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Fiber Tract Stimulation Can Reduce Epileptiform Activity in an *in-vitro* Bilateral Hippocampal Slice Preparation

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Abstract

Mesial temporal lobe epilepsy (MTLE) is a common medically refractory neurological disease that has been treated with electrical stimulation of gray matter with limited success. However, stimulation of a white matter tract connecting the hippocampi could maximize treatment efficacy and extent. We tested low-frequency stimulation (LFS) of a novel target that enables simultaneous targeting of bilateral hippocampi: the ventral hippocampal commissure (VHC) with a novel *in-vitro* slice preparation containing bilateral hippocampi connected by the VHC. The goal of this study is to understand the role of hippocampal interplay in seizure propagation and reduction by commissural fiber tract stimulation. LFS is applied to the VHC as extracellular and intracellular recording techniques are combined with signal processing to estimate several metrics of epilepsy including: (1) total time occupied by seizure activity (%); (2) seizure duration (s); (3) seizures per minute (#); and (4) power in the ictal (V^2Hz^{-1}); as well as (5) interictal spectra (V^2Hz^{-1}). Bilateral epileptiform activity in this preparation is highly correlated between hippocampi. Application of LFS to the VHC reduces all metrics of epilepsy during treatment in an amplitude and frequency dependent manner. This study lends several insights into the mechanisms of bilateral seizure reduction by LFS of the VHC, including that depolarization blocking, LTD/LTP and GABA_A are not involved. Importantly, enhanced post-stimulation 1-Hz spiking correlates with long-lasting seizure reduction and both are heightened by targeting bilateral hippocampi via the VHC. Therefore, stimulating bilateral hippocampi via a single electrode in the VHC may provide an effective MTLE treatment.

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AUTHOR CONTRIBUTIONS

1. Conception and design of the experiments: Equal work from both S. Toprani and DM Durand
2. Collection, analysis and interpretation of data: Mostly carried out by S. Toprani
3. Drafting the article or revising it critically for important intellectual content: Equal involvement from both S. Toprani and DM Durand

Keywords

epilepsy; electrical stimulation; seizure reduction

INTRODUCTION

Epilepsy is a neurological disorder characterized by abnormal electrical activity within the brain, which can result in either generalized or partial seizures. It is a complex disease with varied causes and manifestations that affects more than 50 million people worldwide (Duncan *et al.*, 2006). The most common and medically refractory form of human epilepsy, mesial temporal lobe epilepsy (MTLE), is characterized by seizures of the hippocampus and surrounding structures (King *et al.*, 1995; Swanson, 1995; Barbarosie & Avoli, 1997; Calcagnotto *et al.*, 2000; Avoli *et al.*, 2002; Spencer, 2002). Standard treatments for MTLE include drug therapy and surgery, neither of which is completely effective or risk-free. Only 11–25% of MTLE patients become seizure-free with drug therapy (Jallon, 1997). Although surgery can render 65–75% of selected patients free of seizures, it is only an option for patients with a single, identifiable epileptic focus and traumatic side effects can result (Blume, 2006; Blume & Parrent, 2006). An alternative treatment, deep brain electrical stimulation (DBS), is being tested clinically and shows promise as a safe and effective therapy for medically intractable epilepsy (Durand & Bikson, 2001; Morrell, 2006; Fisher *et al.*, 2010; Morrell, 2011), including MTLE (Velasco *et al.*, 2000; Velasco *et al.*, 2000a; Vonck *et al.*, 2002; Vonck *et al.*, 2005b; Boon *et al.*, 2007). DBS offers several advantages to surgical resection in that (1) it is less invasive; (2) it is reversible; and (3) the treatment protocol can be customized to fit the needs of individual patients (Sunderam *et al.*, 2010). However, this treatment is still recent and undergoing optimization. As a result, there are many stimulation paradigms that are currently being investigated targeting a range of locations including the cerebellum, caudate nucleus, thalamus, and substantia nigra. Current trials for MTLE focus mainly on hippocampal and cortical seizure foci (Theodore & Fisher, 2004; Morrell, 2006; Parrent & Almeida, 2006) utilizing a variety of stimulation patterns and frequencies (Jallon, 1997; Durand & Bikson, 2001; Engel, 2001; Vonck *et al.*, 2005a; Blume & Parrent, 2006; Duncan *et al.*, 2006; Morrell, 2006; Sadler, 2006; Yamamoto *et al.*, 2006) with variable success that does not approach surgical results (Rolston *et al.*). Therefore, there is need for development of specific stimulation parameters as well as identification of an effective target for stimulation.

In this study, we tested a low-frequency electrical stimulation paradigm based on the observation that LFS has been shown to decrease neural excitability. Low-frequency electrical stimulation (LFS) ranging from 0.1 to 10 Hz (Jerger & Schiff, 1995; D'Arcangelo *et al.*, 2005) has shown promising reduction of epileptic activity *in vitro* (Jerger & Schiff, 1995; Durand & Bikson, 2001; Khosravani *et al.*, 2003; Schiller & Bankirer, 2006; Toprani *et al.*, 2008, 2010), in animal (Velisek *et al.*, 2002; Goodman *et al.*, 2005; Ghorbani *et al.*, 2007; Zhang *et al.*, 2009; Kile *et al.*, 2010; Sun *et al.*, 2010; Rashid *et al.*, 2011; Tang & Durand, 2012), and in human models (Kinoshita *et al.*, 2005; Schrader *et al.*, 2006; Yamamoto *et al.*, 2006; Fisher *et al.*, 2010). Moreover, interictal activity, characterized by low-frequency periodic spiking events (Holmes *et al.*, 2000; Bonaventura *et al.*, 2006; Lees *et al.*, 2006; Schiller & Bankirer, 2006), has been suggested as a potential defense mechanism evolved in epileptic brains that protects against ictal onset (Swartzwelder *et al.*, 1987; Barbarosie & Avoli, 1997; Avoli, 2001; Cohen *et al.*, 2002; Cohen *et al.*, 2003; D'Arcangelo *et al.*, 2005). An explanation for this interictal-ictal interplay may be that the interictal events force the hippocampal network to spike at a particular interval or unstable periodic orbit (UPO), thereby making the network more resistant to seizures. This notion is supported by the success of chaos control strategies that use electrical stimulation to entrain

epileptic spiking events (Horgan, 1994; Schiff *et al.*, 1994; Slutzky *et al.*, 2003). Another advantage of LFS is that it inherently requires less power than its high-frequency counterpart, which may result in less tissue and electrode damage. Therefore, LFS, applied at a frequency mimicking interictal events, was chosen for the seizure reduction paradigm.

The LFS paradigm was applied to a clinically neglected target, the hippocampal commissures (HC), which include a large, accessible fiber tract whose stimulation can evoke bilateral hippocampal responses in humans (Gloor *et al.*, 1993; Koubeissi *et al.*, 2009). We chose this fiber tract as a target for seizure reduction by electrical stimulation due to its potential to bilaterally affect large portions of the hippocampi as well as its accessibility. While the hippocampus has the lowest seizure threshold of any brain region and is a well known seizure focus of MTLE (Bikson *et al.*, 2001; Durand & Bikson, 2001; Theodore & Fisher, 2004; Morrell, 2006; Parrent & Almeida, 2006), specific subfields of the hippocampus can be difficult to target stereotactically. Furthermore, direct hippocampal targeting has shown limited efficacy (Jobst *et al.*, 2010), perhaps because hippocampal seizure foci, like cortical foci, are often multiple, evolving, and difficult to characterize (Derchansky *et al.*, 2006). Even if foci can be identified, horizontal spread of synchronized activity often occurs too quickly to curtail with stimulation at the onset zone (Chagnac-Amitai & Connors, 1989). Targeting a decussating axonal tract may curtail seizure spread and reduce the risk of developing generalized seizures (Gastaut, 1970; Engel, 2001; Sadler, 2006). It has been shown that when MTLE events cross over into the contralateral hemisphere, they can usually be traced from the seizure focus to the contralateral hippocampus before evolving further (Lima *et al.*, 1990; Gloor *et al.*, 1993). A case study of rapid epileptic intrahippocampal-propagation with marked seizure amplification in the hippocampus contralateral to the seizure focus highlights the functional role of this fiber tract in humans (Rosenzweig *et al.*). Bilateral hippocampal communication is important in MTLE and changes in hippocampal coherence can predict seizure onset (Meier *et al.*, 2007). Furthermore, the healthy hippocampus in MTLE can evolve into an independent seizure focus through communication with the diseased hippocampus via the hippocampal commissures (HC) (Khalilov *et al.*, 2003), bundles of axons that run in the fimbria-fornix (ff) before decussating to connect bilateral hippocampi (O'Keefe & Nadel, 1978; Demeter *et al.*, 1985, 1990; Wilson *et al.*, 1990; Wilson *et al.*, 1991; Gloor *et al.*, 1993; Vann *et al.*, 2000). Given this evidence that the hippocampi directly communicate and influence one another's activity in animals (Feng & Durand, 2005) and in patients (Lacruz *et al.*, 2007), there may be benefits to broadly targeting both simultaneously as opposed to just the one that is perceived to be the epileptic focus.

The HC consist of a dorsal and ventral hippocampal commissure. The dorsal hippocampal commissure (DHC) is the prominent tract in primates, whereas the ventral hippocampal commissure (VHC) is prominent in rodents (Wilson *et al.*, 1990; Wilson *et al.*, 1991; Gloor *et al.*, 1993). The efficacy of LFS of the VHC has been demonstrated in rats *in vivo* with chemical (Tang & Durand, 2012), electrical (Rashid *et al.*, 2011), and genetic models of epilepsy (Kile *et al.*, 2010). However, an *in-vitro* study is crucial to determine the role of the commissure in mediating seizure reduction by eliminating other possible pathways, such as the corpus callosum or fiber tracts through the cortices. Furthermore, the ability to separate the hippocampi *in vitro* is crucial to determine the contribution of bilateral propagation of epileptic activity to MTLE severity and LFS efficacy. Therefore, the goal of this study is to test *in-vitro* a novel electrical stimulation paradigm for reduction of MTLE seizures, modeled using 4-aminopyridine (4-AP) (Tapia & Sitges, 1982; Perreault & Avoli, 1991; Traub *et al.*, 1996). A chronic open-loop LFS paradigm implemented at a fixed frequency of 1 Hz is applied to the hippocampal commissures for a broad bilateral effect on the hippocampi. The stimulation paradigm is tested in a new *in vitro* slice preparation that

maintains anatomical and functional connectivity of the hippocampi solely through an intact VHC in order to test the bilateral efficacy of the LFS paradigm.

MATERIALS AND METHODS

Ethical approval and animal handling

All procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University. 71 Sprague-Dawley (SD) rats from Charles River (12–21 days) were used for this study. Animals were housed according to IACUC guidelines. All rats were anesthetized using ethyl ether or isoflurane before decapitation for brain harvesting.

Functionally connected bilateral hippocampal slice preparation

The brain was removed and placed in cold (3–4 °C) oxygenated (O₂ 95%, CO₂ 5%) sucrose-rich artificial cerebrospinal fluid (ACSF), consisting of (in mM): 220 sucrose, 3 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 26 NaHCO₃, 2 CaCl₂, and 10 g/L D-glucose (pH 7.45). The cerebellum was detached and the ventral surface of the brain was secured in a vibrating-blade microtome (VT1000S, Leica) containing sucrose-based cold, oxygenated ACSF. A novel bilateral hippocampal slice preparation was developed in which the hippocampi, ECs (for contribution to seizure generation (Barbarosie & Avoli, 1997)), and connecting VHC were preserved after other tissues were carefully dissected away. 750 or 350 µm axial slices were cut and immediately preserved in oxygenated ACSF consisting of (in mM): 124 NaCl, 3.75 KCl, 1.25 KH₂PO₄, 2 MgSO₄, 26 NaHCO₂, 2 CaCl₂, and 10 g/L D-glucose for at least 60 minutes before being transferred to an interface-recording chamber (Harvard Apparatus). Slice viability was confirmed by the presence of distinct, healthy cell layers marked by cresyl violet (CV) staining in select slices and by extracellular field recordings of evoked potentials from CA3 and CA1 larger than 1 mV for all preparations in ACSF (figure 1) that did not diminish over the course of the experiment (figure 5). The axonal anatomy of the VHC was examined histologically for select slices using luxol fast blue (LFB), while the functional connection was established in all preparations by bilateral extracellular evoked potentials (EP) elicited by a single stimulus in the VHC tract (figure 1). Evoked responses in normal ACSF had a mean value of 3 mV $\pm$ 1.7 mV with a single vertex (N=20).

Seizure generation

Epileptic activity was generated using 4-AP in ACSF (100 µM). Alternative seizure models including magnesium-free ACSF (Jones, 1989) and bicuculline methiodide (BMI) in ACSF (10 µM) (Borck & Jefferys, 1999) were also used. Effective seizure generation included regular interictal-like and ictal-like waveforms, as shown in figure 3. Seizures are defined as high-frequency activity (> 10 Hz) lasting at least 1 s of variable amplitude.

Electrical recording

Extracellular field recordings were made using glass microelectrodes (1–4 MΩ) filled with 150mM NaCl. Intracellular sharp electrode recordings were made using glass microelectrodes (90–130 MΩ) filled with 1M KCl. Orthodromic EP and action potential (AP) from VHC stimulation were recorded in the CA1 stratum pyramidale while antidromic EP and AP were simultaneously recorded from the CA3 stratum pyramidale (figure 1). All signals were amplified using an Axoclamp-2A microelectrode amplifier (Axon Instruments), low-pass filtered (5 kHz), and further amplified by a FLA-01 (Cygnus Technology), then stored on a DT-200 digital tape recorder (Microdata Instrument) as well as on computer via an optical data acquisition program (44.1 kHz sampling rate, Audio Companion, Roni Music).

Stimulation parameters

CA3 and CA1 evoked potentials were periodically elicited by stimulation of the VHC using a monopolar tungsten electrode at a rate of 0.33 Hz in monophasic cathodic pulses (100 μ s pulse duration, current intensity to elicit 90% of the maximum evoked potential (figure 1). Pulse trains were generated using a function generator (Grass S88 Stimulator) and converted to a current by stimulus isolator units (A–M systems). The LFS paradigm consisted of monophasic cathodic pulses (100 μ s pulse duration) at a current intensity to elicit 90% of the maximum evoked potential applied at 1 Hz to the VHC while recording intracellularly or extracellularly from CA3 and CA1 (figures 5–7). Stimulus intensities that elicited 0%, 25%, 50%, 75%, and 100% of the maximum evoked potential were also compared (figure 9), as were 0.1 Hz, 0.2 Hz, 0.33 Hz, 0.5 Hz, 2 Hz, 3 Hz, 5 Hz, and 10 Hz stimulation frequencies (figure 10).

Stimulus artifact removal

In order to distinguish spontaneous physiological activity occurring during LFS, it was necessary to remove the stimulus pulse artifacts along with their evoked electrical responses from the recordings. An artifact removal algorithm based on template subtraction utilizing the following logic was implemented (Tang & Durand, 2012): Let $x(n)$, $n = 1:N$ denote recorded data of length N spanning the duration of a stimulus pulse train. Let p_i , $i = 1:I$, where I is the total number of stimulus pulses, be the onset location of the i th stimulus pulse in x . Then for each i th artifact, a template window TW_i is computed as

$$TW_i(n) = \frac{1}{150} \sum_{j=i-75, j \neq i}^{i+75} x_{p_j+n-1}, n=1:SI \quad (1)$$

where $SI = 4000$ is the stimulation interval between two adjacent pulses. The resulting template was subtracted from each window of the original data to reduce unwanted artifacts while retaining physiological non-evoked signals. A 5-second example of an original signal containing artifacts as well as non-evoked epileptic activity is shown in figure 2 along with its template and template-subtracted signal. This method was validated using saline bath experiment (Chiang *et al.*, 2012). The artifact-reduced signal retains non-evoked epileptic activity, but with reduced contamination from the stimulus pulse train, enabling more accurate power spectral analysis of epileptic activity during LFS. The method was validated by examining the coherence of the Fourier transform of artificial signals with artifacts first added and removed (data not shown). Signal power at the stimulation frequency (1 Hz) was negligible (figure 6). The artifact removal algorithm was implemented on stimulation and non-stimulation data prior to power spectral analysis for consistency.

Experimental setup and analysis

To study the effect of the proposed electrical stimulation protocol on hippocampal epileptic activity, we divided animals into two equivalent groups: a control group with no stimulation and a treatment group with stimulation. Epileptic activity was generated in both groups by bath application of 100 μ M 4-AP and changes due to LFS relative to baseline in several measures of epileptic severity, including: (1) percent seizure time (%); (2) seizure duration (seconds); (3) seizures per minute (number); (4) non-evoked power in the ictal spectrum (V^2Hz^{-1}); and (5) non-evoked power in the interictal spectrum (V^2Hz^{-1}) were quantified. The magnitude of change in each of these parameters was compared in treatment and control groups. Furthermore, the difference in the magnitude of any of these parameters within slices before and during LFS was determined.

All data were analyzed using MATLAB (MathWorks) and Lab Chart v7 (AD Instruments). Data is presented as mean $\pm$ SD, where n represents the number of slices from different animals. Two-tailed paired or student's t tests and/or ANOVA ($p < 0.05$) were applied for statistical comparison. Throughout the text, p values are represented as follows: (*) represents changes between treatment and control groups such that (*) indicates $p < 0.05$; (**) indicates $p < 0.01$; (***) indicates $p < 0.001$; and (****) $p < 0.0001$. Similarly, (#) represents changes within slices such that (#) indicates $p < 0.05$; (##) indicates $p < 0.01$; (###) indicates $p < 0.001$; and (####) indicates $p < 0.0001$.

RESULTS

Epileptiform activity in a 4-Aminopyridine *in-vitro* bilateral MTLE model

Although 4-AP is a well known epileptogenic agent (Perreault & Avoli, 1991), it rarely induces hippocampal electrographic seizure-like activity without additional manipulation, such as electrical kindling or fiber-tract cutting. To increase the probability of generating seizure activity, we: (1) included the adjacent EC, which may play a role in hippocampal seizure development (Barbarosie & Avoli, 1997); (2) used thick (750 μ m) slices; and (3) used younger, therefore smaller as well as more seizure-prone, animals (12 to 21 days). With this MTLE model, similar spontaneous epileptic activity was generally observed in CA3 and CA1 pyramidal cells (figure 3A) from 19 slices and lasted indefinitely (> 8 hours). Two types of epileptic activity occurred that were recorded extracellularly (figure 3B) as well as intracellularly (figure 3C): (1) low frequency (< 5 Hz) periodic spikes or bursts that began 10–15 minutes after bath application of 4-AP with variable field potential amplitudes from 0.5 to 10 mV and frequencies between 0.2 and 5 Hz lasting between 30 and 550 ms and (2) high-frequency oscillations (> 10 Hz) of variable amplitude occurring every 1–3 minutes with each episode lasting 3–60 seconds that generally occur regularly 1–3 hours after 4-AP application. These types of epileptic activity could be distinguished by short fast Fourier transform (sFFT) with a 1.5 second time resolution (0.67 Hz frequency resolution) and were classified as (1) interictal and (2) ictal respectively. The mean $\pm$ SD power spectral density relative to baseline signal for ictal and interictal activity are plotted in figure 3D and reveal a peak frequency of around 1 Hz for both types of activity, which have similar spectra at low frequencies (see inset). However, ictal activity has a secondary peak near 50 Hz and contains a broad range of high-frequency components, up to around 300 Hz. Characteristics of ictal and interictal epochs in this model are summarized in figure 3E. From this analysis, we conclude that there are waveforms representative of distinct interictal and ictal epochs in this model and that the average interictal frequency in our MTLE model is nearly 1 Hz. Furthermore, we can define seizures in this model as high-frequency activity (> 10 Hz) lasting at least 1 s of variable amplitude.

Epileptic activity is correlated in bilateral hippocampi that are connected by the VHC

To quantify the cross-talk between hippocampi in the bilateral preparation, neural activity was recorded simultaneously in both hippocampi with and without an intact hippocampal commissure (Fig 4A and B). Cutting the VHC de-correlated left and right hippocampal spontaneous activity, as shown for a 60-second recording from CA1. The cross-covariance in time-matched 60-second segments of left and right CA1 epileptic activity was determined before and after severing the VHC ($n=12$), as shown for the example (figure 4C). Changes in left to right delay and percent maximum covariance from cutting the VHC are presented in figure 4D & E, respectively. With the VHC intact, all left to right lag times were less than ± 30 ms. After cutting the VHC, left to right delays were randomly distributed across the minute-window. The variance of the mean lag was significantly increased when the VHC was cut ($F(11,11) = 4,920,836$, $p < 0.0001$). Furthermore, the maximum covariance after cutting the VHC was $14.20 \pm 11.99\%$ of its value when the VHC was intact, which is a

significant decrease (paired $t(11)=23.55286$, $p<0.0001$). Taken together, these results indicate that there is robust epileptic activity in the new bilateral preparation and that correlated bilateral epileptic activity in this model requires the VHC.

LFS of the VHC reduces bilateral 4-AP induced epileptiform activity

We next studied the hypothesis that VHC stimulation alone is sufficient to generate seizure reduction in bilateral hippocampi. This slice preparation consisting of only bilateral hippocampi and entorhinal cortices connected by the VHC provides an ideal model to test this hypothesis since there is no other pathway that could contribute to the seizure reduction. To test the hypothesis that LFS of the VHC can reduce seizures in bilateral hippocampal slices, epileptic activity was induced by bath application of 100 μM 4-AP. When stable seizure activity was obtained (minimum 30 min), spontaneous epileptic activity from CA3 and CA1 was recorded extracellularly as well as intracellularly in 32 slice preparations as shown in figure 5A. An example of an extracellular trace showing field interictal and ictal activity recorded from CA1 of left and right hippocampi connected by the VHC prior to stimulation is presented in the first panel of figure 5B, with insets showing intracellularly and extracellularly recorded seizures on an expanded time scale. Seizures are marked by dots and can be characterized by increases in power in the higher frequency bands shown for a 15 min data segment. Various parameters of epileptic severity, including: (1) percent seizure time (%); (2) seizure duration (s); (3) seizures per minute (#); (4) power in the ictal (V^2Hz^{-1}); and (5) power in the interictal spectra (V^2Hz^{-1}) were measured over an at least 15-minute baseline period. Half of the slices then received LFS of the VHC for 15 minutes. The stimulus location was chosen for its potential to affect bilateral hippocampal communication (figure 4). The frequency was selected based on experiments showing that the average interictal frequency approximates 1 Hz in the *in vitro* preparations. The duration of the stimulation was set to 15 minutes based on the observation that seizures occur every 97 seconds, on average, in this model (figure 3). The stimulation amplitude was chosen to elicit 90% of the maximum possible evoked response, at which there were no electrophysiological signs of slice deterioration (figure 7).

During stimulation, very little spontaneous activity was evident after stimulus artifacts and evoked responses were removed using template subtraction, as shown in Figure 5B. Examples of intracellular and extracellular evoked responses are shown in the inset on an expanded time scale. The same parameters of epileptic activity were measured bilaterally from 30 seconds after starting LFS in both treatment and control slices. The percent change in each of these parameters between the during-LFS and before-LFS periods for left and right hippocampi of treatment and control slices is shown in figure 5C. In all cases, there were no differences between left and right hippocampi. Therefore, bilateral preparations were pooled for statistical comparison. Changes between groups that did and did not receive LFS were compared (*) as well as those within groups before and during treatment (#). Stimulation of a single focus, the VHC, caused a significant reduction ($p < 0.05$) in all epileptic measures in bilateral hippocampi compared to non-treated controls. Groups were compared using Student's t test. There was a significant reduction ($p < 0.05$) in all measures of epilepsy during LFS compared to the spontaneous activity before LFS within bilateral slices as well, as compared using Student's paired t test. Seizures were almost completely abolished during LFS (99 $\pm$ 2% reduction in percent seizure time). Non-evoked power in the ictal and interictal spectra was significantly decreased by LFS (89 $\pm$ 2% and 90 $\pm$ 2% reduction, respectively). Control groups did not exhibit any significant changes in epileptic activity throughout the experiment (paired t test). These data are summarized in figure 5C.

To compare the effect of LFS of the VHC on a single hippocampus relative to two communicating hippocampi, the VHC was cut in 40 slices, as shown in figure 6A, and the

experimental protocol described for testing LFS efficacy in the intact slice preparation was repeated. As a result of the VHC cut, left and right hippocampal epileptic activity was disassociated. LFS only reduced epileptic activity in hippocampi that received direct axonal stimulation, whereas disconnected hippocampi experienced persistent epileptic activity (figure 6B). In this preparation, axonally-stimulated hippocampi exhibited similar reduction in ictal and interictal activity to that seen with two connected hippocampi ($86 \pm 5\%$ and $82 \pm 6\%$ reduction in ictal and interictal frequency spectra with $98 \pm 3\%$ reduction in percent seizure time during LFS, $p < 0.05$ for all variables), while disconnected hippocampi behaved like non-stimulated controls (figure 6C). Combined, the data presented in figures 5 and 6 shows that this LFS protocol can significantly reduce epileptic activity in bilateral hippocampi, although two communicating hippocampi are not necessary for the effect. Furthermore, the seizure reduction is not a field effect, but rather, is axonally mediated through direct stimulation of the VHC fiber tract.

Seizure reduction does not depend on long-term depression or depolarization block

In order to determine if the effect of low frequency stimulation is mediated by long-term depression (LTD), the effect of the stimulating pulse was monitored throughout the experiments to determine the tissue response to the stimulus. During 1 Hz stimulation of the VHC at amplitudes that elicited 90% of the maximal evoked response, the amplitude of bilateral orthodromic evoked responses as well as the number of evoked action potentials in CA1 was monitored for each stimulus pulse as shown in figure 7A. EP amplitudes were calculated as the difference between the average potential of points (a, b) and the potential at point (c), as shown in the inset of figure 7B. The mean EP amplitude in 4-AP was 5 ± 3.5 mV, which is hyperactive compared to the EP in ACSF with a mean amplitude of 3 ± 1.7 mV. The orthodromic CA1 EP amplitude was normalized per slice and averaged over 19 trials. There were no differences in EP amplitude across the 15 minute stimulation period ($F(779, 12,784) = 0.211$, $p = 1$ using ANOVA), as shown in figure 7B. During LFS, an AP was auto-detected as a post-stimulus peak voltage greater than -20 mV, as shown in the inset of figure 7C. 7 ± 2.7 APs were elicited per stimulus in 4-AP, compared to 2.4 ± 1.2 for an equal stimulus in ACSF. In 4-AP, elicited APs occurred atop a paroxysmal depolarizing shift (PDS), with return to baseline before the next stimulus as shown in figure 7C. The mean number of APs elicited by each pulse did not show differences throughout the LFS protocol ($F(779, 2,337) = 0.799$, $p = 0.999$ using ANOVA). Action potentials were reliably produced by each stimulus. This analysis was performed from 2 minutes after the start of LFS to allow acclimation to stimulation. The mean normalized EPs and number of elicited APs did not change throughout LFS treatment in healthy control slices (ACSF) either (data not shown). The steady amplitude of evoked responses throughout the course of LFS suggests that: (1) slices remain healthy for the entire stimulation period; (2) slices remain responsive to stimulation without significant potentiation of the response in this model; (3) the mechanism of seizure reduction does not rely on long-term depression; and (4) sustained depolarization that would cause “depolarization block” does not occur.

LFS of the VHC does not “block” seizures but requires acclimation for seizure prevention

High frequency stimulation (HFS) used in closed loop systems is designed to block a seizure within a very short time of detection. To determine if LFS could also block seizures instantly, we monitored the response of the system as the onset of stimulation. Unlike HFS, LFS did not block an ongoing seizure right away and sometimes induced a single, short seizure at stimulus onset when using high stimulus amplitudes (such as was used in this study) in epileptic slices. An example of an artifact-removed trace from CA1 during the first 2 minutes of LFS and the sFFT (time resolution of 1.5 s) of that trace are shown in figure 8B with the analogous experiment schematic in figure 8A. Single seizure did occur sometimes at the start of LFS, but completely terminated after 30 seconds in all cases ($N=19$). To

understand the mechanism of this effect, we monitored the extracellular evoked response and the intracellular spike count. The initial seizure, when it occurred, was accompanied by diminished recruitment by stimulation, reflected in smaller EPs and fewer, if any, evoked APs in a given cell (figure 8C & D). The minimum number of evoked action potentials occurred during the post-ictal period. Evoked responses reached steady-state (shown in figure 7) within a minute of starting LFS in all cases. A significant seizure reduction was then achieved for the duration of the stimulation period, as shown (figures 5 and 6). In fact, LFS was tested for up to 2 hours with the same efficacy reported here (not shown). LFS initiation did not induce any seizures in healthy ACSF control slices at any stimulus amplitude. These results suggest that, unlike HFS, LFS does not block seizures quickly but requires a short period of about a minute to take effect.

Seizure reduction by LFS is both amplitude and frequency dependent

In order to determine if the initial seizure could be avoided by lowering the stimulation amplitude, the LFS protocol was repeated at stimulus amplitudes to evoke 25, 50, 75, and 100% of the maximum response ($n=5$ per amplitude tested) while percent reduction in seizures was measured. There was a linear relationship between stimulus amplitude and seizure reduction given by the equation $y = 28x - 11.49$ ($R^2 = 0.998$) (figure 9A1). From the same study, a linear relationship between stimulus amplitude and the frequency of seizure occurrence when turning on LFS was observed that could be described by the equation $y = 28x - 30$ ($R^2 = 0.98$). The initial seizure from turning on LFS could be completely avoided by using lower stimulus amplitudes of LFS, even in epileptic slices (figure 9A2). However, since LFS efficacy linearly correlates to stimulation amplitude, there is a trade-off between maximizing effect and recruiting a seizure when starting LFS.

We then tested the hypothesis that a minimum stimulation frequency must be maintained to achieve seizure reduction by measuring the percent reduction in seizures during LFS at different stimulus frequencies of 0.1, 0.2, 0.33, 0.5, 1, 2, 3, 5, and 10 Hz ($n=5, 4, 6, 8, 10, 7, 4, 5$, and 3, respectively) while otherwise following the described protocol. There were significant differences in seizure reduction among the frequencies tested ($F(10, 47) = 19.93$, $p < 0.0001$ using ANOVA). LFS was more effective at the higher frequency range of low-frequency and that efficacy dropped off as LFS frequency was reduced below 1 Hz (figure 9B).

LFS is effective in several models of epilepsy

We then asked if the suppression of epileptiform activity was resistant to GABA_A blockers and to determine the robustness of the LFS paradigm on seizure reduction, the protocol was repeated in two other seizure models: BMI and magnesium-free ACSF. Percent seizure reduction during LFS was also highly significant in these models and distributed as follows: ($n=14, 9, 5$, respectively): 97% $\pm$ 8% in 4-AP; 97% $\pm$ 6% in BMI; and 94% $\pm$ 12% in low magnesium (figure 10). These results indicate that this LFS paradigm was equally effective in 3 different seizure models ($F(2, 25) = 0.389$, $p = 0.682$ using ANOVA). Moreover, these data show that LFS-induced seizure reduction does not rely on the GABA_A inhibition.

Post-treatment efficacy is enhanced by hippocampal cross-talk

Next, we asked: (1) if there is an after-effect of LFS and (2) whether it is dependent on the presence of two interconnected hippocampi. To determine the after-effect of the stimulation, 15 minutes of 4-AP epileptic activity was recorded from CA3 and CA1 of the left hippocampus extracellularly and intracellularly in 40 preparations where the VHC had been cut just right of the stimulus electrode (figure 11A) and in 32 preparations with the VHC intact (figure 11C). Seizures were identified by spectral analysis (time resolution 1.5

seconds) and are marked by dots. As before, left and right activity was decorrelated by cutting the VHC and highly correlated with the VHC intact (not shown). LFS was applied to half the slices for 15 minutes. Post-LFS epileptic activity was then recorded from all slices for a minimum of 15 minutes up to three hours (figure 11B & D). Changes in pre-LFS and post-LFS epileptic activity were assessed for single and connected hippocampi. The following variables were compared between stimulated slices and non-stimulated controls (*, Student's *t* test) as well as within slices before and after treatment (#, paired *t* test): (1) percent seizure time (%); (2) seizure duration (s); (3) seizures per minute (#); (4) power in the ictal (V^2Hz^{-1}); and (5) power in the interictal spectra (V^2Hz^{-1}) (figure 11E). Connected hippocampi were treated as a unit for statistical comparisons.

Finally, we tested the hypothesis that the VHC played a significant role in the after-stimulation effects. In slices from a single unilateral hippocampus that received LFS, spontaneous activity recurred with both ictal and interictal epochs (figure 11B). However, bilateral-hippocampal treated slices showed decreases in post-LFS seizures, sometimes with complete abolishment of seizures in the post-stimulation period. There was a concurrent increase in interictal activity at the stimulation frequency (figure 11D). Most metrics of epileptic activity decreased post-LFS. Conversely, power in the interictal frequency spectra increased. In single stimulated hippocampi, significant ($p < 0.05$) post-LFS changes from pre-LFS baseline only occurred in seizure duration (11 $\pm$ 24% decrease) and seizure power spectral density (13 $\pm$ 25% decrease) compared to nonstimulated controls (*). However, these were not significant changes compared to pre-LFS baseline within slices (#). In contrast, stimulated bilateral slices exhibited significant changes in all epileptic variables after LFS ($p < 0.05$) both relative to non-stimulated control slices (*) and compared to same-slice pre-LFS conditions (#). While there was a 42 $\pm$ 31% decrease in post-LFS percent seizures compared to pre-LFS baseline and a 44 $\pm$ 27% decrease in power in the ictal spectra, there was a 43 $\pm$ 27% increase in power in the spectra of the interictal activity. All metrics of epilepsy increased over time in non-stimulated controls. While the single-hippocampus data is inconclusively suggestive of post-LFS efficacy, post-stimulation seizure reduction in the bilateral preparation is unequivocal and clearly shows that the commissural tract connecting the two hippocampi contributes significantly to the post-stimulation inhibition of seizure activity. The data also suggest that the seizure reduction effect could be attributed to an increase in interictal activity by the presence of a connection between the two sides.

DISCUSSION

In this study, we present a novel bilateral hippocampal slice preparation to test the hypothesis that a single electrode located within a white matter tract can control seizure activity in both hippocampi simultaneously. The chosen target, the VHC, is a sizable tract that interconnects hippocampi. When stimulated in the presence of 4-AP, seizure reduction is indeed observed bilaterally. The LFS paradigm presented reduced (1) percent seizure time (%); (2) seizure duration (s); (3) seizures per minute (#); (4) non-evoked power in the ictal spectrum (V^2Hz^{-1}); and (5) non-evoked power in the interictal spectrum (V^2Hz^{-1}) during treatment *in vitro* in a dose and frequency dependent manner. The stimulation effect was robust in three different chemical epilepsy models and shown to last following stimulation. While seizure reduction during treatment was nearly complete even when hippocampi were separated by cutting the VHC, the post-stimulation effect was more robust in hippocampi connected by the VHC.

An important component of this study is the introduction of a novel bilateral hippocampal slice preparation where the hippocampi are anatomically and functionally connected through the VHC fiber tract. The VHC has been implicated as a route of secondary generalization of

MTLE (Lima *et al.*, 1990; Wilson *et al.*, 1990; Wilson *et al.*, 1991; Gloor *et al.*, 1993; Feng & Durand, 2005), and as an effective target for seizure reduction by low and high-frequency DBS in acute and chronic *in vivo* studies (Jensen & Durand, 2009; Kile *et al.*, 2010; Rashid *et al.*, 2011; Tang & Durand, 2012). However, other preparations where DBS of the VHC has been tested (*in-vivo* acute and chronic) have included other brain regions that may be involved in the propagation & reduction of seizure activity, such as the corpus callosum or cortical structures, making the role of the VHC difficult to confirm. Furthermore, these studies cannot rule out field effects that may be involved in seizure reduction by DBS. Other bilateral *in vitro* preparations have been developed but include the entire hippocampus (Khalilov *et al.*, 1997; Khazipov *et al.*, 1999; Khalilov *et al.*, 2003). To our knowledge, this is first *in-vitro* slice preparation of bilaterally coupled hippocampi in rats. It is also the first test of DBS on coupled hippocampi *in vitro*. The results of this study strengthen the conclusion of the *in vivo* studies that the VHC is an effective stimulation target for seizure reduction and highlight the importance of targeting bilateral communicating hippocampi for maximizing post-stimulation seizure reduction.

Previous studies have tested low-frequency DBS with the strategy of mimicking interictal frequency for MTLE in isolated unilateral hippocampal rat and mouse *in vitro* models with stimulus targets including: the hippocampus gray matter (Jerger & Schiff, 1995; Barbarosie & Avoli, 1997; D'Arcangelo *et al.*, 2005); the EC (D'Arcangelo *et al.*, 2005); and white matter within the hippocampus proper (Jerger & Schiff, 1995). Seizures have been variably defined in these studies and have included stimulation-induced discharges (D'Arcangelo *et al.*, 2005) and long lasting synchronous discharges of variable durations (Jerger & Schiff, 1995; Barbarosie & Avoli, 1997). The seizure models have required multiple manipulations in addition to chemicals including cutting a hippocampal axon tract, the Schaffer collaterals, and electrical kindling of seizure-like activity (Barbarosie & Avoli, 1997; D'Arcangelo *et al.*, 2005). Stimulus trains have varied from 2 to 15 minutes. Efficacy has varied from shortening an electrically-induced seizure (D'Arcangelo *et al.*, 2005) to reducing the number of seizures (Jerger & Schiff, 1995; Barbarosie & Avoli, 1997), or preventing the transition from interictal to ictal activity (Khosravani *et al.*, 2003). We have utilized a more physiological seizure model with only chemical modulation and with maximized bilateral circuitry kept intact by utilization of thick slices. This is the first demonstration that stimulation of a fiber tract outside of the hippocampus has broad impact on both hippocampi simultaneously, *in vitro*, to control seizures. This is also the first *in vitro* study to thoroughly characterize epileptic activity and seizure reduction based on this combination of variables describing epileptic severity. Previous *in-vitro* studies have reported some effect of LFS on epileptiform activity, but seizure reduction was only achieved during stimulation with complete rebound of epileptic activity following stimulation (Jerger & Schiff, 1995; Barbarosie & Avoli, 1997; D'Arcangelo *et al.*, 2005). In slices, lasting effects of stimulation were only observed in cases where long term depression (LTD) was induced (Albensi *et al.*, 2004; Albensi *et al.*, 2008), which does not occur with this protocol (figure 7). We report some significant ($p < 0.05$) post-stimulation effects even in single-hippocampus preparations in epileptic variables not measured by other studies. However, there is clearly a much more significant ($p < 0.05$) post-stimulation effect that occurs in the intact bilateral preparation that could not have been revealed in the standard hippocampal slice.

Another interesting finding revealed by the intact bilateral model is that the post-stimulation effects of LFS were amplified when stimulating decussating fibers of two communicating hippocampi relative to stimulating VHC fibers connected to a singular hippocampus. Post-treatment effects of LFS *in vivo* are prevalent in the literature (Yang *et al.*, 2006; Kile *et al.*, 2010; Rashid *et al.*, 2011), even when LTD is not concurrently observed (Tang & Durand, 2012). Amygdala-kindled seizures have been abated *in vivo* with same-side or contralateral 1 Hz LFS of the piriform cortex with a lasting effect for 10 days post-stimulation (Yang *et*

al., 2006). The reason for the discrepancy in post-LFS effects *in vitro* and *in vivo* is not known and may be attributable to the presence of hippocampal cross-talk *in vivo*. Currently, all *in vitro* models in which LFS has been tested have contained one hippocampus. This is the first instance of *in vitro* bilaterally functionally connected hippocampi where DBS for epilepsy has been studied. Therefore, we asked if post-LFS antiepileptic effects can occur and if they are dependent on the presence of the connection between the two interconnected hippocampi. The significant ($p < 0.05$) *in vitro* finding of post-treatment LFS efficacy across all variables in the bilateral preparation contrasts with significant post-treatment reduction of only two epileptic variables when hippocampi are separated. It is our hypothesis that two hippocampi that have been entrained together during stimulation will maintain firing at that frequency and will influence each other so that a repetitive interictal-like pattern is retained longer than would occur if a single isolated hippocampus is stimulated.

The mechanism is confirmed by the observation that while ictal activity was inhibited post-stimulation, interictal activity was paradoxically increased. This is in contrast to what was observed during stimulation, namely a reduction of both ictal and interictal activities in connected and single hippocampi. This is not the first study to report enhancement of neural activity in response to stimulation. Entrainment by low amplitude polarizing electric fields has been shown to modulate epileptic seizures (Sunderam *et al.*, 2009) and the tendency of hippocampal networks to become entrained by low-frequency electrical stimuli has been reported (Mason *et al.*, 2010; Reato *et al.*, 2010). However, the current finding is unusual in that enhanced periodic activity continues post-stimulation and appears to be more persistent when there is bilateral hippocampal interplay. Post-stimulation firing imitating LFS frequency was also observed in an *in vivo* study in our laboratory (Tang & Durand, 2012). The mechanism of this post-stimulation patterning and its role in long-lasting seizure reduction after DBS is unclear and requires further investigation. One candidate that may be involved is the slow afterhyperpolarization (sAHP) that follows action potential trains and is involved in facilitation of spike-frequency adaptation (Madison & Nicoll, 1982; Madison & Nicoll, 1984).

Two novel features of the DBS paradigm presented here that are not currently in clinical practice include: (1) stimulation of a white matter tract for bilateral hippocampal targeting and (2) use of low stimulation frequency. There is abundant evidence to support a bilateral approach to refractory epilepsy. Hemispherectomy has been shown to alleviate intractable epilepsy (Limbrick *et al.*, 2009). It has been shown that a mesial temporal lobe epileptic focus begets a contralateral mirror focus (Khalilov *et al.*, 2003). Bilateral hippocampal coherence changes prior to seizures and can be used as a predictive measure (Meier *et al.*, 2007). The importance of simultaneous bilateral targeting was highlighted in a recent paper that compared stimulation of bilateral anterior nucleus of the thalamus (ANT) to unilateral ANT stimulation and found that amygdala-kindled generalized seizures in rats could only be significantly reduced with bilateral, and not unilateral, stimulation (Zhong *et al.*). The DHC, the human counterpart to the VHC in rodents, has recently been shown to be functional in communicating stimulated evoked potentials simultaneously to bilateral hippocampi (Koubeissi *et al.*, 2009) and in transferring epileptic activity between hippocampi in humans (Rosenzweig *et al.*). It follows that the DHC may be an ideal target for bilateral MTLE seizure reduction by DBS. Preliminary experiments carried out in human patients with temporal lobe epilepsy have shown that 90% seizure suppression could be achieved (Koubeissi *et al.*, 2012). Recent clinical trials have tested high-frequency, closed loop systems of DBS for epilepsy (Fisher *et al.*, 2010; Morrell, 2011). However, increasing experimental and clinical evidence suggests that LFS can also reduce or prevent seizure activity (Velisek *et al.*, 2002; Chkhenkeli *et al.*, 2004; Goodman *et al.*, 2005; Ghorbani *et al.*, 2007) and may be preferable since the low duty cycle can improve battery life and decrease

side-effects. A concern with LFS may be the hyperexcitability of epileptic tissue at the onset of the stimulation, as seen in this study (figure 9). Previous studies have reported the presence of seizures during the first minute of LFS that are eradicated for the rest of its duration (Barbarosie & Avoli, 1997). Furthermore, in-session seizures during low-frequency transcranial magnetic stimulation in patients with epilepsy have been analyzed and found to be the same or shorter than baseline seizures with no effect on overall neurological outcome on follow-up (Rotenberg *et al.*, 2009). Nonetheless, the initial seizure can be avoided by using lower stimulation amplitudes (figure 10). Indeed, this initial seizure was not observed in a recent chronic *in vivo* study from our laboratory using an analogous setup that demonstrated seizure reduction by LFS of the VHC, probably due to lower current density at the recording sites (Rashid *et al.*, 2011).

Conceivably the largest hurdle to implementation of novel antiepileptic DBS paradigms is a lack of understanding of the mechanisms of seizure reduction. Long term depression (LTD) has been proposed as a mechanism of antiepileptic DBS (Albensi *et al.*, 2004; Schiller & Bankirer, 2006). However, LTD is not observed during this stimulation protocol since the EPs did not significantly change in amplitude during LFS (figure 7). This is corroborated by a similar VHC stimulation protocol tested *in vivo* (Tang & Durand, 2012). Intracellular recordings indicate the presence of paroxysmal depolarization shift (PDS) following each stimulus as shown in figure 7 during which the occurrence of spontaneous spikes is severely limited. However, the inter-stimulus interval during which seizures are inhibited lasts longer than the duration of the PDS so that cannot explain the effect in its entirety. Furthermore, there is no evidence of depolarization block as cell potentials return to baseline between stimuli and cells respond reliably to stimuli. This paradigm is not effective at very low frequencies (less than 1 Hz) as shown in figure 10, suggesting that the “protection” offered by LFS may be short-lived and limited in duration to around 1 second. Given the paradigm’s efficacy in the presence of BMI, we can conclude that GABA_A is probably not involved, as reported by Schiller and Bankirer (Schiller & Bankirer, 2006). The LFS paradigm is equally effective under the condition of enhanced glutamatergic signaling produced by the magnesium-free seizure model, suggesting inhibition of glutamatergic neurotransmission is not a key player. Finally, there seems to be an antiepileptic effect from increased, regular interictal activity, which is enhanced by bilateral hippocampal interplay, suggesting that pacing the hippocampus at 1 Hz may be protective. This could occur through induction of long-lasting hyperpolarization approximating 1 second, which could be mediated by GABA_B signaling or the slow after-hyperpolarization (sAHP), both of which are recruited by repetitive electrical stimulation.

The described MTLE model has been optimized for studying mechanisms of seizure reduction by DBS in a bilateral hippocampal network by avoiding additional network cutting or electrical kindling, which are often necessary in chemical seizure models (Barbarosie & Avoli, 1997; Avoli, 2001; Avoli *et al.*, 2002; Schiller & Bankirer, 2006). We utilized bath application of 100 μ M 4-AP, which antagonizes the A-type potassium current, for its ability to enhance synaptic activity without bias for any particular neurotransmitter (Tapia & Sitges, 1982; Perreault & Avoli, 1991; Traub *et al.*, 1996). The combination of these tools enables us to begin to understand the implications of hippocampal cross-talk in epileptic development, progression, and treatment. We are also carrying out experiments to further understand the mechanisms of seizure reduction by LFS of the VHC, which is the logical next step in this study.

CONCLUSIONS

Although DBS is a promising treatment for MTLE, current gray matter targets have met with limited success. We developed a new *in-vitro* slice preparation containing both

hippocampi connected by the VHC to study the effect of low frequency electrical stimulation of a novel white matter target, the VHC, on bilateral epileptic activity. Epileptic activity spreads across the VHC and LFS of this single target effectively reduces several metrics of epileptic severity in bilateral hippocampi in an amplitude and frequency-dependent manner in this model without causing depolarization block, LTD, or requiring GABA_A receptors. Post-stimulation seizure reduction is observed and requires connected hippocampi. It is correlated with an increase in low-frequency interictal bursting at the stimulation frequency that persists post-stimulation. This stimulation paradigm is significant in that it enables utilization of a single stimulation electrode in the commissural white matter to simultaneously target bilateral hippocampi. These findings highlight the importance of low frequency stimulation of the hippocampal commissures as a non-invasive treatment solution for MTLE patients. Furthermore, the behavior of cells during stimulation and the unexpected interplay of bilateral hippocampi in post-stimulation seizure reduction lend important mechanistic insights about DBS at low frequencies for epilepsy.

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Abbreviations

4-AP	4-aminopyridine
ACSF	artificial cerebral spinal fluid
ANT	anterior nucleus of thalamus
AP	action potential
BMI	bicuculline methiodide
CV	cresyl-violet
DBS	deep brain stimulation
DHC	dorsal hippocampal commissure
EC	entorhinal cortex
EP	evoked potential
ff	fimbria-fornix
HC	hippocampal commissures
HFS	high frequency stimulation
IACUC	Institutional Animal Care and Use Committee
LFB	luxol fast blue
LFS	low frequency stimulation
MTLE	mesial temporal lobe epilepsy
PDS	paroxysmal depolarizing shift
SD	Sprague-Dawley
sAHP	slow after-hyperpolarization
UPO	unstable periodic orbit

VHC ventral hippocampal commissure

REFERENCES

- Albensi BC, Ata G, Schmidt E, Waterman JD, Janigro D. Activation of long-term synaptic plasticity causes suppression of epileptiform activity in rat hippocampal slices. *Brain Res.* 2004; 998:56–64. [PubMed: 14725968]
- Albensi BC, Toupin JD, Oikawa K, Oliver DR. Controlled pulse delivery of electrical stimulation differentially reduces epileptiform activity in Mg²⁺-free-treated hippocampal slices. *Brain Res.* 2008; 1226:163–172. [PubMed: 18582443]
- Avoli, Massimo, D'Antuno M, Louvel J, Kohling R, Biagini G, Pumain R, D'Arcangelo G, Tancredi V. Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. *Progress in Neurobiology.* 2002; 68:167–207. [PubMed: 12450487]
- Avoli M. Do interictal discharges promote or control seizures? Experimental evidence from an in vitro model of epileptiform discharge. *Epilepsia.* 2001; 42(Suppl 3):2–4. [PubMed: 11520313]
- Barbarosie M, Avoli M. CA3-driven hippocampal-entorhinal loop controls rather than sustains in vitro limbic seizures. *J Neurosci.* 1997; 17:9308–9314. [PubMed: 9364076]
- Bikson M, Lian J, Hahn PJ, Stacey WC, Sciortino C, Durand DM. Suppression of epileptiform activity by high frequency sinusoidal fields in rat hippocampal slices. *J Physiol.* 2001; 531:181–191. [PubMed: 11179402]
- Blume WT. Focal seizures: intractability and semiology. *Adv Neurol.* 2006; 97:17–25. [PubMed: 16383111]
- Blume WT, Parrent AG. Assessment of patients with intractable epilepsy for surgery. *Adv Neurol.* 2006; 97:537–548. [PubMed: 16383161]
- Bonaventura CD, Vaudano AE, Carni M, Pantano P, Nucciarelli V, Garreffa G, Maraviglia B, Prencipe M, Bozzao L, Manfredi M, Giallonardo AT. EEG/fMRI Study of Ictal and Interictal Epileptic Activity: Methodological Issues and Future Perspectives in Clinical Practice. *Epilepsia.* 2006; 47:52–58. [PubMed: 17239107]
- Boon P, Vonck K, De Herdt V, Van Dycke A, Goethals M, Goossens L, Van Zandijcke M, De Smedt T, Dewaele I, Achten R, Wadman W, Dewaele F, Caemaert J, Van Roost D. Deep Brain Stimulation in Patients with Refractory Temporal Lobe Epilepsy. *Epilepsia.* 2007; 48:1551–1560. [PubMed: 17726798]
- Borck C, Jefferys JGR. Seizure-like events in disinhibited ventral slices of adult rat hippocampus. *J Neurophysiol.* 1999; 82:2130–2142. [PubMed: 10561393]
- Calcagnotto ME, Barbarosie M, Avoli M. Hippocampus-entorhinal cortex loop and seizure generation in the young rodent limbic system. *J Neurophysiol.* 2000; 83:3183–3187. [PubMed: 10805716]
- Chagnac-Amitai Y, Connors BW. Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. *J Neurophysiol.* 1989; 61:747–758. [PubMed: 2542471]
- Chiang C, Lin C, Ju M, Durand D. High-frequency stimulation can suppress globally seizures induced by 4-AP in the rat hippocampus: An acute in vivo study. *Brain Stimulation.* 2012 In Press.
- Chkhenkeli S, Sramka M, Lortkipanidze G, Rakviashvili T, Bregvadze E, Magalashvili G. Electrophysiological effects and clinical results of direct brain stimulation for intractable epilepsy. *Clin Neurol Neurosurg.* 2004; 106:318–329. [PubMed: 15297008]
- Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the Origin of Interictal Activity in Human Temporal Lobe Epilepsy in Vitro. *Science.* 2002; 298:1418–1421. [PubMed: 12434059]
- Cohen I, Navarro V, Huberfeld G, Clemenceau S, Baulac M, Miles R. Response to Comment on "On the Origin of Interictal Activity in Human Temporal Lobe Epilepsy in Vitro". *Science.* 2003; 301:463d.
- D'Arcangelo G, Panuccio G, Tancredi V, Avoli M. Repetitive low-frequency stimulation reduces epileptiform synchronization in limbic neuronal networks. *Neurobiol Dis.* 2005; 19:119–128. [PubMed: 15837567]

- Demeter S, Rosene DL, Hoesen GWV. Interhemispheric Pathways of the Hippocampal Formation, Presubiculum, and Entorhinal and Posterior Parahippocampal Cortices in the Rhesus Monkey: The Structure and Organization of the Hippocampal Commissures. *The Journal of Comparative Neurology*. 1985; 233:30–47. [PubMed: 3980771]
- Demeter S, Rosene DL, Hoesen GWV. Fields of Origin and Pathways of the Interhemispheric Commissures in the Temporal Lobe of Macaques. *The Journal of Comparative Neurology*. 1990; 302:29–53. [PubMed: 2086614]
- Derchansky M, Rokni D, Rick JT, Wennberg R, Bardakjian BL, Zhang L, Yarom Y, Carlen PL. Bidirectional multisite seizure propagation in the intact isolated hippocampus: the multifocality of the seizure "focus". *Neurobiol Dis*. 2006; 23:312–328. [PubMed: 16815026]
- Duncan JS, Sander JW, Sisodiya SM, Walker MC. Adult epilepsy. *Lancet*. 2006; 367:1087–1100. [PubMed: 16581409]
- Durand DM, Bikson M. Suppression and control of epileptiform activity by electrical stimulation: a review. *Proceedings of the IEEE*. 2001; 89:1065–1082.
- Engel J. Mesial Temporal Lobe Epilepsy: What Have We Learned? *Neuroscientist*. 2001; 7:340–352. [PubMed: 11488399]
- Feng Z, Durand DM. Propagation of low calcium non-synaptic induced epileptiform activity to the contralateral hippocampus in vivo. *Brain Res*. 2005; 1055:25–35. [PubMed: 16087166]
- Fisher R, Salanova V, Witt T, Worth R, Henry T, Gross R, Oommen K, Osorio I, Nazzaro J, Labar D, Kaplitt M, Sperling M, Sandok E, Neal J, Handforth A, Stern J, DeSalles A, Chung S, Shetter A, Bergen D, Bakay R, Henderson J, French J, Baltuch G, Rosenfeld W, Youkilis A, Marks W, Garcia P, Barbaro N, Fountain N, Bazil C, Goodman R, McKhann G, Babu Krishnamurthy K, Papavassiliou S, Epstein C, Pollard J, Tonder L, Grebin J, Coffey R, Graves N. Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. *Epilepsia*. 2010; 51:899–908. [PubMed: 20331461]
- Gastaut H. Clinical and Electroencephalographical Classification of Epileptic Seizures. *Epilepsia*. 1970; 11:102–113. [PubMed: 5268244]
- Ghorbani P, Mohammad-Zadeh M, Mirnajafi-Zadeh J, Fathollahi Y. Effect of different patterns of low-frequency stimulation on piriform cortex kindled seizures. *Neurosci Lett*. 2007; 425:162–166. [PubMed: 17868994]
- Gloor P, Salanova V, Olivier A, Quesney LF. The human dorsal hippocampal commissure. An anatomically identifiable and functional pathway. *Brain*. 1993; 116(Pt 5):1249–1273. [PubMed: 8221057]
- Goodman JH, Berger RE, Tcheng TK. Preemptive low-frequency stimulation decreases the incidence of amygdala-kindled seizures. *Epilepsia*. 2005; 46:1–7. [PubMed: 15660762]
- Holmes MD, Kutsy RL, Ojemann GA, Wilensky AJ, Ojemann LM. Interictal, unifocal spikes in refractory extratemporal epilepsy predict ictal origin and postsurgical outcome. *Clin Neurophysiol*. 2000; 111:1802–1808. [PubMed: 11018495]
- Horgan J. Brain storm. Controlling chaos could help treat epilepsy. *Scientific American*. 1994; 271:24. [PubMed: 7997864]
- Jallon P. The problem of intractability: the continuing need for new medical therapies in epilepsy. *Epilepsia*. 1997; 38(Suppl 9):S37–S42. [PubMed: 9578544]
- Jensen AL, Durand DM. High frequency stimulation can block axonal conduction. *Experimental Neurology*. 2009; 220:57–70. [PubMed: 19660453]
- Jerger K, Schiff SJ. Periodic pacing an in vitro epileptic focus. *J Neurophysiol*. 1995; 73:876–879. [PubMed: 7760143]
- Jobst BC, Darcey TM, Thadani VM, Roberts DW. Brain stimulation for the treatment of epilepsy. *Epilepsia*. 2010; 51(Suppl 3):88–92. [PubMed: 20618409]
- Jones R. Ictal epileptiform events induced by removal of extracellular magnesium in slices of entorhinal cortex are blocked by baclofen. *Exp Neurol*. 1989; 104:155–161. [PubMed: 2651139]
- Khalilov I, Esclapez M, Medina I, Aggoun D, Lamsa K, Leinekugel X, Khazipov R, Ben-Ari Y. A novel in vitro preparation: the intact hippocampal formation. *Neuron*. 1997; 19:743–749. [PubMed: 9354321]

- Khalilov I, Holmes GL, Ben-Ari Y. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. *Nature. Neuroscience.* 2003; 6:1079–1085. [PubMed: 14502289]
- Khazipov R, Desfreres L, Khalilov I, Ben-Ari Y. Three-independent-compartment chamber to study in vitro commissural synapses. *J Neurophysiol.* 1999; 81:921–924. [PubMed: 10036300]
- Khosravani H, Carlen PL, Valazquez JLP. The Control of Seizure-Like Activity in the Rat Hippocampal Slice. *Biophysical Journal.* 2003; 84:687–695. [PubMed: 12524321]
- Kile K, Tian N, Durand D. Low frequency stimulation decreases seizure activity in a mutation model of epilepsy. *Epilepsia.* 2010; 51:1745–1753. [PubMed: 20659150]
- King D, Spencer SS, McCarthy G, Luby M, Spencer DD. Bilateral hippocampal atrophy in medial temporal lobe epilepsy. *Epilepsia.* 1995; 36:905–910. [PubMed: 7649130]
- Kinoshita M, Ikeda A, Matsushashi M, Matsumoto R, Hitomi T, Begum T, Usui K, Takayama M, Mikuni N, Miyamoto S, Hashimoto N, Shibasaki H. Electric cortical stimulation suppresses epileptic and background activities in neocortical epilepsy and mesial temporal lobe epilepsy. *Clin Neurophysiol.* 2005; 116:1291–1299. [PubMed: 15978492]
- Koubeissi, M.; Durand, D.; Kahriman, E.; Syed, T.; Miller, J.; Luders, H. Low frequency electrical stimulation of white matter tracts in intractable mesial temporal lobe epilepsy; American Academy, Annual Conference; 2012.
- Koubeissi, MZ.; Durand, DM.; Rashid, S.; Luders, H.; Maciunas, R. In vivo demonstration of connectivity between dorsal hippocampal commissure and bilateral mesial temporal structures; Proceedings of the 63rd Annual Meeting of the American Epilepsy Society; Boston, MA. 2009.
- Lacruz ME, Garcia Seoane JJ, Valentin A, Selway R, Alarcon G. Frontal and temporal functional connections of the living human brain. *Eur J Neurosci.* 2007; 26:1357–1370. [PubMed: 17767512]
- Lees G, Stohr T, Errington AC. Stereoselective effects of the novel anticonvulsant lacosamide against 4-AP induced epileptiform activity in rat visual cortex in vitro. *Neuropharmacology.* 2006; 50:98–110. [PubMed: 16225894]
- Lima, VMFd; Pijn, JP.; Filipe, CN.; Silva, FLd. The role of hippocampal commissures in the interhemispheric transfer of epileptiform afterdischarges in the rat: a study using linear and non-linear regression analysis. *Electroencephalography and clinical Neurophysiology.* 1990; 76:520–539. [PubMed: 1701120]
- Limbrick D, Narayan P, Powers A, Ojemann J, Park T, Bertrand M, Smyth M. Hemispherotomy: efficacy and analysis of seizure recurrence. *J Neurosurg Pediatr.* 2009; 4:323–332. [PubMed: 19795963]
- Madison DV, Nicoll RA. Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. *Nature.* 1982; 299:636–638. [PubMed: 6289127]
- Madison DV, Nicoll RA. Control of the repetitive discharge of rat CA1 pyramidal neurons in vitro. *J Physiol.* 1984; 354:319–331. [PubMed: 6434729]
- Mason J, Voss L, Jacobson G, Elbohouty M, Sleight JW, Steyn-Ross M, Steyn-Ross A, Peixoto N. Changes in frequency of seizure-like events in stimulated cortical slices. *Conf Proc IEEE Eng Med Biol Soc.* 2010; 2010:2041–2044. [PubMed: 21096430]
- Meier R, Haussler U, Aertsen A, Deransart C, Depaulis A, Egert U. Short-term changes in bilateral hippocampal coherence precede epileptiform events. *NeuroImage.* 2007; 38:138–149. [PubMed: 17761441]
- Morrell M. Brain stimulation for epilepsy: can scheduled or responsive neurostimulation stop seizures? *Curr Opin Neurol.* 2006; 19:164–168. [PubMed: 16538091]
- Morrell MJ. Responsive cortical stimulation for the treatment of medically intractable partial epilepsy. *Neurology.* 2011; 77:1295–1304. [PubMed: 21917777]
- O'Keefe, J.; Nadel, L., editors. *The Hippocampus as a Cognitive Map.* Oxford: Oxford University Press; 1978.
- Parrent A, Almeida CS. Deep brain stimulation and cortical stimulation in the treatment of epilepsy. *Adv Neurol.* 2006; 97:563–572. [PubMed: 16383164]
- Perreault P, Avoli M. Physiology and pharmacology of epileptiform activity induced by 4-aminopyridine in rat hippocampal slices. *J Neurophysiol.* 1991; 65:771–785. [PubMed: 1675671]

- Rashid S, Pho G, Czigler M, Werz MA, Durand DM. Low frequency stimulation of ventral hippocampal commissures reduces seizures in a rat model of chronic temporal lobe epilepsy. *Epilepsia*. 2011; 53:147–156. [PubMed: 22150779]
- Reato D, Rahman A, Bikson M, Parra LC. Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. *J Neurosci*. 2010; 30:15067–15079. [PubMed: 21068312]
- Rolston JD, Desai SA, Laxpati NG, Gross RE. Electrical Stimulation for Epilepsy: Experimental Approaches. *Neurosurgery clinics of North America*. 22:425–442. [PubMed: 21939841]
- Rosenzweig I, Beniczky S, Brunnhuber F, Alarcon G, Valentin A. The dorsal hippocampal commissure: when functionality matters. *J Neuropsychiatry Clin Neurosci*. 23:E45–E48. [PubMed: 21948928]
- Rotenberg A, Bae E, Muller P, Jr JR, Bourgeois B, Blum A, Pascual-Leone A. In-session seizures during low-frequency repetitive transcranial magnetic stimulation in patients with epilepsy. *Epilepsy Behav*. 2009; 16:353–355. [PubMed: 19747883]
- Sadler RM. The syndrome of mesial temporal lobe epilepsy with hippocampal sclerosis: clinical features and differential diagnosis. *Adv Neurol*. 2006; 97:27–37. [PubMed: 16383112]
- Schiff SJ, Jerger K, Duong DH, Chang T, Spano ML, Ditto WL. Controlling chaos in the brain. *Nature*. 1994; 370:615–620. [PubMed: 8065447]
- Schiller Y, Bankirer Y. Cellular mechanisms underlying antiepileptic effects of low- and high-frequency electrical stimulation in acute epilepsy in neocortical brain slices in-vitro. *J Neurophysiol*. 2006; 97:1887–1902. [PubMed: 17151229]
- Schrader LM, Stern JM, Wilson CL, Fields TA, Salamon N, Nuwer MR, Vespa PM, Fried I. Low frequency electrical stimulation through subdural electrodes in a case of refractory status epilepticus. *Clin Neurophysiol*. 2006; 117:781–788. [PubMed: 16458067]
- Slutzky MW, Cvitanovic P, Mogul DJ. Manipulating epileptiform bursting in the rat hippocampus using chaos control and adaptive techniques. *IEEE Trans Biomed Eng*. 2003; 50:559–570. [PubMed: 12769432]
- Spencer Susan S. Neural Networks in Human Epilepsy: Evidence of and Implications for Treatment. *Epilepsia*. 2002; 43:219–227. [PubMed: 11906505]
- Sun HL, Zhang SH, Zhong K, Xu ZH, Zhu W, Fang Q, Wu DC, Hu WW, Xiao B, Chen Z. Mode-dependent effect of low-frequency stimulation targeting the hippocampal CA3 subfield on amygdala-kindled seizures in rats. *Epilepsy Res*. 2010; 90:83–90. [PubMed: 20392604]
- Sunderam S, Chernyy N, Peixoto N, Mason JP, Weinstein SL, Schiff SJ, Gluckman BJ. Seizure entrainment with polarizing low-frequency electric fields in a chronic animal epilepsy model. *J Neural Eng*. 2009; 6 046009.
- Sunderam S, Gluckman B, Reato D, Bikson M. Toward rational design of electrical stimulation strategies for epilepsy control. *Epilepsy & Behavior*. 2010; 17:6–22. [PubMed: 19926525]
- Swanson TH. The Pathophysiology of Human Mesial Temporal Lobe Epilepsy. *Journal of Clinical Neurophysiology*. 1995; 12:15–22.
- Swartzwelder H, Lewis D, Anderson W, Wilson W. Seizure-like events in brain slices: suppression by interictal activity. *Brain Res*. 1987; 410:362–366. [PubMed: 3594246]
- Tang Y, Durand DM. A novel electrical stimulation paradigm for the suppression of epileptiform activity in an in vivo model of mesial temporal lobe status epilepticus. *IJNS*. 2012; 22:12500–12501. 12514.
- Tapia R, Sitges M. Effect of 4-aminopyridine on transmitter release in synaptosomes. *Brain Res*. 1982; 250:291–299. [PubMed: 6129030]
- Theodore WH, Fisher RS. Brain stimulation for epilepsy. *Lancet Neurol*. 2004; 3:111–118. [PubMed: 14747003]
- Toprani S, Najm I, Durand D. Seizure Suppression by Low Frequency Electrical Stimulation of the Fimbria in Combined Hippocampus-Entorhinal Cortex Slices in Rats. *Annals of Neurology*. 2008; 64:S4–S64.
- Toprani S, Najm I, Durand D. Fiber tract stimulation for bilateral hippocampal seizure prevention. 2010. Neuroscience Meeting Planner. 2010 Program No. 657.29.

- Traub RD, Borck C, Colling SB, Jefferys JGR. On the Structure of Ictal Events in Vitro. *Epilepsia*. 1996; 37:879–891. [PubMed: 8814102]
- Vann SD, Brown MW, Erichsen JT, Aggleton JP. Using Fos Imaging in the Rat to Reveal the Anatomical Extent of the Disruptive Effects of Fornix Lesions. *The Journal of Neuroscience*. 2000; 20:8144–8152. [PubMed: 11050137]
- Velasco A, Velasco M, Velasco F, Menes D, Gordon F, Rocha L, Briones M, I M. Subacute and chronic electrical stimulation of the hippocampus on intractable temporal lobe seizures: preliminary report. *Arch Med Res*. 2000; 31:316–328. [PubMed: 11036183]
- Velasco M, Velasco F, Velasco A, Boleaga B, Jimenez F, Brito F, Marquez I. Subacute electrical stimulation of the hippocampus blocks intractable temporal lobe seizures and paroxysmal EEG activities. *Epilepsia*. 2000a; 41
- Velisek L, Veliskova J, Stanton PK. Low-frequency stimulation of the kindling focus delays basolateral amygdala kindling in immature rats. *Neurosci Lett*. 2002; 326:61–63. [PubMed: 12052538]
- Vonck K, Boon P, Achten E, De Reuck J, Caemaert J. Long-term amygdalohippocampal stimulation for refractory temporal lobe epilepsy. *Annals of Neurology*. 2002; 52:556–565. [PubMed: 12402252]
- Vonck K, Boon P, Claeys P, Dedeurwaerdere S, Achten R, Van Roost D. Long-term deep brain stimulation for refractory temporal lobe epilepsy. *Epilepsia*. 2005a; 46(Suppl 5):98–99. [PubMed: 15987261]
- Vonck K, Boon P, Claeys P, Dedeurwaerdere S, Achten R, Van Roost D. Long-term Deep Brain Stimulation for Refractory Temporal Lobe Epilepsy. *Epilepsia*. 2005b; 46:98–99. [PubMed: 15987261]
- Wilson CL, Isokawa M, Babb TL, Crandall PH. Functional connections in the human temporal lobe. I. Analysis of limbic system pathways using neuronal responses evoked by electrical stimulation. *Exp Brain Res*. 1990; 82:279–292. [PubMed: 2286232]
- Wilson CL, Isokawa M, Babb TL, Crandall PH, Levesque MF, Engel J Jr. Functional connections in the human temporal lobe. II. Evidence for a loss of functional linkage between contralateral limbic structures. *Exp Brain Res*. 1991; 85:174–187. [PubMed: 1884756]
- Yamamoto J, Ikeda A, Kinoshita M, Matsumoto R, Satow T, Takeshita K, Matsushashi M, Mikuni N, Miyamoto S, Hashimoto N, Shibasaki H. Low-frequency electric cortical stimulation decreases interictal and ictal activity in human epilepsy. *Seizure*. 2006; 15:17–24. [PubMed: 17113794]
- Yang LX, Jin CL, Zhu-Ge ZB, Wang S, Wei EQ, Bruce IC, Chen Z. Unilateral low-frequency stimulation of central piriform cortex delays seizure development induced by amygdaloid kindling in rats. *Neuroscience*. 2006; 138:1089–1096. [PubMed: 16427743]
- Zhang SH, Sun HL, Fang Q, Zhong K, Wu DC, Wang S, Chen Z. Low-frequency stimulation of the hippocampal CA3 subfield is anti-epileptogenic and anti-ictogenic in rat amygdaloid kindling model of epilepsy. *Neurosci Lett*. 2009; 455:51–55. [PubMed: 19429105]
- Zhong XL, Lv KR, Zhang Q, Yu JT, Xing YY, Wang ND, Tan L. Low-frequency stimulation of bilateral anterior nucleus of thalamus inhibits amygdala-kindled seizures in rats. *Brain Res Bull*. 2009; 86:422–427. [PubMed: 21893168]

HIGHLIGHTS

- We present a slice preparation of bilateral hippocampi connected by a fiber tract.
- Stimulation with one fiber-tract electrode reduces bilateral hippocampal seizures.
- Seizure reduction does not involve depolarization block, LTD, or GABA_A receptors.
- Post-stimulation seizure reduction is observed and requires connected hippocampi.
- It is correlated with an increase in low-frequency interictal bursting.

Figure 1. Bilateral hippocampal slice preparation

An example of a bilateral slice preparation that has been stained with cresyl violet for nuclei and luxol fast blue for myelinated axons of the VHC is shown to the left with a schematic of typical recording and stimulating parameters on the right. To test the functional connection between left and right hippocampi, stimulation was applied to three distinct locations along the VHC axon tract (1–3) with simultaneous left and right CA1 recordings (Rec L, Rec R) in ACSF solution. The evoked potentials recorded from left and right CA1 during stimulation at each location are shown with truncated stimulus artifacts marked by dots.

Figure 2. Artifact removal

Template subtraction reduces artifacts and evoked responses in the signal while maintaining spontaneous physiological activity. A representative example of 5 seconds of raw data from CA1 in an epileptic slice during stimulation at 1 Hz is shown in the top panel. Stimulation artifacts are marked by dots and followed by evoked responses. There is also non-evoked spontaneous epileptic activity in the trace. Templates were constructed as described in the text with the goal of capturing stimulus artifacts and evoked responses only and are shown in the middle panel for each second of data. All analysis was performed on template-subtracted signals like the example shown in the third panel. The location of artifacts is denoted by dots. The template-subtraction paradigm selectively reduced artifacts.

Figure 3. 4-AP-induced epileptiform activity

(A) recorded from CA3 and CA1 hippocampal subfields of a combined hippocampal-EC preparation is shown as recorded (B) from an extracellular electrode (C) as well as from an intracellular electrode. The epileptiform activity included distinct ictal and interictal epochs. (D) Mean $\pm$ SD sFFT (time resolution 1.5 s, frequency resolution 0.67 Hz) of 4-AP-induced activity relative to slice-baseline showed that both types of epileptiform activity had peak amplitudes near 1 Hz, as shown in the inset. However, ictal activity had a secondary peak near 50 Hz with high amplitude activity at a range of frequencies approaching up to 300 Hz. (E) Ictal and interictal activities in this model could be distinguished by: (1) mean

spectral frequency; (2) mean duration; and (3) mean period. **** indicates $p < 0.0001$ using paired (t) test.

A Experimental conditions **B** 4-AP induced epileptiform activity **C** Cross covariance of left and right hippocampal activity **D** Lag with intact and cut VHC **E** % of max covariance after cut

Figure 4. Epileptic activity synchronizes across the hippocampal commissures in this model (A) 4-AP-induced epileptiform activity was recorded from CA3 and CA1 of bilateral hippocampi with the VHC intact and after it was cut. (B) One minute of left and right field potentials from CA1 before and after the VHC was severed show epileptiform activity that appears to synchronize across the VHC that is no longer correlated in the absence of the VHC. (C) The left and right cross covariance shows highly correlated bilateral epileptiform activity only when there is an intact VHC. (D) Left to right delay was from 12 bilateral *in vitro* models in which the VHC was subsequently cut showed a significant increase in variance of the mean lag with VHC cutting ($F(11,11)=4,920,836$, $p<0.0001$). With VHC intact, all left-right lags were less than 30 ms. However, left and right activity was independent after the VHC was cut with a broad range of lag times. (E) There was a significant decrease in mean % maximum covariance resulting from severing the VHC

(paired $t(11)=23.55286$, $p<0.0001$). **** indicates $p<0.0001$. Box plots represent data mean $\pm$ SD.

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Figure 5. LFS reduced bilateral seizures

(A) Epileptiform activity induced by 4-AP before and during LFS was recorded bilaterally from CA3 and CA1 extracellularly and intracellularly. (B) Interictal and ictal activity occurred prior to stimulation. Ictal activity is marked by dots (magnified in inset from intracellular and extracellular recordings) and characterized by high frequencies. sFFTs were performed on 15-minute segments of data (time resolution 1.5 s, frequency resolution 0.67 Hz) and amplitudes were normalized to account for slice variability. During stimulation, very little spontaneous activity was evident after template subtraction. Examples of evoked responses are shown in the inset. (C) Changes in parameters of epileptiform activity, including (1) percent seizure time; (2) seizure duration; (3) seizures per minute; (4) power in

the ictal; and (5) power in the interictal spectra were compared between groups that did and did not receive LFS (*) as well as within groups before and during treatment (#) for the left and right hippocampus. There was highly significant bilateral reduction in all epileptic measures during stimulation.

* $p < 0.05$; # $p < 0.05$;
** $p < 0.01$; ## $p < 0.01$;
*** $p < 0.001$; and ### $p < 0.001$; and
**** $p < 0.0001$ #### $p < 0.0001$

Figure 6. The effect of LFS requires axonal connection

(A) 4-AP-induced epileptiform before and during LFS is recorded from bilateral CA3 and CA1 extracellularly and intracellularly after the VHC has been severed to the right of stimulus. (B) Non-correlated interictal and ictal activity occurred in each hippocampus prior to stimulation. Seizures are marked by dots and characterized by high frequency. sFFTs were performed on 15-minute segments of data (time resolution 1.5 s, frequency resolution 0.67 Hz) and amplitudes were normalized to account for slice variability. During stimulation, very little spontaneous activity was evident after template subtraction for the left hippocampus, while epileptiform activity persisted in the right hippocampus. (C) Changes in parameters of epileptiform activity, including (1) percent seizure time; (2)

seizure duration; (3) seizures per minute; (4) power in the ictal; and (5) power in the interictal spectra are compared between groups that did and did not receive LFS (*) as well as within groups before and during treatment (#) for the left and right hippocampus. There was highly significant reduction in all epileptic measures in hippocampi that received direct axonal stimulation only.

* $p < 0.05$; # $p < 0.05$;
** $p < 0.01$; ## $p < 0.01$;
*** $p < 0.001$; and ### $p < 0.001$; and
**** $p < 0.0001$ #### $p < 0.0001$

A Experimental conditions **B** Mean amplitude of CA1 EPs during LFS with 4-AP **C** Mean number of CA1 APs evoked by LFS with 4-AP **Figure 7. Evoked responses during LFS**

The LFS paradigm, applied to the VHC while recording from bilateral CA1 as shown in (A), did not induce significant changes in evoked responses over treatment course. (B) The average normalized evoked potential amplitude $\pm$ SD per 1 Hz stimulus did not change across the 15-minute stimulation paradigm ($F(779, 12,784) = 0.211$, $p = 1$ using ANOVA). The amplitudes of evoked potentials elicited during LFS in the 4-AP seizure model were determined as the average potential of (a, b) minus the potential of (c), as shown in the inset. Evoked potentials in 4-AP displayed hyperactivity, as shown. Measurements were taken every second and are shown after 2 minutes allow acclimation to stimulation. (C) Similarly, the mean number of action potentials elicited by each LFS pulse $\pm$ SD remained relatively

consistent throughout the LFS protocol ($F(779, 2,337) = 0.799, p = 0.999$ using ANOVA). Responses to LFS with potential amplitudes above -20 mV following each stimulus were counted as action potentials as shown in the inset. Measurements are shown after 2 minutes to allow acclimation to stimulation and are taken at one-second intervals to count each stimulus. Multiple action potentials atop a paroxysmal depolarizing shift (PDS) were elicited by each LFS pulse in 4-AP as shown.

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Figure 8. Acclimation to LFS

(A) CA1 evoked responses and non-evoked activities were recorded during LFS. (B) Seizure can occur at the start of LFS but terminates within 30 seconds, as shown in a template-subtracted trace of the first 2 minutes of LFS and its corresponding normalized power spectral density (time resolution 1.5 s, frequency resolution 0.67 Hz). (C) The average normalized EP amplitude $\pm$ SD increased gradually over this time and reached steady state around 30 s after LFS was started. EP amplitudes were measured every second and calculated as the average potential of (a, b) minus the potential of (c), as shown in the inset. (D) The mean number of action potentials elicited by each LFS pulse $\pm$ SD reached steady

state 1 minute after LFS was turned on. Responses to LFS above -20 mV following each stimulus were counted as action potentials as shown in the inset.

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A1 Amplitude dependence of LFS **A2** Amplitude dependence of start-seizure **B** Frequency dependence of LFS **Figure 9. Amplitude and frequency dependence of LFS**

(A1) The LFS protocol was repeated at stimulus amplitudes to evoke 25, 50, 75, and 100% of the maximum response ($n=5$ per amplitude tested) while percent reduction in seizures was measured. There was a linear relationship between stimulus amplitude and seizure reduction given by the equation $y = 28x - 11.49$ ($R^2 = 0.998$). (A2) For the protocol described, the percent of trials in which seizure occurred when LFS was turned on increased linearly with stimulus amplitude and could be described by the equation $y = 28x - 30$ ($R^2 = 0.98$). (B) % reduction in seizure time during LFS was measured at stimulus frequencies of 0.1, 0.2, 0.33, 0.5, 1, 2, 3, 5, and 10 Hz ($n=5, 4, 6, 8, 10, 7, 4, 5$, and 3, respectively). A significant effect of % reduction in seizure time was found for changes in LFS frequency using ANOVA with $F(10, 47) = 19.93$. **** indicates $p < 0.0001$.

Figure 10. LFS in different seizure models

LFS efficacy was not different in 3 distinct seizure models ($F(2, 25) = .389$, $p = 0.682$ using ANOVA). Seizure reduction during electrical stimulation across groups was distributed as follows ($n = 14, 9, 5$, respectively): 97% $\pm$ 8% in 100 μ M 4-AP; 97% $\pm$ 6% in 10 μ M BMI; and 94% $\pm$ 12% in magnesium-free ACSF.

Figure 11. Interconnected hippocampi exhibit significant post-LFS seizure reduction

(A) 4-AP-induced epileptiform activity before LFS and after its termination was recorded from CA3 and CA1 extracellularly and intracellularly from slices where the VHC was cut. Stimulation was applied to the axons just left of the cut. (B) In the left hippocampus, interictal and ictal activity occurred prior to stimulation as well as after it was turned off. Seizures are marked by dots and characterized by high frequency. Normalized sFFT were performed on 15-minute segments of data (time resolution 1.5 s, frequency resolution 0.67 Hz) and are shown for each trace. (C) The protocol was repeated for intact bilateral slice preparations. (D) In the left hippocampus, interictal and ictal activity (dots) occurred prior to VHC stimulation. Post-LFS ictal activity was variable in the bilateral preparation and in

some instances did not occur at all. Post-LFS interictal activity followed the frequency of LFS, as shown in the insets from intracellular and extracellular recordings. (E) Changes in parameters of epileptiform activity between pre-treatment and post-treatment levels, including: (1) percent seizure time; (2) seizure duration; (3) seizures per minute; (4) power in the ictal; and (5) power in the interictal spectra were compared between groups that did and did not receive LFS (*) as well as within groups before and after treatment or sham-treatment (#). This data is presented for the axonally stimulated hippocampus in the cut preparation (left panel) and for both hippocampi of the intact preparation (right panel).

* $p < 0.05$	# $p < 0.05$
** $p < 0.01$	## $p < 0.01$
*** $p < 0.001$	### $p < 0.001$
**** $p < 0.0001$	#### $p < 0.0001$