ratios, are simultaneously analyzed to distinguish the occurrence of a seizure from normal activity (Armstrong et al., 2013). Manual tuning of the detection thresholds and of the filter parameters is necessary to ensure the low occurrence of false-positive or missed triggers. Once a seizure starts, a brief integration period (<2 seconds) is usually sufficient to detect the pathological activity and respond. Using closed-loop optogenetic techniques, it was possible to drastically decrease seizure duration (57±14% reduction) in

epileptic animals through inhibition of hippocampal excitatory cells (Krook-Magnuson et al., 2014). Likewise, on-demand inhibition of thalamocortical cells in a stroke-induced model of focal cortical epilepsy has also been shown to be effective at suppressing spontaneous seizure activity (Paz et al., 2013). Like the new surgical technique of RNS as described above, these experimental models operated as a closed-loop system, but the advantage to an optogenetic system is that the optical fiber specifically activates a target set of neurons, whereas the RNS electrode activates all nearby neurons.

Clinical implications of optogenetic studies

Optogenetic-based seizure control has not only been helpful in teasing apart important microcircuits involved in ictal activity, but it has also enhanced our understanding of the therapeutic mechanisms underlying current clinical treatments such as electrical stimulation. It is unclear how clinically used electrical stimulation such as DBS and RNS interfere with ictogenenesis, due to the fact both of these techniques rely on global stimulation with nontransparent effects on individual cells or connections. Optogenetics can shed light on these mechanisms by allowing cell-specific stimulations in animal models, the effects of which can then be compared with those of the global stimulation generated by DBS. For example, in a 4-aminopyridine model of acute seizures, optogenetic stimulation of interneurons, at frequencies standardly used with DBS (Koubeissi et al., 2013), was sufficient to recapitulate the seizure suppressive effect observed when all neuronal cells were stimulated (Chiang et al., 2014; Ladas et al., 2015). Mechanistically, inhibitory cells appeared to initially induce a paradoxical excitatory bursting in pyramidal cells, which caused synchronization, and then ultimately suppression of pyramidal cell activity (Ladas et al., 2015). These results emphasize the role of

interneurons in network synchronization, and suggests that induction of the inhibitory network is a crucial aspect of DBS.

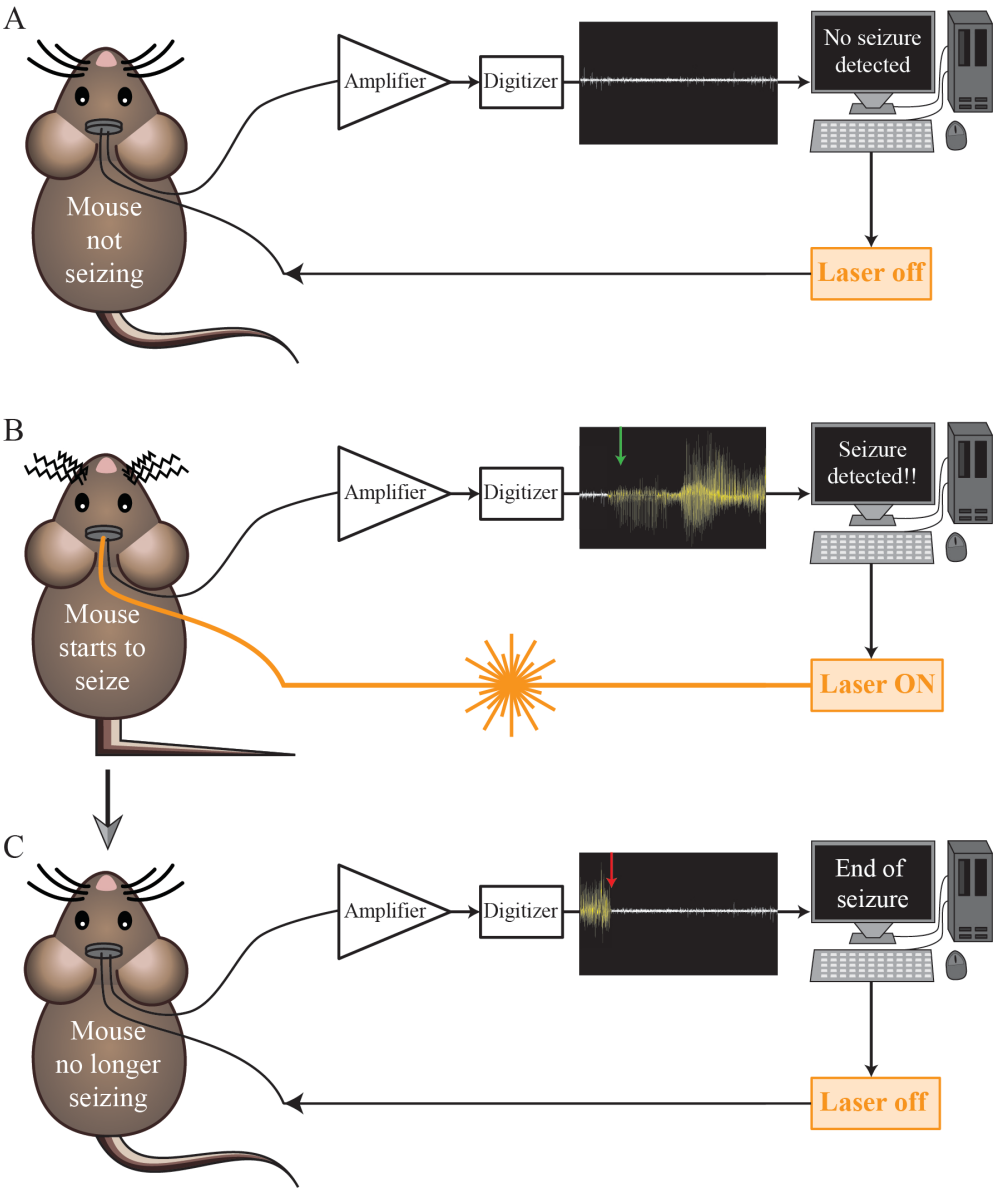

Figure 4.3. Closed-loop optogenetic seizure intervention in epileptic mouse models. An optrode (optical fiber + electrode) is implanted into the brain. Electrocorticographic (ECoG) activity from the rodent brain is recorded in real time. (A) At the resting state, no seizure is detected, and the optical fiber is not illuminated. (B) When the animal begins to seize, the hyperactivity and hypersynchrony is detected by the recording software (green arrow), and a predetermined pulse of light is delivered via the optical fiber to modulate the firing activity of opsin-expressing neurons and thereby stop the seizure. (C) The seizure terminates (red arrow), and the light is turned off.

On-demand seizures: characterizing circuit dynamics and pinpointing seizure propagation networks with optogenetics

Beyond its use as a tool to control seizures, an unexpectedly powerful application of optogenetics is in the generation of "artificial," light-inducible seizures. There are many ways to trigger seizures with optogenetic techniques. For example, patterned bursting in random networks of cultured ChR2-infected hippocampal neurons can be induced with pulsed optogenetic activation of the entire culture plate (El Hady et al., 2013). Additionally, pulsed stimulation leading to activation of ChR2 in rat hippocampus can lead to the successful induction of seizure-like afterdischarges (Osawa et al., 2013).

However, if the ultimate goal is treatment of epilepsy, what is the utility of causing or triggering seizures? From a microcircuit standpoint, finding neurons that can provoke seizures or cause hyperexcitatory activity may reveal information about mechanisms of ictogenesis. This strategy was instrumental in demonstrating the hippocampal dentate gyrus's place as a powerful node in the epileptic circuit, as excitation of granule cells increases seizure activity in mice (Krook-Magnuson et al., 2015), as well as in revealing that seizures caused by optical activation of PV-positive interneurons may be partially due to a transient breakdown in GABAergic signaling (Ellender et al., 2014), as described above.

In addition to pinpointing "guilty" elements in neuronal circuits, light-generated seizures have also been used as a way to create uniform seizures with well-defined initiation conditions. One of the most daunting challenges of epilepsy research lies in the heterogeneity and diversity of seizures and epilepsies. Even within a given epilepsy model, not all seizures are the same: seizures can differ in the location of initiation as well as in subsequent microcircuit dynamics. In fact, studies using two-photon microscopy or microelectrode arrays have shown that, in

experimental models as well as in human patients with epilepsy, such variability can occur even between sequential convulsive events within the same individual (Feldt Muldoon et al., 2013; Keller et al., 2010; Sabolek et al., 2012; Truccolo et al., 2011). For example, two-photon imaging of network dynamics looked at the activity of neurons in the hippocampal granule cell layer during epileptiform events. It was found that for epileptic animals, clusters of spatially localized cells were simultaneously recruited during large-scale network events (Feldt Muldoon et al., 2013). However, these synchronous neuronal clusters did not fire during each epileptiform event, and the clusters that were recruited varied from one event to another. This seizure-to-seizure variability can be a confounding factor when interpreting experimental data, as seizures that appear similar may in fact involve different pathways and respond differently to optogenetic perturbations. Therefore, optogenetically generated seizures which begin with a predetermined population of neurons offer an attractive approach to the investigation of underlying mechanisms, since they often have more reproducible and more stereotypic characteristics. These more tightly regulated, light-triggered seizures will be especially useful for investigating how and which networks are recruited upon seizure initiation.

An interesting study that took advantage of optogenetic seizure generation also utilized functional MRI to visualize network activity on a large scale (Weitz et al., 2014). High-frequency optogenetic stimulation of excitatory cells in the intermediate hippocampus led to widespread propagation of activity to cortical and subcortical brain regions. On the other hand, when cells in the dorsal hippocampus were excited, the activity remained restricted to the hippocampus. Since the rodent dorsal hippocampus is equivalent to the human posterior hippocampus, this result has interesting clinical implications. In human temporal lobe epilepsy, prior studies have shown that the posterior hippocampus is much less epileptogenetic and

undergoes far less severe anatomical and neurochemical perturbations than other areas of the hippocampus (Babb et al., 1984; Dam, 1980; King and Marsan, 1977; King et al., 1997). These results also highlight that the combination of optogenetics and functional MRI represents a promising method for the investigation of large-scale functional networks and for mapping brain regions that are more susceptible or resistant to seizure propagation.

Microcircuits affecting seizure dynamics or seizure dynamics affecting microcircuits?

Thanks to optogenetics, we are gaining a more complete picture of the circuit components involved in epileptic activity. However, it is also becoming increasingly clear that a static approach to circuit dissection might not be sufficient to develop a thorough understanding of the disorder. Seizures are inherently dynamic and so the effect of a given optogenetic perturbation does not depend solely on which connections are controlled, but also on the location of intervention with respect to the seizure focus as well as on the time of intervention with respect to ongoing brain processes.

One of the most notable illustrations of the need for awareness of the spatial context of an intervention concerns the role of interneurons in seizure activity. In a cortical slice model of focal epilepsy, exciting parvalbumin (PV)-positive interneurons had opposite effects on seizure activity depending on the proximity of the cells to the seizure focus. When PV interneurons at the seizure focus were stimulated, this induced a post-inhibitory rebound spiking in pyramidal cells, ultimately enhancing neuronal synchrony and promoting seizure generation. In contrast, activation of PV cells distant from the focus instead blocked ictal propagation and significantly shortened the seizure duration (Sessolo et al., 2015). Therefore, stimulation of even the same

type of GABAergic neurons can have antagonistic effects on epileptiform activity depending on the exact location of the stimulation with respect to the seizure focus.

Likewise, the temporal context of the light intervention with respect to the network dynamics has been shown to be important. For instance, optogenetic stimulation of GABAergic neurons in entorhinal cortical slices perfused with the pro-convulsant compound 4-aminopyridine was sufficient to elicit interictal spikes or preictal discharges that were followed by tonic-clonic seizure-like events (Yekhlef et al., 2015). However, stimulation of the same neurons after the seizure had already begun did not alter the ongoing seizure activity. Even more interestingly, a recent report revealed the outcome of activating principal cells is strongly correlated to the brain state prior to stimulation. Light delivery to ChR2-expressing cortical pyramidal cells was shown to trigger convulsive activity in a rodent model of absence seizures (Wagner et al., 2015). Interestingly, seizures were not induced during every trial, and the occurrence of a seizure could be predicted by the power of the local field potential (LFP) oscillations immediately prior to the stimulation. Increased LFP power directly correlated with increased probability of seizure occurrence. This result corresponds well with the observation that in animal models as well as in humans, seizures occur mostly during certain behavioral states, such as drowsiness or light sleep. Additionally, it has been shown that optogenetic intervention to terminate seizures is much more effective in behavioral states exhibiting theta rhythm (such as slow-wave sleep) than in non-theta states (such as waking or REM sleep) (Ewell et al., 2015).

Taken together, the results of these optogenetic studies clearly highlight the fact that the potential of a given targeted microcircuit for seizure control or induction is not a static property of the microcircuit itself, but is also a function of the state of network and the overall brain

activity. In light of these results, it is expected that our understanding and treatment of epilepsy will greatly benefit from assays that combine optogenetics (as a way to engage or disengage specific neuronal populations) with technologies that can report on network dynamics during and in between seizures. Advances are being made in functional MRI (as discussed earlier) as well as in electrical recording capabilities. Studies using miniaturized electrocorticography (ECoG) arrays have demonstrated the ability to precisely record temporally and spatially discrete signals (Richner et al., 2014; Wang et al., 2010). A novel optrode has also been constructed, in which the electrode portion of the optrode is a multi-electrode array, which allows for seizure detection in a larger spatial area (Zhang et al., 2009). Genetic Absence Epilepsy Rats from Strasbourg (GAERS) rats were injected with virus, so that they would express ChR2 in neocortical neurons. The MEA-optrode construct was subsequently implanted above the injection site, and this device was successfully used to detect and stop spontaneous absence seizures. Another technique that exists but has not yet been used in the context of epilepsy is fiber photometry, which allows the recording of spatially and genetically well-defined neurons by measuring Ca^{2+} transients (Grosenick et al., 2015; Gunaydin et al., 2014).

Importantly, better understanding of the relationship between the network state and the effect of optogenetic manipulation of specific neuronal connections or populations will likely open doors for more efficient seizure control schemes. Closed-loop seizure control, for instance, could be improved by integrating information, in real-time, from EEG recordings with a wider array of signals, such as fMRI or multielectrode array recordings. Rather than simply applying light intervention based on a binary signal (seizure/no seizure), one could personalize the response to each seizure in an activity-guided manner.

Future directions

The final common goal for all epilepsy research is not just to understand the circuits involved or to stop seizures in mice, but to apply these lessons to humans in order to treat epilepsy patients, especially the thirty percent who are refractory to AEDs. The obvious "holy grail" of translating optogenetic techniques into humans would be a closed-loop implantable system using on-demand light stimulation to stop seizures. Under ideal circumstances, the seizure would be aborted while it was only still electrographic, i.e. without any behavioral manifestations. Overall, the system setup would be similar to DBS or RNS and would work like so: an optrode would be permanently implanted into the patient's brain at their specific seizure focus. The optrode lead would then be tunneled under the skin and connected to a power source/light source, which could be implanted either in the skull (like RNS) or under the skin in a subclavicular location (like DBS and VNS). The seizure-detecting microchip would likely be housed with the power source and light source. The electrode portion of the optrode would detect the beginning of the seizure and then the light would be activated through the optical fiber portion of the optrode at a particular frequency and duration to stop the progression of the seizure. Ideally, like RNS, this human closed-loop optogenetic system would be customizable as far as seizure detection parameters and light activation parameters (Figure 4.3).

Unfortunately, there are major hurdles that lie between where we are today (closed-loop system in rodents) and the finish line. Technical challenges abound, and there are several questions regarding feasibility that remain to be addressed. First of all, given that the volume of the human brain is over one thousand times larger than the mouse brain, will the volume of tissue that can be illuminated by an optical fiber be sufficient to stop an entire human epileptic network? If a large volume of tissue is successfully activated, will this end up heating and

damaging the neurons (Pavlov et al., 2013)? In order for the system to be fully implantable, a laser or LED must be engineered, along with an associated power source, which is small and lightweight enough to be implantable. The power source will have to have sufficient power to last a few years before replacement, or be rechargeable through the skin. The optical fiber will have to be flexible enough to hold up to the patient's head and neck movement without breaking. The optrode will have to be non-immunogenic.

Scientific challenges remain as well. To induce opsin expression in the human brain, genetically based Cre recombinase-loxP systems will not be usable. Two other possibilities do exist with current technology to induce opsin expression in human neurons: viral vectors to induce opsin expression under specific promoters, and injection of pre-transfected neuronal stem cells. Will either of these techniques be safe and successful with human tissue? Will institutional review boards approve the injection of mutational viruses or engineered stem cells into human patients with a non-fatal disease? If a viral method is chosen, will transfection be stable over decades of the patient's life (Fisher, 2012)?

There are some answers from animal studies which suggest that the answer to at least some of the above questions is "yes." Although we are not aware of any human studies, there have been a few papers demonstrating feasibility of functional opsins in primates. Recent studies have demonstrated the feasibility of functional expression of various opsins in macaque cortex, with appropriate electrophysiological responses, stable expression over months, and a labeled volume of 1 mm^3 per injection (Diester et al., 2011; Han et al., 2009). Demonstrating a behavioral effect of optogenetic activation of neurons seems to be more challenging in primates. However, it has been shown that by activating the frontal eye fields or visual cortex of macaques with ChR2, functional saccades could be induced or altered (Gerits et al., 2012; Jazayeri et al.,

2012). Other promising advances include the fabrication of MRI-compatible carbon fiber optrodes, which would improve the clinical utility in human subjects (Duffy et al., 2015). Computer modeling studies in meso-scale model of the human cortex also demonstrated feasibility of using optogenetic control to suppress seizures (Selvaraj et al., 2013).

Although injecting stem cells into human brain that could then be activated by light to stop seizures might seem like futuristic technology, it might not be so far-fetched. It has been shown that implanted embryonic medial ganglionic eminence (MGE) cells into the hippocampus of pilocarpine-treated epileptic mice develop into functional inhibitory interneurons, and that this treatment greatly reduces the seizure frequency in these animals (Hunt et al., 2013). In pilocarpine-induced temporal lobe epilepsy in mice, ChR2-expressing MGE-cells were successfully transplanted into the dentate gyrus. Mice receiving these transplants had fewer seizures, and light-induced IPSCs were recorded in native dentate granule cells (Henderson et al., 2014). In another study, long-term self-renewing neuroepithelial stem cells (ltNES) were successfully generated from human fibroblasts and then injected into the hippocampus of nude rats. By stimulating ChR2-labeled rat neurons, this group was able to evoke EPSPs in the human ltNES-derived neurons (Avaliani et al., 2014). To move this into a translational application, one would want to have the human stem-cell derived neurons express ChR2. In this way, it might be feasible to inject human ChR2-expressing stem cells that would develop into GABAergic neurons into the seizure focus. An optrode would be implanted simultaneously. Once the stem cells matured into functional neurons, the optrode would use closed-loop technology to detect the seizure and then optogenetically activate a large quantity of GABAergic neurons, thereby shutting down the seizure before any behavioral manifestation occurs. This would be a clever way to get the ChR2 cells into human brain without having to use a viral vector (Figure 4.4).

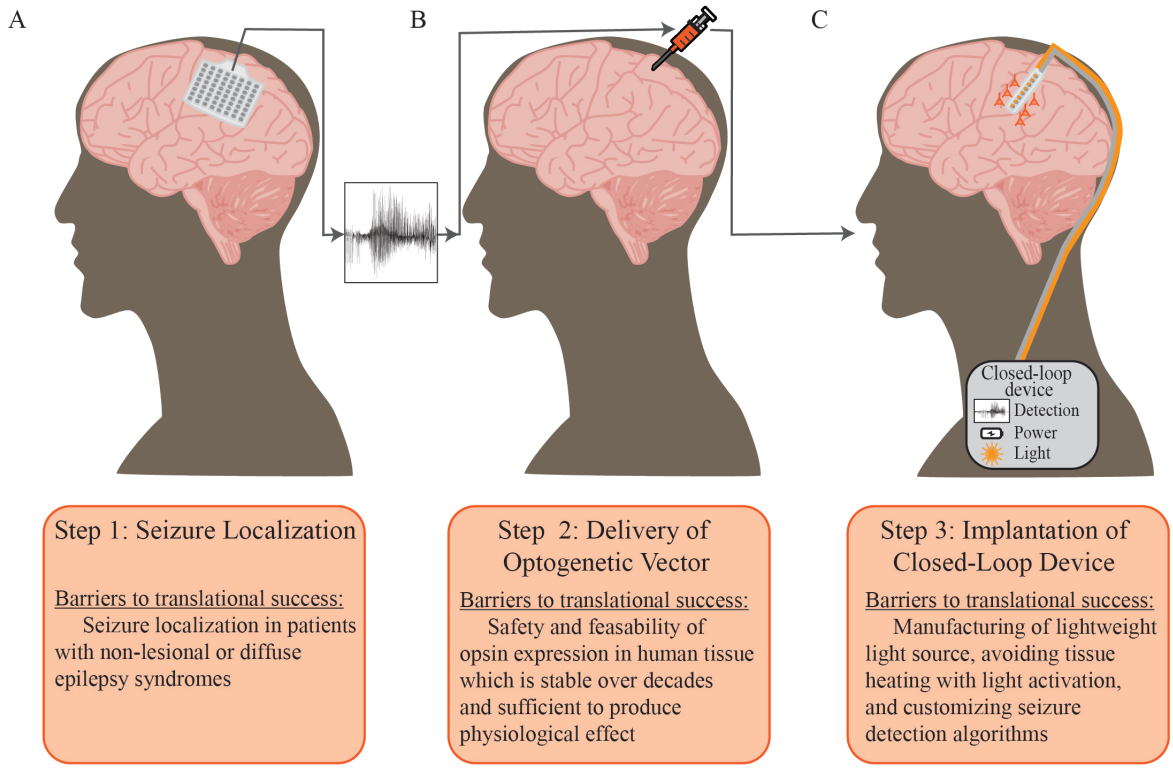

Figure 4.4. A theoretical model of closed-loop optogenetic seizure control in humans. (A) The first step would be localization of the seizure focus. This could be performed via standard phase II monitoring (see text), but in the future this step could possibly be avoided in some patients if long-distance targets, or targets based on seizure semiology or genetics are identified. (B) The next step is to deliver the optogenetic vector to the seizure focus. This could be accomplished with a viral vector carrying an opsin gene, which would then transfect the patient's own neurons, or by pretransfecting neurons (or neuronal precursors) with opsins, which would then functionally integrate into the patient's brain. (C) An optrode construct (gray denotes electrodes for recording, orange denotes optical fibers) is implanted in the brain at the seizure focus or remote target. The electrical leads and optical fibers are tunneled under the skin to the "control box" which is implanted in a subclavicular location. This control box would contain a seizure-detecting microchip, a power source, and a light source. Its function would be to detect seizures quickly and then turn on the light source to activate or silence specific neurons, thereby stopping the seizure. As noted in the figure, ethical concerns with such a system include the safety of injecting optogenetic viral vectors or transfected neuronal precursors into the human brain. Technical challenges include being able to optogenetically activate a sufficient volume of tissue to cause a physiological response, the stability of optogenetic expression in neurons over decades of the patient's life, manufacturing a compact and lightweight light source, customizing seizure detection algorithms, and avoiding tissue heating with light activation.

It is possible that, due to all of these technical and ethical hurdles, optogenetics will never be directly translated into human clinical use. However, this does not diminish the importance or relevance of using optogenetic techniques to study seizures in animal models. As discussed above, optogenetics present a unique tool for determining what changes can lead to dysfunctional networks. Understanding more about the mechanisms of epileptogenesis, seizure initation, and pathologic seizure circuitry could open up avenues to develop other treatments for epilepsy. For example, if a particular interneuronal subtype can be manipulated to stop seizures, perhaps a biologic drug could be developed to similarly modulate these neurons in humans. If clinically relevant models of epilepsy are studied and a stimulation target is found which can stop the seizure, perhaps an RNS electrode could be placed into that target. Finally, if seizure models are studied which are based on human genetic epilepsies, perhaps new drug targets may be found as well.

CHAPTER 5

Conclusions and Future Directions

This work provides insight into the role of the hippocampus in normal brain processes and how pathological alterations in the circuitry can lead to seizures and cognitive impairments in temporal lobe epilepsy. In chapter 1, we discussed various microcircuit alterations that occur in epilepsy, and how network theory and computational modeling can help unravel and better understand these complex changes. We also described findings using electrophysiology and two-photon imaging that reveal the complex interplay of different neurons and connections during seizure-like activity. In chapters 2 and 3, we focused on the hippocampal dentate gyrus and shed light on two very important cell populations in the region, hilar mossy cells and granule cells. Mossy cells have long been an enigmatic cell population in the hippocampus, as a large hurdle was to be able to selectively target the cells. Using a combination of optogenetics, real-time seizure detection and intervention, and behavioral tests, we showed that mossy cells are critical in controlling seizure propagation throughout the brain as well as in learning and memory. We also found that restoration of the dentate gyrus to a hyperpolarized state by photoinhibiting granule cells was able to successfully curtail electrographic seizures. Finally, in chapter 4, we discussed the currently available treatments for epilepsy, and explore how optogenetic techniques can enable the development of more therapeutical approaches or may one day become itself a treatment for epilepsy.

These findings open exciting new avenues for future work investigating exactly how these neurons and circuits behave during normal brain processes as well as during seizures.

As Eve Marder, Professor at Brandeis University, perfectly put it, "Optogenetics reveals what neurons *can* do, not what they *do* do." There is increasing evidence for the heterogeneity of cell populations, even within the same family. This has been the case, for example, during once-thought repetitive states such as seizures, where it was found that even for sequential seizures or seizure-like events, different populations of neurons were recruited each time (Feldt et al., 2011; Feldt Muldoon et al., 2013; Keller et al., 2010; Truccolo et al., 2011). Therefore, it would be informative to specifically measure and record the activity of the various cell populations, including mossy cells and granule cells of the hippocampal dentate gyrus, in epilepsy as well as during normal behavior. New methods and technologies are continuously being developed that would allow such measurements, such as fiber photometry (Gunaydin et al., 2014) and two-photon microscopy (Danielson et al., 2017). Fiber photometry, with its advantages of being light-weight, stable, and minimally disruptive, would be useful monitoring of cell dynamics during exploratory behavioral processes requiring freely moving animals, or for chronic recordings to study seizure activity.

Finally, it will be important and useful to take such studies a step further and investigate how different cell populations interact during normal and abnormal brain processes. We have shown that mossy cells play a key role in the propagation of seizure activity, but exactly how their activity influences the activity of other cells in the dentate gyrus during seizures remains an outstanding question. Likewise, we have found that mossy cells are necessary for the encoding of spatial information and other studies have pointed to the involvement of granule cells in contextual memory encoding (Kheirbek et al., 2013; Zhuo et al., 2016). An important next step will be to understand how these cells interact with each other, as well as with other cells in the hippocampus, to encode information. With the continual and rapid technological advancements,

it is becoming possible to simultaneous manipulate and record from specific and different neuronal populations. Such findings may have wide implications and may open new avenues in the development of new therapeutical approaches for epilepsy as well as for the comorbid cognitive impairments often observed in this neurological disorder.

REFERENCES

Alme, C.B., Buzzetti, R.A., Marrone, D.F., Leutgeb, J.K., Chawla, M.K., Schaner, M.J., Bohanick, J.D., Khoboko, T., Leutgeb, S., Moser, E.I., et al. (2010). Hippocampal granule cells opt for early retirement. Hippocampus *20*, 1109–1123.

Aravanis, A.M., Wang, L.-P., Zhang, F., Meltzer, L.A., Mogri, M.Z., Schneider, M.B., and Deisseroth, K. (2007). An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. J. Neural Eng. *4*, S143-56.

Armstrong, C., Krook-Magnuson, E., Oijala, M., and Soltesz, I. (2013). Closed-loop optogenetic intervention in mice. Nat. Protoc. *8*, 1475–1493.

Avaliani, N., Sørensen, A.T., Ledri, M., Bengzon, J., Koch, P., Brüstle, O., Deisseroth, K., Andersson, M., and Kokaia, M. (2014). Optogenetics Reveal Delayed Afferent Synaptogenesis on Grafted Human-Induced Pluripotent Stem Cell-Derived Neural Progenitors. Stem Cells *32*, 3088–3098.

Babb, T.L., Brown, W.J., Pretorius, J., Davenport, C., Lieb, J.P., and Crandall, P.H. (1984). Temporal lobe volumetric cell densities in temporal lobe epilepsy. Epilepsia *25*, 729–740.

Banerjee, P.N., Filippi, D., and Allen Hauser, W. (2009). The descriptive epidemiology of epilepsy—A review. Epilepsy Res. *85*, 31–45.

Bartus, R.T., Weinberg, M.S., and Samulski, R.J. (2014). Parkinson's disease gene therapy: success by design meets failure by efficacy. Mol. Ther. *22*, 487–497.

Ben-Ari, Y. (2001). Developing networks play a similar melody. Trends Neurosci. *24*, 353–360.

Bentley, J.N., Chestek, C., Stacey, W.C., and Patil, P.G. (2013). Optogenetics in epilepsy. Neurosurg. Focus *34*, E4.

Berényi, A., Belluscio, M., Mao, D., Buzsáki, G., Berenyi, a., Belluscio, M., Mao, D., and Buzsaki, G. (2012). Closed-loop control of epilepsy by transcranial electrical stimulation. Science *337*, 735–737.

Berglind, F., Ledri, M., Sørensen, A.T., Nikitidou, L., Melis, M., Bielefeld, P., Kirik, D., Deisseroth, K., Andersson, M., and Kokaia, M. (2014). Optogenetic inhibition of chemically induced hypersynchronized bursting in mice. Neurobiol. Dis. *65C*, 133–141.

Berndt, A., Lee, S.Y., Ramakrishnan, C., and Deisseroth, K. (2014). Structure-guided transformation of channelrhodopsin into a light-activated chloride channel. Science *344*, 420–424.

Beudel, M., and Brown, P. (2015). Adaptive deep brain stimulation in Parkinson's disease. Parkinsonism Relat. Disord. 1–4.

Bezaire, M.J., and Soltesz, I. (2013). Quantitative assessment of CA1 local circuits: knowledge base for interneuron-pyramidal cell connectivity. Hippocampus *23*, 751–785.

Blümcke, I., Suter, B., Behle, K., Kuhn, R., Schramm, J., Elger, C.E., and Wiestler, O.D. (2000). Loss of hilar mossy cells in Ammon's horn sclerosis. Epilepsia *41 Suppl 6*, S174-80.

Bonifazi, P., Goldin, M., Picardo, M. a, Jorquera, I., Cattani, a, Bianconi, G., Represa, a, Ben-Ari, Y., and Cossart, R. (2009). GABAergic hub neurons orchestrate synchrony in developing hippocampal networks. Science *326*, 1419–1424.

Boyden, E.S. (2011). Optogenetics: using light to control the brain. Cerebrum *2011*, 16.

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. Nat. Neurosci. *8*, 1263–1268.

Bragin, A., Engel, J., Wilson, C.L., Vizentin, E., and Mathern, G.W. (1999). Electrophysiologic analysis of a chronic seizure model after unilateral hippocampal KA injection. Epilepsia *40*, 1210–1221.

Brodie, M.J., Shorvon, S.D., Canger, R., Halász, P., Johannessen, S., Thompson, P., Wieser, H.G., and Wolf, P. (1997). Commission on European Affairs: appropriate standards of epilepsy care across Europe.ILEA. Epilepsia *38*, 1245–1250.

Brun, V.H., Otnass, M.K., Molden, S., Steffenach, H.-A., Witter, M.P., Moser, M.-B., and Moser, E.I. (2002). Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry. Science *296*, 2243–2246.

Buckmaster, P.S., Wenzel, H.J., Kunkel, D.D., and Schwartzkroin, P.A. (1996). Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. J. Comp. Neurol. *366*, 270–292.

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. Nat. Rev. Neurosci. *10*, 186–198.

Buzsáki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. Science *304*, 1926–1929.

Buzsáki, G., Geisler, C., Henze, D. a, and Wang, X.-J. (2004). Interneuron Diversity series: Circuit complexity and axon wiring economy of cortical interneurons. Trends Neurosci. *27*, 186–193.

Buzsáki, G., Anastassiou, C. a, and Koch, C. (2012). The origin of extracellular fields and

currents--EEG, ECoG, LFP and spikes. Nat. Rev. Neurosci. *13*, 407–420.

Buzsàki, G., and Eidelberg, E. (1981). Commissural projection to the dentate gyrus of the rat: evidence for feed-forward inhibition. Brain Res. *230*, 346–350.

Case, M., and Soltesz, I. (2011). Computational modeling of epilepsy. Epilepsia *52 Suppl 8*, 12–15.

Chawla, M.K., Guzowski, J.F., Ramirez-Amaya, V., Lipa, P., Hoffman, K.L., Marriott, L.K., Worley, P.F., McNaughton, B.L., and Barnes, C.A. (2005). Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience. Hippocampus *15*, 579–586.

Chiang, C.-C., Ladas, T.P., Gonzalez-Reyes, L.E., and Durand, D.M. (2014). Seizure Suppression by High Frequency Optogenetic Stimulation Using In Vitro and In Vivo Animal Models of Epilepsy. Brain Stimul. *7*, 890–899.

Choi, H., Heiman, G., Pandis, D., Cantero, J., Resor, S.R., Gilliam, F.G., and Hauser, W.A. (2008). Seizure remission and relapse in adults with intractable epilepsy: A cohort study. Epilepsia *49*, 1440–1445.

Chow, B.Y., Han, X., Dobry, A.S., Qian, X., Chuong, A.S., Li, M., Henninger, M.A., Belfort, G.M., Lin, Y., Monahan, P.E., et al. (2010). High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature *463*, 98–102.

Chuong, A.S., Miri, M.L., Busskamp, V., Matthews, G. a C., Acker, L.C., Sørensen, A.T., Young, A., Klapoetke, N.C., Henninger, M. a, Kodandaramaiah, S.B., et al. (2014). Noninvasive optical inhibition with a red-shifted microbial rhodopsin. Nat. Neurosci. *17*, 1123–1129.

Coulter, D. (2004). Functional Consequences of Hilar Mossy Cell Loss in TLE: Proepileptic or Antiepileptic? Jbjs.org.

Coulter, D., Yue, C., Ang, C.W., Weissinger, F., Goldberg, E., Hsu, F.-C., Carlson, G.C., and Takano, H. (2011). Hippocampal microcircuit dynamics probed using optical imaging approaches. J. Physiol. *589*, 1893–1903.

Curry, D.J., Gowda, A., McNichols, R.J., and Wilfong, A. a. (2012). MR-guided stereotactic laser ablation of epileptogenic foci in children. Epilepsy Behav. *24*, 408–414.

Cymerblit-Sabba, A., and Schiller, Y. (2010). Network dynamics during development of pharmacologically induced epileptic seizures in rats in vivo. J. Neurosci. *30*, 1619–1630.

Dam, A. (1980). Epilepsy and neuron loss in the hippocampus. Epilepsia *21*, 617–629.

Dani, A., Huang, B., Bergan, J., Dulac, C., and Zhuang, X. (2010). Superresolution imaging of

chemical synapses in the brain. Neuron *68*, 843–856.

Danielson, N.B., Turi, G.F., Ladow, M., Chavlis, S., Petrantonakis, P.C., Poirazi, P., and Losonczy, A. (2017). In Vivo Imaging of Dentate Gyrus Mossy Cells in Behaving Mice. Neuron *93*, 552–559.e4.

Deisseroth, K. (2011). Optogenetics. Nat. Methods *8*, 26–29.

Dhakal, K.R., Gu, L., Shivalingaiah, S., Dennis, T.S., Morris-Bobzean, S.A., Li, T., Perrotti, L.I., and Mohanty, S.K. (2014). Non-scanning fiber-optic near-infrared beam led to two-photon optogenetic stimulation in-vivo. PLoS One *9*, e111488.

Diester, I., Kaufman, M.T., Mogri, M., Pashaie, R., Goo, W., Yizhar, O., Ramakrishnan, C., Deisseroth, K., and Shenoy, K. V (2011). An optogenetic toolbox designed for primates. Nat. Neurosci. *14*, 387–397.

Drane, D.L., Loring, D.W., Voets, N.L., Price, M., Ojemann, J.G., Willie, J.T., Saindane, A.M., Phatak, V., Ivanisevic, M., Millis, S., et al. (2015). Better object recognition and naming outcome with MRI-guided stereotactic laser amygdalohippocampotomy for temporal lobe epilepsy. Epilepsia *56*, 101–113.

Dudok, B., Barna, L., Ledri, M., Szabo, S.I., Szabaditis, E., Pinter, B., Woodhams, S.G., Henstridge, C.M., Balla, G.Y., Nyilas, R., et al. (2014). Cell type-specific STORM superresolution imaging reveals nanoscale organization of cannabinoid signaling at hippocampal GABAergic synapses. Nat. Neurosci. *in press*.

Duffy, B.A., Choy, M., Chuapoco, M.R., Madsen, M., and Lee, J.H. (2015). MRI compatible optrodes for simultaneous LFP and optogenetic fMRI investigation of seizure-like afterdischarges. Neuroimage *123*, 173–184.

Duncan, J.S., Sander, J.W., Sisodiya, S.M., and Walker, M.C. (2006). Adult epilepsy. Lancet *367*, 1087–1100.

Dyhrfjeld-Johnsen, J., Santhakumar, V., Morgan, R.J., Huerta, R., Tsimring, L., and Soltesz, I. (2007). Topological determinants of epileptogenesis in large-scale structural and functional models of the dentate gyrus derived from experimental data. J. Neurophysiol. *97*, 1566–1587.

Ellender, T.J., Raimondo, J. V, Irkle, A., Lamsa, K.P., and Akerman, C.J. (2014). Excitatory effects of parvalbumin-expressing interneurons maintain hippocampal epileptiform activity via synchronous afterdischarges. J. Neurosci. *34*, 15208–15222.

Esquenazi, Y., Kalamangalam, G.P., Slater, J.D., Knowlton, R.C., Friedman, E., Morris, S.-A., Shetty, A., Gowda, A., and Tandon, N. (2014). Stereotactic laser ablation of epileptogenic periventricular nodular heterotopia. Epilepsy Res. *108*, 547–554.

Ewell, L.A., Liang, L., Armstrong, C., Soltész, I., Leutgeb, S., and Leutgeb, J.K. (2015). Brain State Is a Major Factor in Preseizure Hippocampal Network Activity and Influences Success of Seizure Intervention. J. Neurosci. *35*, 15635–15648.

Feldt, S., Bonifazi, P., and Cossart, R. (2011). Dissecting functional connectivity of neuronal microcircuits: experimental and theoretical insights. Trends Neurosci. *34*, 225–236.

Feldt Muldoon, S., Soltesz, I., and Cossart, R. (2013). Spatially clustered neuronal assemblies comprise the microstructure of synchrony in chronically epileptic networks. Proc. Natl. Acad. Sci. *110*, 3567–3572.

Fisher, R.S. (2012). Therapeutic devices for epilepsy. Ann. Neurol. *71*, 157–168.

Fisher, R., Salanova, V., Witt, T., Worth, R., Henry, T., Gross, R., Oommen, K., Osorio, I., Nazzaro, J., Labar, D., et al. (2010). Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. Epilepsia *51*, 899–908.

Fornito, A., Zalesky, A., and Breakspear, M. (2013). Graph analysis of the human connectome: promise, progress, and pitfalls. Neuroimage *80*, 426–444.

Fries, P. (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. Annu. Rev. Neurosci. *32*, 209–224.

Gabriel, S., Njunting, M., Pomper, J.K., Merschhemke, M., Sanabria, E.R.G., Eilers, A., Kivi, A., Zeller, M., Meencke, H.-J., Cavalheiro, E.A., et al. (2004). Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. J. Neurosci. *24*, 10416–10430.

Gerits, A., Farivar, R., Rosen, B.R., Wald, L.L., Boyden, E.S., and Vanduffel, W. (2012). Optogenetically Induced Behavioral and Functional Network Changes in Primates. Curr. Biol. *22*, 1722–1726.

Ghosh, K.K., Burns, L.D., Cocker, E.D., Nimmerjahn, A., Ziv, Y., Gamal, A. El, and Schnitzer, M.J. (2011). Miniaturized integration of a fluorescence microscope. Nat. Methods *8*, 871–878.

GoodSmith, D., Chen, X., Wang, C., Kim, S.H., Song, H., Burgalossi, A., Christian, K.M., and Knierim, J.J. (2017). Spatial Representations of Granule Cells and Mossy Cells of the Dentate Gyrus. Neuron *93*, 677–690.e5.

Gradinaru, V., Mogri, M., Thompson, K.R., Henderson, J.M., and Deisseroth, K. (2009). Optical deconstruction of parkinsonian neural circuitry. Science *324*, 354–359.

Gradinaru, V., Zhang, F., Ramakrishnan, C., Mattis, J., Prakash, R., Diester, I., Goshen, I., Thompson, K.R., and Deisseroth, K. (2010). Molecular and cellular approaches for diversifying and extending optogenetics. Cell *141*, 154–165.

Grosenick, L., Marshel, J.H., and Deisseroth, K. (2015). Closed-Loop and Activity-Guided Optogenetic Control. Neuron *86*, 106–139.

Gunaydin, L.A., Grosenick, L., Finkelstein, J.C., Kauvar, I. V., Fenno, L.E., Adhikari, A., Lammel, S., Mirzabekov, J.J., Airan, R.D., Zalocusky, K. a., et al. (2014). Natural neural projection dynamics underlying social behavior. Cell *157*, 1535–1551.

El Hady, A., Afshar, G., Bröking, K., Schlüter, O.M., Geisel, T., Stühmer, W., and Wolf, F. (2013). Optogenetic stimulation effectively enhances intrinsically generated network synchrony. Front. Neural Circuits *7*, 167.

Halabisky, B., Parada, I., Buckmaster, P.S., and Prince, D.A. (2010). Excitatory input onto hilar somatostatin interneurons is increased in a chronic model of epilepsy. J. Neurophysiol. *104*, 2214–2223.

Han, X., Qian, X., Bernstein, J.G., Zhou, H., Franzesi, G.T., Stern, P., Bronson, R.T., Graybiel, A.M., Desimone, R., and Boyden, E.S. (2009). Millisecond-Timescale Optical Control of Neural Dynamics in the Nonhuman Primate Brain. Neuron *62*, 191–198.

Hawasli, A.H., Bagade, S., Shimony, J.S., Miller-Thomas, M., and Leuthardt, E.C. (2013). Magnetic Resonance Imaging-Guided Focused Laser Interstitial Thermal Therapy for Intracranial Lesions. Neurosurgery *73*, 1007–1017.

Heck, C.N., King-Stephens, D., Massey, A.D., Nair, D.R., Jobst, B.C., Barkley, G.L., Salanova, V., Cole, A.J., Smith, M.C., Gwinn, R.P., et al. (2014). Two-year seizure reduction in adults with medically intractable partial onset epilepsy treated with responsive neurostimulation: Final results of the RNS System Pivotal trial. Epilepsia *55*, 432–441.

Heinemann, U., Beck, H., Dreier, J.P., Ficker, E., Stabel, J., and Zhang, C.L. (1992). The dentate gyrus as a regulated gate for the propagation of epileptiform activity. Epilepsy Res. Suppl. *7*, 273–280.

Henderson, K.W., Gupta, J., Tagliatela, S., Litvina, E., Zheng, X., Van Zandt, M.A., Woods, N., Grund, E., Lin, D., Royston, S., et al. (2014). Long-Term Seizure Suppression and Optogenetic Analyses of Synaptic Connectivity in Epileptic Mice with Hippocampal Grafts of GABAergic Interneurons. J. Neurosci. *34*, 13492–13504.

Hirtz, D., Thurman, D.J., Gwinn-Hardy, K., Mohamed, M., Chaudhuri, a. R., and Zalutsky, R. (2007). How common are the "common" neurologic disorders? Neurology *68*, 326–337.

Hochbaum, D.R., Zhao, Y., Farhi, S.L., Klapoetke, N., Werley, C. a, Kapoor, V., Zou, P., Kralj, J.M., Maclaurin, D., Smedemark-Margulies, N., et al. (2014). All-optical electrophysiology in mammalian neurons using engineered microbial rhodopsins. Nat. Methods *11*.

Houser, C.R. (1990). Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. Brain Res. *535*, 195–204.

Houser, C.R. (1999). Neuronal loss and synaptic reorganization in temporal lobe epilepsy. Adv. Neurol. *79*, 743–761.

Howard, A., and Neu, A. (2007). Opposing modifications in intrinsic currents and synaptic inputs in post-traumatic mossy cells: evidence for single-cell homeostasis in a hyperexcitable network. J. Neurophysiol. 2394–2409.

Hsu, T.-T., Lee, C.-T., Tai, M.-H., and Lien, C.-C. (2015). Differential Recruitment of Dentate Gyrus Interneuron Types by Commissural Versus Perforant Pathways. Cereb. Cortex 1–13.

Hu, H., Gan, J., and Jonas, P. (2014). Fast-spiking, parvalbumin+ GABAergic interneurons: From cellular design to microcircuit function. Science (80-.). *345*, 1255263–1255263.

Hunt, R.F., Girskis, K.M., Rubenstein, J.L., Alvarez-Buylla, A., and Baraban, S.C. (2013). GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. Nat. Neurosci. *16*, 692–697.

Hyde, R. a, and Strowbridge, B.W. (2012). Mnemonic representations of transient stimuli and temporal sequences in the rodent hippocampus in vitro. Nat. Neurosci. *15*, 1430–1438.

Jazayeri, M., Lindbloom-Brown, Z., and Horwitz, G.D. (2012). Saccadic eye movements evoked by optogenetic activation of primate V1. Nat. Neurosci. *15*, 1368–1370.

Ji, G., and Neugebauer, V. (2012). Modulation of medial prefrontal cortical activity using in vivo recordings and optogenetics. Mol. Brain *5*, 36.

Jinde, S., Zsiros, V., Jiang, Z., Nakao, K., Pickel, J., Kohno, K., Belforte, J.E., and Nakazawa, K. (2012). Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron *76*, 1189–1200.

Keller, C.J., Truccolo, W., Gale, J.T., Eskandar, E., Thesen, T., Carlson, C., Devinsky, O., Kuzniecky, R., Doyle, W.K., Madsen, J.R., et al. (2010). Heterogeneous neuronal firing patterns during interictal epileptiform discharges in the human cortex. Brain *133*, 1668–1681.

Kheirbek, M.A., Drew, L.J., Burghardt, N.S., Costantini, D.O., Tannenholz, L., Ahmari, S.E., Zeng, H., Fenton, A.A., and Hen, R. (2013). Differential Control of Learning and Anxiety along the Dorsoventral Axis of the Dentate Gyrus. Neuron *77*, 955–968.

King, D.W., and Marsan, C.A. (1977). Clinical features and ictal patterns in epileptic patients with EEG temporal lobe foci. Ann. Neurol. *2*, 138–147.

King, D., Bronen, R.A., Spencer, D.D., and Spencer, S.S. (1997). Topographic distribution of seizure onset and hippocampal atrophy: Relationship between MRI and depth EEG. Electroencephalogr. Clin. Neurophysiol. *103*, 692–697.

Kleen, J., Scott, R., Lenck-Santini, P.-P., and Holmes, G.L. (2012). Cognitive and Behavioral Co-Morbidities of Epilepsy. In Jasper's Basic Mechanisms of the Epilepsies, pp. 1–19.

Klein, S., Bankstahl, M., and Löscher, W. (2015). Inter-individual variation in the effect of antiepileptic drugs in the intrahippocampal kainate model of mesial temporal lobe epilepsy in mice. Neuropharmacology *90*, 53–62.

Kokaia, M. (2011). Light-activated channels in acute seizures. Epilepsia *52 Suppl 8*, 16–18.

Koubeissi, M.Z., Kahriman, E., Syed, T.U., Miller, J., and Durand, D.M. (2013). Low-frequency electrical stimulation of a fiber tract in temporal lobe epilepsy. Ann Neurol *74*, 223–231.

Kramer, M.A., Eden, U.T., Kolaczyk, E.D., Zepeda, R., Eskandar, E.N., and Cash, S.S. (2010). Coalescence and fragmentation of cortical networks during focal seizures. J. Neurosci. *30*, 10076–10085.

Krook-Magnuson, E., and Soltesz, I. (2015). Beyond the hammer and the scalpel: selective circuit control for the epilepsies. Nat. Neurosci. *18*, 331–338.

Krook-Magnuson, E., Armstrong, C., Oijala, M., and Soltesz, I. (2013). On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. Nat. Commun. *4*, 1376.

Krook-Magnuson, E., Szabo, G.G., Armstrong, C., Oijala, M., and Soltesz, I. (2014). Cerebellar Directed Optogenetic Intervention Inhibits Spontaneous Hippocampal Seizures in a Mouse Model of Temporal Lobe Epilepsy. eNeuro *1*, 1376.

Krook-Magnuson, E., Armstrong, C., Bui, A., Lew, S., Oijala, M., and Soltesz, I. (2015). In vivo evaluation of the dentate gate theory in epilepsy. J. Physiol. *593*, 2379–2388.

Kros, L., Eelkman Rooda, O.H.J., Spanke, J.K., Alva, P., van Dongen, M.N., Karapatis, A., Tolner, E. a., Strydis, C., Davey, N., Winkelman, B.H.J., et al. (2015). Cerebellar output controls generalized spike-and-wave discharge occurrence. Ann. Neurol. *77*, 1027–1049.

Kwan, P., and Sander, J.W. (2004). The natural history of epilepsy: an epidemiological view. J. Neurol. Neurosurg. Psychiatry *75*, 1376–1381.

Kwan, P., Schachter, S.C., and Brodie, M.J. (2011). Drug-Resistant Epilepsy. N. Engl. J. Med. *365*, 919–926.

Kwon, O.-Y., and Park, S.-P. (2014). Depression and anxiety in people with epilepsy. J. Clin. Neurol. *10*, 175–188.

Ladas, T.P., Chiang, C.-C., Gonzalez-Reyes, L.E., Nowak, T., and Durand, D.M. (2015). Seizure reduction through interneuron-mediated entrainment using low frequency optical stimulation. Exp. Neurol. *269*, 120–132.

Laxer, K.D., Trinka, E., Hirsch, L.J., Cendes, F., Langfitt, J., Delanty, N., Resnick, T., and Benbadis, S.R. (2014). The consequences of refractory epilepsy and its treatment. Epilepsy Behav. *37*, 59–70.

Ledri, M., Madsen, M.G., Nikitidou, L., Kirik, D., and Kokaia, M. (2014). Global Optogenetic Activation of Inhibitory Interneurons during Epileptiform Activity. J. Neurosci. *34*, 3364–3377.

Leutgeb, J.K., Leutgeb, S., Moser, M., and Moser, E.I. (2007). Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus. *315*.

Lewis, E.C., Weil, A.G., Duchowny, M., Bhatia, S., Ragheb, J., and Miller, I. (2015). MR-guided laser interstitial thermal therapy for pediatric drug-resistant lesional epilepsy. Epilepsia 1590–1598.

Liang, S.-F., Liao, Y.-C., Shaw, F.-Z., Chang, D.-W., Young, C.-P., and Chiueh, H. (2011). Closed-loop seizure control on epileptic rat models. J. Neural Eng. *8*, 45001.

Lin, H., Holmes, G., Kubie, J., and Muller, R. (2009). Recurrent seizures induce a reversible impairment in a spatial hidden goal task. Hippocampus *19*, 817–827.

Loring, D.W., Kapur, R., Meador, K.J., and Morrell, M.J. (2015). Differential neuropsychological outcomes following targeted responsive neurostimulation for partial-onset epilepsy. Epilepsia *56*, 1836–1844.

Lothman, E.W., Stringer, J.L., and Bertram, E.H. (1992). The dentate gyrus as a control point for seizures in the hippocampus and beyond. Epilepsy Res. Suppl. *7*, 301–313.

Lytton, W.W., Hellman, K.M., and Sutula, T.P. (1998). Computer models of hippocampal circuit changes of the kindling model of epilepsy. Artif. Intell. Med. *13*, 81–97.

Maejima, T., Wollenweber, P., Teusner, L.U.C., Noebels, J.L., Herlitze, S., and Mark, M.D. (2013). Postnatal Loss of P/Q-Type Channels Confined to Rhombic-Lip-Derived Neurons Alters Synaptic Transmission at the Parallel Fiber to Purkinje Cell Synapse and Replicates Genomic Cacna1a Mutation Phenotype of Ataxia and Seizures in Mice. J. Neurosci. *33*, 5162–5174.

Mahn, M., Prigge, M., Ron, S., Levy, R., and Yizhar, O. (2016). Biophysical constraints of optogenetic inhibition at presynaptic terminals. Nat. Neurosci. *19*, 554–556.

Mansouri, A., Fallah, A., and Valiante, T. a (2012). Determining surgical candidacy in temporal lobe epilepsy. Epilepsy Res. Treat. *2012*, 706917.

Margerison, J.H., and Corsellis, J.A. (1966). Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. Brain *89*, 499–530.

McIsaac, R.S., Engqvist, M.K.M., Wannier, T., Rosenthal, A.Z., Herwig, L., Flytzanis, N.C., Imasheva, E.S., Lanyi, J.K., Balashov, S.P., Gradinaru, V., et al. (2014). Directed evolution of a far-red fluorescent rhodopsin. Proc. Natl. Acad. Sci. *111*, 13034–13039.

Medvid, R., Ruiz, A., Komotar, R.J., Jagid, J.R., Ivan, M.E., Quencer, R.M., and Desai, M.B. (2015). Current Applications of MRI-Guided Laser Interstitial Thermal Therapy in the Treatment of Brain Neoplasms and Epilepsy: A Radiologic and Neurosurgical Overview. Am. J. Neuroradiol.

Menendez de la Prida, L., and Sanchez-Andres, J. V (2000). Heterogeneous populations of cells mediate spontaneous synchronous bursting in the developing hippocampus through a frequency-dependent mechanism. Neuroscience *97*, 227–241.

van Mierlo, P., Papadopoulou, M., Carrette, E., Boon, P., Vandenberghe, S., Vonck, K., and Marinazzo, D. (2014). Functional brain connectivity from EEG in epilepsy: Seizure prediction and epileptogenic focus localization. Prog. Neurobiol. *121C*, 19–35.

Miocinovic, S., Somayajula, S., Chitnis, S., and Vitek, J.L. (2013). History, Applications, and Mechanisms of Deep Brain Stimulation. JAMA Neurol. *70*, 163.

Mohanraj, R., Norrie, J., Stephen, L.J., Kelly, K., Hitiris, N., and Brodie, M.J. (2006). Mortality in adults with newly diagnosed and chronic epilepsy: a retrospective comparative study. Lancet Neurol. *5*, 481–487.

Morgan, R.J., and Soltesz, I. (2008). Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. Proc. Natl. Acad. Sci. U. S. A. *105*, 6179–6184.

Mormann, F., Kreuz, T., Andrzejak, R.G., David, P., Lehnertz, K., and Elger, C.E. (2003). Epileptic seizures are preceded by a decrease in synchronization. Epilepsy Res. *53*, 173–185.

Myers, C., and Scharfman, H. (2009). A role for hilar cells in pattern separation in the dentate gyrus: a computational approach. Hippocampus *19*, 321–337.

Nelson, T.S., Suhr, C.L., Freestone, D.R., Lai, A., Halliday, A.J., McLean, K.J., Burkitt, A.N., and Cook, M.J. (2011). Closed-loop seizure control with very high frequency electrical stimulation at seizure onset in the GAERS model of absence epilepsy. Int. J. Neural Syst. *21*, 163–173.

Netoff, T.I., and Schiff, S.J. (2002). Decreased neuronal synchronization during experimental seizures. J. Neurosci. *22*, 7297–7307.

Noe, K., Sulc, V., Wong-Kisiel, L., Wirrell, E., Van Gompel, J.J., Wetjen, N., Britton, J., So, E., Cascino, G.D., Marsh, W.R., et al. (2013). Long-term Outcomes After Nonlesional Extratemporal Lobe Epilepsy Surgery. JAMA Neurol. *70*, 1–6.

Nune, G., DeGiorgio, C., and Heck, C. (2015). Neuromodulation in the Treatment of Epilepsy. Curr. Treat. Options Neurol. *17*, 43.

Oesterhelt, D., and Stoeckenius, W. (1971). Rhodopsin-like protein from the purple membrane of Halobacterium halobium. Nat. New Biol. *233*, 149–152.

Oesterhelt, D., and Stoeckenius, W. (1973). Functions of a New Photoreceptor Membrane. Proc. Natl. Acad. Sci. *70*, 2853–2857.

Orosz, I., McCormick, D., Zamponi, N., Varadkar, S., Feucht, M., Parain, D., Griens, R., Vallée, L., Boon, P., Rittey, C., et al. (2014). Vagus nerve stimulation for drug-resistant epilepsy: a European long-term study up to 24 months in 347 children. Epilepsia *55*, 1576–1584.

Osawa, S., Iwasaki, M., Hosaka, R., Matsuzaka, Y., Tomita, H., Ishizuka, T., Sugano, E., Okumura, E., Yawo, H., Nakasato, N., et al. (2013). Optogenetically Induced Seizure and the Longitudinal Hippocampal Network Dynamics. PLoS One *8*, e60928.

Osorio, I., Frei, M.G., Sunderam, S., Giftakis, J., Bhavaraju, N.C., Schaffner, S.F., and Wilkinson, S.B. (2005). Automated seizure abatement in humans using electrical stimulation. Ann. Neurol. *57*, 258–268.

Pavlov, I., Kaila, K., Kullmann, D.M., and Miles, R. (2013). Cortical inhibition, pH and cell excitability in epilepsy: what are optimal targets for antiepileptic interventions? J. Physiol. *591*, 765–774.

Paz, J.T., and Huguenard, J.R. (2015). Optogenetics and epilepsy: past, present and future. Epilepsy Curr. *15*, 34–38.

Paz, J.T., Bryant, A.S., Peng, K., Fenno, L., Yizhar, O., Frankel, W.N., Deisseroth, K., and Huguenard, J.R. (2011). A new mode of corticothalamic transmission revealed in the Gria4(-/-) model of absence epilepsy. Nat. Neurosci. *14*, 1167–1173.

Paz, J.T., Davidson, T.J., Frechette, E.S., Delord, B., Parada, I., Peng, K., Deisseroth, K., and Huguenard, J.R. (2013). Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. Nat. Neurosci. *16*, 64–70.

Penfield, W., and Jasper, H. (1954). Epilepsy and the Functional Anatomy of the Human Brain (Boston: Little Brown).

Peng, Z., Zhang, N., Wei, W., Huang, C.S., Cetina, Y., Otis, T.S., and Houser, C.R. (2013). A reorganized GABAergic circuit in a model of epilepsy: evidence from optogenetic labeling and stimulation of somatostatin interneurons. J. Neurosci. *33*, 14392–14405.

Picot, M.-C., Baldy-Moulinier, M., Daurs, J.-P., Dujols, P., and Crespel, A. (2008). The prevalence of epilepsy and pharmacoresistant epilepsy in adults: A population-based study in a

Western European country. Epilepsia *49*, 1230–1238.

Racine, R.J. (1972). Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. *32*, 281–294.

Ratzliff, A., Santhakumar, V., Howard, A., and Soltesz, I. (2002). Mossy cells in epilepsy: rigor mortis or vigor mortis? Trends Neurosci. *25*, 140–144.

Ratzliff, A.D.H., Howard, A.L., Santhakumar, V., Osapay, I., and Soltesz, I. (2004). Rapid deletion of mossy cells does not result in a hyperexcitable dentate gyrus: implications for epileptogenesis. J. Neurosci. *24*, 2259–2269.

Richner, T.J., Thongpang, S., Brodnick, S.K., Schendel, A.A., Falk, R.W., Krugner-Higby, L.A., Pashaie, R., and Williams, J.C. (2014). Optogenetic micro-electrocorticography for modulating and localizing cerebral cortex activity. J. Neural Eng. *11*, 16010.

Rochefort, N.L., Jia, H., and Konnerth, A. (2008). Calcium imaging in the living brain: prospects for molecular medicine. Trends Mol. Med. *14*, 389–399.

Rossignol, E., Kruglikov, I., van den Maagdenberg, A.M.J.M., Rudy, B., and Fishell, G. (2013). CaV 2.1 ablation in cortical interneurons selectively impairs fast-spiking basket cells and causes generalized seizures. Ann. Neurol. *74*, 209–222.

Sabolek, H.R.R., Swiercz, W.B.B., Lillis, K.P.P., Cash, S.S.S., Huberfeld, G., Zhao, G., Ste. Marie, L., Clemenceau, S., Barsh, G., Miles, R., et al. (2012). A Candidate Mechanism Underlying the Variance of Interictal Spike Propagation. J. Neurosci. *32*, 3009–3021.

Sanchez, R.M., Ribak, C.E., and Shapiro, L.A. (2012). Synaptic connections of hilar basal dendrites of dentate granule cells in a neonatal hypoxia model of epilepsy. Epilepsia *53 Suppl 1*, 98–108.

Santhakumar, V., Aradi, I., and Soltesz, I. (2005). Role of mossy fiber sprouting and mossy cell loss in hyperexcitability: a network model of the dentate gyrus incorporating cell types and axonal topography. J. Neurophysiol. *93*, 437–453.

Scharfman, H.E. (2016). The enigmatic mossy cell of the dentate gyrus. Nat. Rev. Neurosci. *17*, 562–575.

Schneider, C.J., Cuntz, H., and Soltesz, I. (2014). Linking Macroscopic with Microscopic Neuroanatomy Using Synthetic Neuronal Populations. PLoS Comput. Biol. *10*, e1003921.

Selvaraj, P., Sleigh, J.W., Freeman, W.J., Kirsch, H.E., and Szeri, A.J. (2013). Open loop optogenetic control of simulated cortical epileptiform activity. J. Comput. Neurosci. *36*, 515–525.

Senzai, Y., and Buzsáki, G. (2017). Physiological Properties and Behavioral Correlates of Hippocampal Granule Cells and Mossy Cells. Neuron *93*, 691–704.e5.

Sessolo, M., Marcon, I., Bovetti, S., Losi, G., Cammarota, M., Ratto, G.M., Fellin, T., and Carmignoto, G. (2015). Parvalbumin-Positive Inhibitory Interneurons Oppose Propagation But Favor Generation of Focal Epileptiform Activity. J. Neurosci. *35*, 9544–9557.

Sloviter, R.S., Zappone, C.A., Harvey, B.D., Bumanglag, A. V, Bender, R.A., and Frotscher, M. (2003). "Dormant basket cell" hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. J. Comp. Neurol. *459*, 44–76.

Smith, S.J.M. (2005). EEG in the diagnosis, classification, and management of patients with epilepsy. J. Neurol. Neurosurg. Psychiatry *76 Suppl 2*, ii2-7.

Sorokin, J.M., Davidson, T.J., Frechette, E., Abramian, A.M., Deisseroth, K., Huguenard, J.R., and Paz, J.T. (2017). Bidirectional Control of Generalized Epilepsy Networks via Rapid Real-Time Switching of Firing Mode. Neuron *93*, 194–210.

Sporns, O. (2013). Structure and function of complex brain networks. Dialogues Clin. Neurosci. *15*, 247–262.

Sporns, O. (2014). Contributions and challenges for network models in cognitive neuroscience. Nat. Neurosci. *17*, 652–660.

Stam, C.J. (2014). Modern network science of neurological disorders. Nat. Rev. Neurosci. *15*, 683–695.

Sugaya, Y., Yamazaki, M., Uchigashima, M., Kobayashi, K., Watanabe, M., Sakimura, K., and Kano, M. (2016). Crucial Roles of the Endocannabinoid 2-Arachidonoylglycerol in the Suppression of Epileptic Seizures. Cell Rep. *16*, 1405–1415.

Sukhotinsky, I., Chan, A.M., Ahmed, O.J., Rao, V.R., Gradinaru, V., Ramakrishnan, C., Deisseroth, K., Majewska, A.K., and Cash, S.S. (2013). Optogenetic Delay of Status Epilepticus Onset in an In Vivo Rodent Epilepsy Model. PLoS One *8*, e62013.

Tashiro, A., Makino, H., and Gage, F.H. (2007). Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. J. Neurosci. *27*, 3252–3259.

Taylor, R.S., Sander, J.W., Taylor, R.J., and Baker, G.A. (2011). Predictors of health-related quality of life and costs in adults with epilepsy: A systematic review. Epilepsia *52*, 2168–2180.

Tejada, J., and Roque, A.C. (2014). Computational models of dentate gyrus with epilepsy-induced morphological alterations in granule cells. Epilepsy Behav.

Telesford, Q.K., Simpson, S.L., Burdette, J.H., Hayasaka, S., and Laurienti, P.J. (2011). The brain as a complex system: using network science as a tool for understanding the brain. Brain Connect. *1*, 295–308.

Téllez-Zenteno, J.F., Ronquillo, L.H., Moien-Afshari, F., and Wiebe, S. (2010). Surgical outcomes in lesional and non-lesional epilepsy: A systematic review and meta-analysis. Epilepsy Res. *89*, 310–318.

Testa, I., Urban, N.T., Jakobs, S., Eggeling, C., Willig, K.I., and Hell, S.W. (2012). Nanoscopy of living brain slices with low light levels. Neuron *75*, 992–1000.

Thomas, E.A., Reid, C.A., and Petrou, S. (2010). Mossy fiber sprouting interacts with sodium channel mutations to increase dentate gyrus excitability. Epilepsia *51*, 136–145.

Thurman, D.J., Hesdorffer, D.C., and French, J.A. (2014). Sudden unexpected death in epilepsy: assessing the public health burden. Epilepsia *55*, 1479–1485.

Tønnesen, J., Sørensen, A.T., Deisseroth, K., Lundberg, C., Kokaia, M., Tonnesen, J., Sorensen, A.T., Deisseroth, K., Lundberg, C., Kokaia, M., et al. (2009). Optogenetic control of epileptiform activity. Proc. Natl. Acad. Sci. U. S. A. *106*, 12162–12167.

Truccolo, W., Donoghue, J. a, Hochberg, L.R., Eskandar, E.N., Madsen, J.R., Anderson, W.S., Brown, E.N., Halgren, E., and Cash, S.S. (2011). Single-neuron dynamics in human focal epilepsy. Nat. Neurosci. *14*, 635–641.

Vogel-Ciernia, A., Matheos, D.P., Barrett, R.M., Kramár, E. a, Azzawi, S., Chen, Y., Magnan, C.N., Zeller, M., Sylvain, A., Haettig, J., et al. (2013). The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory. Nat. Neurosci. *16*, 552–561.

Volz, F., Bock, H.H., Gierthmuehlen, M., Zentner, J., Haas, C. a, and Freiman, T.M. (2011). Stereologic estimation of hippocampal GluR2/3- and calretinin-immunoreactive hilar neurons (presumptive mossy cells) in two mouse models of temporal lobe epilepsy. Epilepsia *52*, 1579–1589.

Wagner, F.B., Truccolo, W., Wang, J., and Nurmikko, A. V. (2015). Spatiotemporal dynamics of optogenetically induced and spontaneous seizure transitions in primary generalized epilepsy. J. Neurophysiol. *113*, 2321–2341.

Wang, X.J., and Buzsáki, G. (1996). Gamma oscillation by synaptic inhibition in a hippocampal interneuronal network model. J. Neurosci. *16*, 6402–6413.

Wang, J., Borton, D.A., Zhang, J., Burwell, R.D., and Nurmikko, A. V (2010). A neurophotonic device for stimulation and recording of neural microcircuits. 2010 Annu. Int. Conf. IEEE Eng. Med. Biol. *2010*, 2935–2938.

Watts, D.J., and Strogatz, S.H. (1998). Collective dynamics of "small-world" networks. Nature *393*, 440–442.

Weitz, A.J., Fang, Z., Lee, H.J., Fisher, R.S., Smith, W.C., Choy, M., Liu, J., Lin, P., Rosenberg, M., and Lee, J.H. (2014). Optogenetic fMRI reveals distinct, frequency-dependent networks recruited by dorsal and intermediate hippocampus stimulations. Neuroimage *107C*, 229–241.

Wiebe, S., Blume, W.T., Girvin, J.P., and Eliasziw, M. (2001). A Randomized, Controlled Trial of Surgery for Temporal-Lobe Epilepsy. N. Engl. J. Med. *345*, 311–318.

Willie, J.T., Laxpati, N.G., Drane, D.L., Gowda, A., Appin, C., Hao, C., Brat, D.J., Helmers, S.L., Saindane, A., Nour, S.G., et al. (2014). Real-Time Magnetic Resonance-Guided Stereotactic Laser Amygdalohippocampotomy for Mesial Temporal Lobe Epilepsy. Neurosurgery *74*, 569–585.

Wilt, B.A., Burns, L.D., Wei Ho, E.T., Ghosh, K.K., Mukamel, E.A., and Schnitzer, M.J. (2009). Advances in light microscopy for neuroscience. Annu. Rev. Neurosci. *32*, 435–506.

World Health Organization (WHO) (2015). "Epilepsy."

Wykes, R.C., Heeroma, J.H., Mantoan, L., Zheng, K., MacDonald, D.C., Deisseroth, K., Hashemi, K.S., Walker, M.C., Schorge, S., and Kullmann, D.M. (2012). Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. Sci. Transl. Med. *4*, 161ra152.

Yekhlef, L., Breschi, G.L., Lagostena, L., Russo, G., and Taverna, S. (2015). Selective activation of parvalbumin- or somatostatin-expressing interneurons triggers epileptic seizurelike activity in mouse medial entorhinal cortex. J. Neurophysiol. *113*, 1616–1630.

Yizhar, O., Fenno, L.E., Davidson, T.J., Mogri, M., and Deisseroth, K. (2011). Optogenetics in neural systems. Neuron *71*, 9–34.

Zaccara, G., and Perucca, E. (2014). Interactions between antiepileptic drugs, and between antiepileptic drugs and other drugs. Epileptic Disord. *16*, 409–431.

Zhang, W., and Buckmaster, P.S. (2009). Dysfunction of the dentate basket cell circuit in a rat model of temporal lobe epilepsy. J. Neurosci. *29*, 7846–7856.

Zhang, J., Laiwalla, F., Kim, J.A., Urabe, H., Van Wagenen, R., Song, Y.-K., Connors, B.W., and Nurmikko, A.V. (2009). A microelectrode array incorporating an optical waveguide device for stimulation and spatiotemporal electrical recording of neural activity. In 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, (IEEE), pp. 2046–2049.

Zhang, W., Huguenard, J.R., and Buckmaster, P.S. (2012). Increased excitatory synaptic input to

granule cells from hilar and CA3 regions in a rat model of temporal lobe epilepsy. J. Neurosci. *32*, 1183–1196.

Zhang, W., Thamattoor, A.K., LeRoy, C., and Buckmaster, P.S. (2015). Surviving mossy cells enlarge and receive more excitatory synaptic input in a mouse model of temporal lobe epilepsy. Hippocampus *25*, 594–604.

Zhuo, J.-M., Tseng, H.-A., Desai, M., Bucklin, M.E., Mohammed, A.I., Robinson, N.T., Boyden, E.S., Rangel, L.M., Jasanoff, A.P., Gritton, H.J., et al. (2016). Young adult born neurons enhance hippocampal dependent performance via influences on bilateral networks. Elife *5*.

Ziv, Y., Burns, L.D., Cocker, E.D., Hamel, E.O., Ghosh, K.K., Kitch, L.J., El Gamal, A., and Schnitzer, M.J. (2013). Long-term dynamics of CA1 hippocampal place codes. Nat. Neurosci. *16*, 264–266.