Experimental Neurology 294 (2017) 68–77

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Effects of hippocampal low-frequency stimulation in idiopathic non-human primate epilepsy assessed via a remote-sensing-enabled neurostimulator

Thomas A. Wozny [a], Witold J. Lipski [a], Ahmad Alhourani [a], Efstathios D. Kondylis [a], Arun Antony [b,c], R. Mark Richardson [a,c,d,*]

[a] Brain Modulation Lab, Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA 15213, United States
[b] Department of Neurology, University of Pittsburgh, Pittsburgh, PA 15213, United States
[c] University of Pittsburgh Comprehensive Epilepsy Center, Pittsburgh, PA 15213, United States
[d] University of Pittsburgh Brain Institute, Pittsburgh, PA 15213, United States

ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form 28 April 2017
Accepted 6 May 2017
Available online 7 May 2017

Keywords:
Epilepsy
Hippocampus
Local field potential
Non-human primate
Electrical stimulation
Low-frequency stimulation
Open-loop stimulation
Closed-loop stimulation
Deep brain stimulation
Sensing-enabled neurostimulator

ABSTRACT

Individuals with pharmacoresistant epilepsy remain a large and under-treated patient population. Continued technologic advancements in implantable neurostimulators have spurred considerable research efforts directed towards the development of novel antiepileptic stimulation therapies. However, the lack of adequate preclinical experimental platforms has precluded a detailed understanding of the differential effects of stimulation parameters on neuronal activity within seizure networks. In order to chronically monitor seizures and the effects of stimulation in a freely-behaving non-human primate with idiopathic epilepsy, we employed a novel simultaneous video-intracranial EEG recording platform using a state-of-the-art sensing-enabled, rechargeable clinical neurostimulator with real-time seizure detection and wireless data streaming capabilities. Using this platform, we were able to characterize the electrographic and semiologic features of the focal-onset, secondarily generalizing tonic-clonic seizures stably expressed in this animal. A series of acute experiments exploring low-frequency (2 Hz) hippocampal stimulation identified a pulse width (150 μs) and current amplitude (4 mA) combination which maximally suppressed local hippocampal activity. These optimized stimulation parameters were then delivered to the seizure onset-side hippocampus in a series of chronic experiments. This long-term testing revealed that the suppressive effects of low-frequency hippocampal stimulation 1) diminish when delivered continuously but are maintained when stimulation is cycled on and off, 2) are dependent on circadian rhythms, and 3) do not necessarily confer seizure protective effects.

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1. Introduction

Epilepsy is one of the most common neurological disorders, affecting approximately 0.4–1% of the global population. Mesial temporal lobe epilepsy (MTLE) is the most common form of focal epilepsy and the most likely to be resistant to medical therapy, with at least one third of patients failing either mono or poly pharmacotherapy (see Duncan et al., 2006 for summary). For this population, resective surgery has long remained the only definitive treatment option, offering roughly two thirds of patients freedom from debilitating seizures (Spencer et al., 2005; Tellez-Zenteno et al., 2005; Wiebe et al., 2001; Wieser et al., 2003). Individuals who are not seizure free after surgery, and those unsuitable for resective surgery due either to lack of a single identifiable seizure focus or unacceptably high risk for neurologic deficit secondary to resection, have historically been without adequate treatment options.

To address this unmet therapeutic need and to build upon prior successes in the treatment of movement disorders, direct brain stimulation for epilepsy has been the subject of considerable research effort. In contrast to resective surgery, direct brain stimulation is a less invasive intervention that is highly customizable for meeting the evolving treatment needs of an individual patient. Numerous neuroanatomical targets for electrical stimulation have been explored for the treatment of MTLE in humans, including the vagus nerve (DeGiorgio et al., 2000), anterior nucleus of the thalamus (Fisher et al., 2010; Lee et al., 2012; Lehtimaki et al., 2015; Salanova et al., 2015), fimbria/fornix (Koubeissi et al., 2013),

Abbreviations: DBS, deep brain stimulation; EEG, electroencephalogram; ERP, event-related potential; FFT, fast Fourier transform; HFS, high-frequency stimulation; LFP, local field potential; LFS, low-frequency stimulation; LL, line-length; MRI, magnetic resonance imaging; MTLE, mesial temporal lobe epilepsy; NHP, non-human primate; RNS, Responsive Neurostimulation; SANTE, Stimulation of the Anterior Nucleus of the Thalamus for Epilepsy.

* Corresponding author at: Department of Neurological Surgery, University of Pittsburgh Medical Center, 200 Lothrop St., Pittsburgh, PA 15213, United States.
E-mail address: richardsonrm@upmc.edu (R.M. Richardson).

http://dx.doi.org/10.1016/j.expneurol.2017.05.003
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and the mesial temporal lobe itself (Boëx et al., 2011; Cukiert et al., 2014; Lim et al., 2016; McLachlan et al., 2010; Min et al., 2013; Tellez-Zenteno et al., 2006; Velasco et al., 2007; Vonck et al., 2002, 2013; see Han et al., 2014 for review). Two large-scale, long-term clinical trials of direct brain stimulation therapies for epilepsy have already been conducted: 1) the Stimulation of the Anterior Nucleus of the Thalamus for Epilepsy (SANTE) trial (Fisher et al., 2010; Salanova et al., 2015) delivered stimulation in an open-loop paradigm, in which predetermined stimulation is delivered regardless of brain state, whereas 2) the Responsive Neurostimulation (RNS) Pivotal Study (Bergey et al., 2015; Heck et al., 2014) utilized closed-loop stimulation, which is modified in response to ongoing monitoring of neural activity, delivered to clinically-determined, patient-specific cortical and/or deep brain targets. While promising results of approximately 69% (SANTE) and 60% (RNS) reduction in seizure frequency after five years were reported, only a minority of participants became seizure-free.

Numerous obstacles hinder the rapid optimization of electrical stimulation therapies. Perhaps one of the greatest barriers to advancement is the vast, multi-dimensional space of stimulation parameters to be explored. Frequency, pulse width, and amplitude (Fig. 1A) are among the most commonly operationalized variables. Each of these parameters differentially effects neuronal activity and, in addition to cumulative stimulation ON time, contributes to the total electrical current requirements. Further limiting improvements in stimulation therapies is the lack of adequate platforms for stimulation testing – many rodent models of epilepsy have been developed but the translational utility of these models remains a subject of debate. On the other hand, while non-human primates (NHP) better recapitulate human neurophysiology and functional neuroanatomy, there is a relative paucity of NHP research in epilepsy, and testing in humans is often constrained by financial, logistical, and ethical considerations. As a result of these obstacles, the preponderance of stimulation studies in epilepsy have followed the precedent established by early work in movement disorders surgery demonstrating the acutely suppressive effects of high-frequency stimulation (HFS, >60 Hz) on neuronal synchrony. Low-frequency stimulation (LFS, <10 Hz) has consequently remained under-explored despite its theoretically lower current requirements and its demonstrated success in reducing seizure frequency and severity in multiple rodent models (summarized in Han et al., 2014). Further indicating the need for continued investigation, recent studies have demonstrated reductions in interictal epileptiform activity and seizure frequency as well as improvements in cognitive functioning with LFS (Koubeissi et al., 2013; Lim et al., 2016; Toprani and Durand, 2013; Toprani et al., 2013; Wang et al., 2016).

An additional challenge to the advancement of stimulation therapies is the absence of suitable biomarkers indexing stimulation efficacy, which are observable at short latencies. While stimulation programing in Parkinson's and essential tremor benefits, for instance, from the near immediate reduction in tremor amplitude following HFS, the gold standard for measuring therapeutic benefit in epilepsy remains the observed seizure frequency. Quantifying this effect necessitates long-term follow-up resulting in lengthy research trials and extended periods of clinical device programming. Indeed, clinical stimulation programming is currently conducted through an iterative process of individual parameter adjustment followed by observation of the change's effect on seizure rate. During this protracted process, patients may receive suboptimal seizure control and experience accelerated battery consumption, with some groups reporting implanted device lifespans as short as nine months (Lee et al., 2015). Closed-loop stimulation also relies on short-latency biomarkers to modulate stimulation delivery. While the RNS device has demonstrated success with power-based measures of local-field potential (LFP) activity in general, and the measure of line-length in particular, identification of signal features which better distinguish pathologic from physiologic brain states stands to improve the accuracy of stimulation delivery.

In order to investigate the effects of LFS in the mesial temporal lobe of an NHP with idiopathic epilepsy, we used a next-generation sensing-enabling brain stimulation device with wireless telemetry capability (Freestone et al., 2013; Stypulkowski et al., 2013, 2014) to create a chronic, simultaneous video-intracranial EEG monitoring platform. This implanted rechargeable clinical neurostimulator capable of simultaneous stimulation and real-time, wireless LFP recording allowed for long-term recordings in the freely behaving animal. We built upon our prior study in this animal (Lipski et al., 2015) by further characterizing both the electrographic and semiologic correlates of the NHP's clinical seizures. Next, a series of acute experiments explored the differential effects of pulse width and current amplitude on the neural response to LFS. The results from these acute experiments were then used to guide stimulation parameter selection for further exploration in a set of chronic experiments. We hypothesized that this approach would reveal potential avenues for expediting the optimization of stimulation therapies on a patient-specific basis.

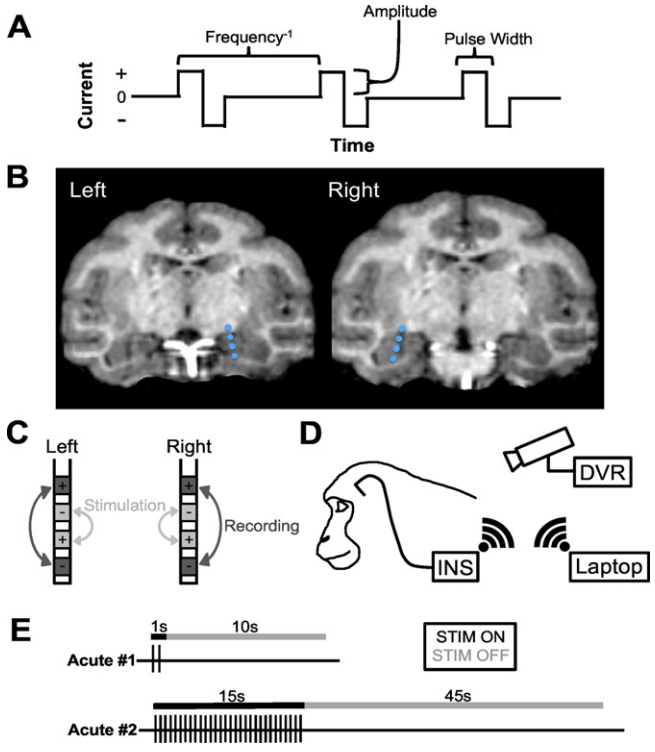

Fig. 1. Experimental design. Panel A is a schematic representation summarizing stimulation parameters of interest. Panel B shows the preoperative 3 T MRI with electrode contact locations represented by blue circles, in the two coronal planes that best matched the lead trajectories, overlaid from the intraoperative MRI (not shown). Panel C depicts the recording-stimulating bipolar montage (arrows indicating paired electrode contacts) used for both depth electrodes. "+" and "−" represent cathode and anode, respectively. The experimental setup is summarized in panel D, with intracranial electrodes connected to an implanted neurostimulator (INS) which wirelessly communicates with a laptop outside of the animal's enclosure. In parallel, a digital video recording (DVR) system continuously records the animal's behavior. Organization of single trials of stimulation trains in acute experiments 1 & 2 is shown in panel E – the x-axis is time and vertical lines indicate stimulation pulses.

2. Materials and methods

2.1. Animal

One eight-year-old male NHP (*Macaca mulatta*; 10 kg) exhibiting spontaneous, recurrent seizures for >3 years was studied. Prior to inclusion in this study, the NHP was raised in captivity as part of a behavioral study in which no other animals developed epilepsy. No pharmacologic,

surgical, or genetic manipulations were employed in order to induce seizures in the NHP studied. A high-resolution, 3-T magnetic resonance imaging (MRI) conducted prior to initial surgery did not reveal any neuroanatomical abnormalities. At diagnosis, the animal briefly received levetiracetam for seizure management but had not received any antiepileptic medications for >3 years prior the start of this study. The NHP was maintained on a 12 h light/dark cycle and cared for in a manner consistent with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). Additionally, all experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

2.2. Implanted device

The NHP was chronically implanted (>2 years) with two clinical deep brain stimulation (DBS) electrodes (model 3389, Medtronic, Minneapolis, MN), one in each hippocampus. DBS electrodes were implanted using interventional 3 T MRI, which allowed real-time visual confirmation of lead placement in the body of the hippocampus, as previously described (Lipski et al., 2015). The transfrontal approach resulted in contact placement across the dorsal-ventral axis of the hippocampus, with the deepest contact in the parahippocampal gyrus, the middle two contacts in the hippocampus, and the most proximal contact in the temporal stem (Fig. 1B). Prior to the start of the study, the NHP's indwelling pulse generator (previously the Activa PC + S, Medtronic, Inc.) was replaced with an Activa RC + S (investigational device, Medtronic, Inc.). The stimulation and recording features of this device were controlled wirelessly using a laptop running a custom-designed user interface written in LabVIEW (National Instruments). When needed, the RC + S battery was recharged transcutaenously using a wireless inductive charger while the animal was anesthetized with ketamine (Ketaset, 5–10 mg/kg), since fully recharging the battery from empty required approximately 1 h.

2.3. Neural recordings and stimulation

All recordings were performed while the animal was freely behaving and >24 h after the most recent administration of ketamine to minimize any confounding medication effects. All LFP recordings were sampled at either 1000 Hz (acute experiments) or 500 Hz (spontaneous recordings and chronic experiments). LFPs were obtained using bipolar referencing between the most distal and the most proximal contact on each DBS electrode, thus yielding one LFP time series per hippocampus. Constant-current electrical stimulation consisted of biphasic, square-wave pulses delivered unilaterally in a bipolar fashion using the two contacts located between the bipolar recording pair on each DBS electrode (the more distal contact of the stimulating pair was always the cathode; Fig. 1C). Because simultaneous recording and stimulation through the same electrode contacts was not technically possible, separate contact pairs had to be used for the two functions. The bipolar recording-stimulating montage depicted in Fig. 1C was used because it provided the highest quality recordings by minimizing stimulation artifact. The reason for this benefit in signal quality is largely due to electrode geometry. More specifically, the location of the stimulating contacts relative to recording contacts directed the majority of stimulation current to pass between, as opposed to across, the recording contacts. The location of recording contacts symmetrically "outside" the stimulating pair additionally maximized the cancellation of the stimulation dipole. Despite this benefit in recording quality, additional denoising was still required to prevent saturation of the recording channels, particularly at higher current amplitudes. To address this problem, the built-in "blanking" feature on the RC + S was used to transiently electrically decouple the recording circuitry from the implanted electrodes during delivery of each individual stimulation pulse. Blanking was empirically varied between 1.5 and 2.5 ms duration for each stimulation parameter combination in order to minimize artifact while still leaving a sufficient voltage deflection in the LFP recording so that stimulation pulses could be detected offline for pulse-locked analyses.

2.4. Spontaneous seizure monitoring and online detector programming

A night-vision enabled, closed-circuit digital video recording system was used to continuously record the NHP's spontaneous behavior for a total of 11 weeks (Fig. 1D). All video was visually reviewed for the presence of clinically overt seizure activity with the aid of 16-times playback speed and motion detection software. After clinical seizures were manually identified, video segments including preictal, ictal, and postictal periods were epoched and scored based on semiologic characteristics. A subset of epoched clinical seizures were additionally reviewed by a board-certified neurologist subspecializing in epilepsy to verify the accuracy of seizure scoring. During periods of spontaneous seizure monitoring, timed LFP recordings were streamed and saved to a nearby laptop (Fig. 1D). Timed rather than continuous LFP recordings were used to allow for longer monitoring periods between battery recharges as wireless data streaming is the most current-intensive process performed by the Activa RC + S (11 h of cumulative streaming per charge).

Also during this time, an online electrographic seizure detector implementing a sliding window FFT (256-point with 50% overlap; 500 Hz sampling) using the integrated circuitry of the RC + S was empirically programmed to accurately detect video-confirmed seizures. Based on our prior experience with the Activa PC + S in this NHP (Lipski et al., 2015), right hippocampal LFP power within the 25 ± 2.5 Hz band was used as the signal of interest in the detector. Programming involved adjustment of the 1) band power threshold and 2) minimum contiguous suprathreshold duration. Online detections triggered a ~2 min recording centered on the detection event to be saved from the looping buffer of the RC + S to its internal memory for subsequent download to the laptop at the next scheduled recording.

2.5. Experimental design

To constrain the immense number of possible stimulation parameter combinations to be tested, a stimulation frequency of 2 Hz was used for all experiments given that 1) 2 Hz stimulation previously has been shown to effectively suppress ictal activity (Toprani and Durand, 2013), 2) the considerable inter-pulse interval of 500 ms allowed for analysis of the neural response to individual pulses, and 3) 2 Hz is the lowest frequency (least current-intensive) that the RC + S could reliably deliver using native settings.

Acute experiments were used to characterize the differential effects of pulse width and current amplitude on hippocampal LFP responses to LFS. Each acute experiment consisted of a series of ~90 min stimulation sessions in which pulse trains were delivered in a trial-wise fashion using the cycling feature of the RC + S to turn stimulation on and off for set time intervals (Fig. 1E). All stimulation parameters were held constant within an experimental session, and all sessions were conducted during the lights ON period of the day.

For chronic experiments, the antiepileptic potential of a given stimulation parameter combination was inferred by its ability to transiently suppress LFP energy in acute experiments. This measure was chosen because LFP energy is increased during interictal discharges and seizures, suppressed by HFS previously shown to terminate seizures, and elevated during times of day and pharmacologic states when seizures are most likely to occur in this animal specifically (Lipski et al., 2015). Each chronic testing session lasted approximately two weeks, during which clinical seizure activity was detected using manual video review (as described above for seizure detection during unstimulated seizure monitoring). Additionally, LFP recording segments were obtained for 30 s every 90 min (chronic experiment 1) or for 120 s every 180 min (chronic experiment 2). An a priori threshold of 50% reduction in clinical seizure rate during chronic stimulation compared to the unstimulated condition was used to classify a seizure suppressive effect as meaningful.

2.6. Artifact detection

All analyses were conducted offline using a combination of custom-written scripts and built-in functions in MATLAB (The MathWorks Inc., Natick, MA). The timing of stimulation pulses was identified offline using the following procedure: 1) The first temporal derivative of the LFP ipsilateral to stimulation was plotted and a threshold manually set so as to detect all stimulation artifacts. The extrema of the raw LFP for each threshold crossing was then used as a timestamp to mark putative stimulation artifacts. 2) Because this first step could not always be completely specific for stimulation artifact, a multi-dimensional feature space was created to aid in classification by aligning epochs from 10 ms before to 20 ms after each timestamp, computing the second temporal derivative of each epoch, and conducting a time-domain principal component analysis across all detections. The eigenvalue of each detection for the first three principal components was displayed using a 3-dimensional scatter plot. This feature clearly separated endogenous neural events from stimulation artifact into two distinct clusters so that artifacts could be isolated. 3) Finally, pre-screened artifact detections were confirmed by visually reviewing the raw time series with timestamps marked and any false positive or negative detections corrected.

2.7. LFP analysis

All analyses for acute experiments were conducted in a stimulation pulse-wise manner in which measures were aggregated across stimulation trains with respect to a pulse's ordinal position within each train (i.e. the first pulse from each train was grouped together, etc.). Because neural recordings were wirelessly streamed prior to being permanently saved, occasional periods of brief signal loss resulted in sporadic interruption of LFP recordings. Entire stimulation trains were excluded from analysis if data loss occurred anywhere within the time window from 500 ms before the first pulse to 500 ms after the last pulse of the train. Remaining stimulation trains were then epoched into 500 ms windows beginning at the delivery of each stimulation pulse. An additional train-wise baseline window was segmented using the 500 ms of data immediately preceding the first pulse of each train.

Event-related potentials (ERPs) were calculated using the first pulse in each train from the first acute experiment by averaging across pulses after baseline mean subtraction. To assess the effect of stimulation on ongoing background LFP energy, stimulation artifact and ERP activity were removed by template subtraction prior to energy estimation. Briefly, a template for each ordinal pulse was created by averaging all pulses of that type. This template was then linearly subtracted from each pulse epoch, leaving only activity not phase-locked to stimulation. LFP energy was quantified using line-length (LL), which is defined as the sum of the rectified first temporal derivative of a signal over a given time segment. A 50 ms sliding window and a step size of one sample point was used to provide a fine scale of temporal resolution while still offering stable estimates of local energy. LL was chosen as measure of signal energy because its use is well established in the field of epilepsy research for its ability to capture both large amplitude, low-frequency activity (e.g. interictal spikes) and low amplitude, high-frequency activity, which has been associated with ictogenesis in the mesial temporal lobe (Bragin et al., 2010; Jacobs et al., 2010; Kondylis et al., 2014; Staba et al., 2007). Additionally, LL is the most commonly used measure of LFP energy for closed-loop seizure detection in the RNS device. Statistically significant changes in LL from baseline were determined using a Wilcoxon rank-sum test calculated at every time point and evaluated at a critical value of 0.05 Bonferroni corrected for the number of time points tested. To ensure that extremely brief fluctuations in LL would not be falsely interpreted as meaningful, an additional constraint was imposed which required changes in LL meet the statistical threshold for at least 50 ms contiguously to be considered significant.

For chronic LFP recordings, stimulation pulses were similarly epoched into 500 ms windows beginning with pulse delivery and excluding data loss, however, pulses were aggregated within recording segment to provide a sufficient sample number within each data streaming session. A multiple linear regression was used to model the relative effect of stimulation duration and time of day on LFP LL. Because time of day is a circular variable, it was converted into radians (1 day = 2π radians) and then transformed using both the sine and cosine function to allow for an arbitrary phase delay when being modeled. "A multiple linear regression equation was used to model unilateral line-length (LL), with time represented in units of hours (24-hour clock) and modeled both as total time from start of recording session (X_{TT}) and as the time of day (X_{ToD}). All time variables were scaled by a coefficient (B_{2-4}) and the entire equation was fit with a constant offset (B_1)."

$$LL = B_1 + B_2{}^{*}X_{TT} + B_3{}^{*}\sin\left(\frac{\pi}{12}{}^{*}X_{ToD}\right) + B_4{}^{*}\cos\left(\frac{\pi}{12}{}^{*}X_{ToD}\right) \quad (1)$$

Each model was compared to a model containing only a constant term using an ANOVA at a critical value of 0.05 to assess statistical significance. Significant models were then subjected to post-hoc *t*-tests (critical value of 0.05) to assess which variables specifically contributed to modeling of LL variance.

Frequency analysis for chronic experiment 2 was conducted with data identically epoched and processed (i.e. template subtracted) as with LL analyses. The amplitude of individual frequency components for each post-stimulation pulse time segment was estimated using a fast Fourier transform (FFT) to optimize frequency resolution. All FFT amplitudes within a recording segment were then averaged across stimulation pulses to generate a stable representation of frequency content for a given recording. Each frequency component from these averaged FFTs was then correlated with the radian normalized time of day using the circular-linear correlation function available through the CircStat Toolbox (Berens, 2009). To identify significant circadian-frequency band relationships, a cluster-based Monte Carlo method was used. For each iteration of this procedure, the FFT to time of day relationship was randomly shuffled and the circular-linear correlation re-computed at each frequency. The newly generated surrogate FFT correlation was thresholded at a critical value of 0.05 and the number of contiguous frequency bins meeting the significance criteria were counted and retained. This process was repeated 1000 times to generate a null distribution of frequency band sizes. Finally, the original unshuffled FFT correlation was similarly thresholded at a critical value of 0.05 and only contiguous frequency bands exceeding the 95th percentile of the null distribution of band sizes were retained as significant clusters.

3. Results

3.1. Spontaneous seizure monitoring reveals a single seizure type

A total of 11 weeks of continuous video recording was conducted to characterize spontaneous seizures in an NHP with idiopathic epilepsy. During this time, 49 clinical seizures were captured yielding an average seizure rate of 4.5 seizures/week. Most seizures occurred from sleep in the early morning, around the time when lights were first turned on (Fig. 2). All 49 seizures followed a single, stereotyped semiologic pattern characterized initially by 1) jaw opening, rhythmic blinking, and arching of the back and tail immediately followed by or concurrent with 2) left head turn and left forelimb flexion which ultimately resulted in 3) loss of postural stability and generalized tonic-clonic movements in all extremities. An additional set of behaviors, consistent with the aura of focal onset seizures, were observed prior to overt seizure activity. For seizures occurring from sleep, the animal would first awaken, assume a seated position, and stare for 5–15 s before a seizure began. Similarly, seizures occurring during wakefulness were often preceded by the animal looking or turning to the left and staring briefly.

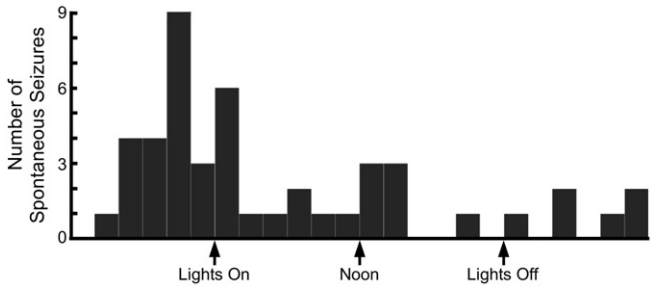

Fig. 2. Spontaneous seizures. Histogram of video-confirmed spontaneous seizure occurrence observed during baseline monitoring.

During this monitoring period, an online electrographic seizure detector running on the RC + S was being programmed empirically. We found that lowering the detector's power threshold was most useful for maximizing sensitivity whereas increasing the duration of time that power values must remain suprathreshold afforded improved specificity. By the final iteration of programming, the detector successfully captured concurrent LFP recordings for 8 video-confirmed seizures. All 8 of these seizures demonstrated right hippocampal onset with subsequent spread to the left hippocampus. Furthermore, periods of large amplitude, rhythmic spiking in bilateral hippocampi corresponded to the generalized clonic phase of seizures. Notably, the circadian pattern, overall rate of occurrence, and electrographic characteristics of seizures observed in this experiment are consistent with prior studies in this animal (Lipski et al., 2015). Taken together, these findings provide strong evidence that this primate's generalized tonic-clonic seizures are of focal onset, right hemisphere dominant, and stably expressed across long periods.

3.2. Acute experiment 1: high duty cycle LFS modulates local hippocampal LFP energy

In the first set of acute experiments, 1 s duration pulse trains of 2 Hz stimulation (2 pulses per train) were delivered with a 10 s inter-train delay. A total of 18 stimulation sessions explored all combinations of 50, 150, and 250 μs pulse-widths with 1, 2, and 3 mA current amplitudes administered unilaterally to the right and left hippocampus separately. Variable and considerable residual stimulation artifact precluded reliable estimation of ERPs ipsilateral to stimulation, however, contralateral LFPs exhibited minimal stimulation contamination. Analysis of this contralateral activity revealed that the amplitude of hippocampal ERPs was positively correlated with both pulse-width and current amplitude, such that concurrent use of higher settings for both parameters reliably evoked contralateral ERPs for both right and left-sided stimulation (Fig. 3A).

Despite ERP evidence of connectivity between implanted regions of both hippocampi, contralateral background LFP energy was not significantly modulated by any stimulation setting tested (data not shown). Ipsilateral background LFP energy was significantly suppressed by stimulation at amplitudes above 1 mA concurrent with pulse-widths >50 μs. Net LFP suppression across the duration of the analysis window was greatest for each hippocampus at the highest stimulation setting tested. While LFP responses to these higher duty cycle pulses were typified by transient LFP energy suppression followed by a gradual return to baseline on the left, the right exhibited an additional post-suppression supra-baseline rebound before returning to baseline energy values at all but the highest stimulation settings. Interestingly, this hyper-excitable response varied considerably between the first and second pulse in the stimulation train (Fig. 3B).

3.3. Acute experiment 2: LFP suppression increases with stimulation train duration

Given the finding that higher stimulation settings were more effective at suppressing LFP energy and that LFP responses evolved even over as little as two pulses, a second set of experiments explored higher stimulation settings delivered for longer durations. Specifically, pulse-widths of 150, 250, and 350 μs and current amplitudes of 2, 3, and 4 mA were delivered unilaterally to each hippocampus for 15 s (30 pulses per train) with a 45 s inter-train delay. Of note, three of the nine highest duty cycle parameter combinations for each side could not be tested because the animal exhibited signs of discomfort and agitation at these high settings. The etiology of this effect is unknown, but could include current propagation to the dura or insula, or activation of the seizure network.

As before, LFP energy in each hippocampus was transiently suppressed by ipsilateral stimulation but rebounded to above baseline levels at some parameter combinations on the right only (Fig. 4A). When averaging LFP energy across the analysis window, unilateral LFS increasingly suppressed ipsilateral hippocampal LFP energy

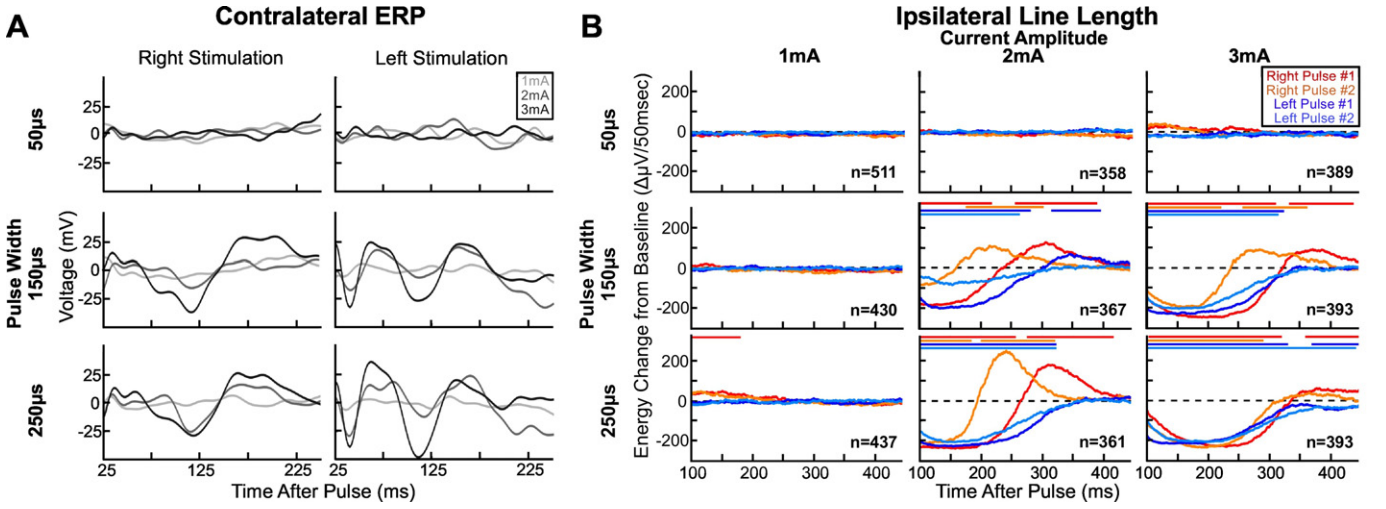

Fig. 3. Acute experiment 1. The current amplitude and pulse-width dependent nature of event-related potentials (ERP) in the hippocampus contralateral to right and left-sided stimulation is shown in panel A. Panel B depicts the median effect for two pulses of 2 Hz stimulation on LFP energy ipsilateral to stimulation for various pulse widths and current amplitudes. Periods of significant modulation in the LFP from baseline are denoted by horizontal lines above each plot. The number of stimulation trains used to construct each plot is indicated in the lower right corner.

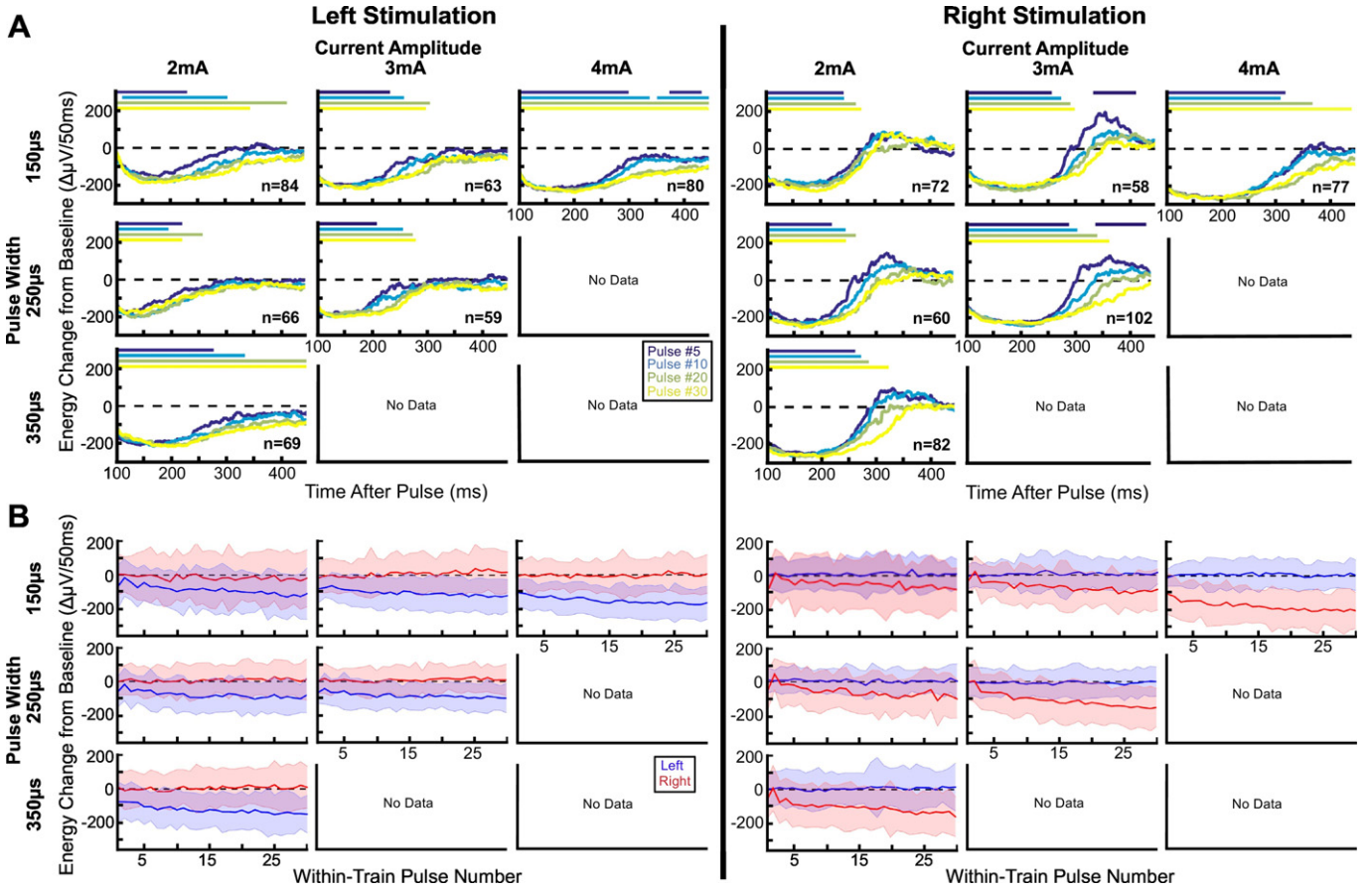

Fig. 4. Acute experiment 2. Modulation of LFP energy (line-length) for 30 pulse trains at increased stimulation settings is summarized. The time course of ipsilateral changes is shown for selected pulses in panel A. Periods of significant modulation in the LFP from baseline are denoted by horizontal lines above each plot. The number of stimulation trains used to construct each plot is indicated in the lower right corner. Panel B shows the bilateral effects to unilateral stimulation over the entire pulse train by averaging across time within each pulse epoch. The median ± the interquartile range is indicated by the dark colored trace and lighter shaded region, respectively.

with increasing pulse number. No appreciable effect was observed on the contralateral side for either left or right stimulation (Fig. 4B). Of the settings tested, only 4 mA–150 µs was able to significantly suppress LFP energy for the entirety of the analysis window by the last stimulation pulse for each hippocampus (Fig. 4A).

3.4. Chronic experiment 1: LFP responses quickly habituate to continuous LFS

In order to test the hypothesis that LFS at maximally LFP energy-suppressive settings delivered specifically to the seizure onset side hippocampus could reduce the rate of clinical seizures, 2 Hz stimulation at 4 mA and 150 µs was administered to the right hippocampus. Stimulation was delivered for 12 days continuously since acute experiments demonstrated increasing suppression with increasing stimulation duration. Continuous video recording revealed that 9 semiologically typical seizures occurred during this stimulation session resulting in an average seizure rate of 5.3 seizures/week, which is not reduced from spontaneous rates (Fig. 5A & B).

To better understand this lack of efficacy in seizure suppression, scheduled LFP recordings were analyzed using multiple linear regression modeling of the effect of total stimulation duration and time of day on net LFP energy modulation for each hippocampus separately (Eq. (1)). While the model of left hippocampal (non-seizure onset side) LFP energy was not significant ($F = 1.82$, $p = 0.14$), the model of right-sided (seizure onset side) LFP energy was highly significant ($F = 29$, $p = 2E-15$). Post-hoc testing on the right revealed significant contributions of both duration of stimulation and time of day (all $p < 0.025$) such that the initial LFP energy suppression continually decayed towards normal levels (Fig. 5A) and LFP energy was greatest during early morning when seizures were most common (Fig. 5B).

The finding that LFP energy within the stimulated hippocampus was dependent on multiple temporal variables prompted a retrospective analysis of unstimulated LFP recordings to assess whether these relationships were present at baseline or the result of stimulation. Two approximately 2 week-duration scheduled-interval recording sessions of unstimulated LFP activity were analyzed using an identical approach to that used for stimulated recordings (i.e. data were epoched into sequential 500 ms windows, template subtracted, and LFP energy calculated using line-length) to ensure results were comparable between recording conditions. Interestingly, neither time from start of recording nor time of day had a significant effect on LFP energy of either hippocampus in these unstimulated recordings (all $p > 0.05$; data not shown) suggesting that the observed temporal relationships are specific to the stimulated condition.

3.5. Chronic experiment 2: LFS cycling prevents LFP habituation

Since the LFP energy suppressive effects of LFS diminished over time with continuous stimulation but was sustained for each stimulation train of cycling LFS throughout the ~90 min of acute experiments, chronic stimulation testing was repeated at the same stimulation settings except that 15 s ON/45 s OFF cycling was used instead of continuous stimulation. LFP data collected over the 14 days of cycling stimulation was analyzed using multiple linear regression as before.

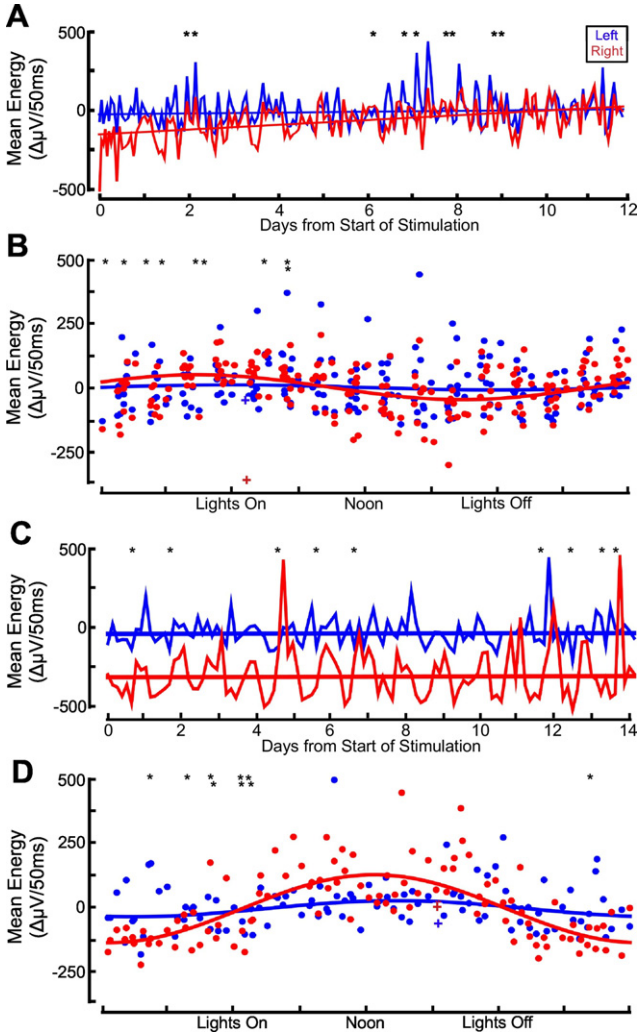

Fig. 5. Line-length analysis of chronic experiments. Bilateral LFP energy measured during stimulation in chronic experiment #1 (A & B) and #2 (C & D) is plotted with respect to duration of stimulation (A & C) and time of day (B & D) and regression fits are overlaid. For panels B and D, the first recording at the start of stimulation is indicated with a red/blue plus sign, and the effect of stimulation duration has been removed using regression for better visualization of circadian patterns. Black asterisks denote clinical seizures. Mean energy is normalized such that zero equals the average spontaneous energy observed during unstimulated baseline recordings.

Models of LFP energy calculated specifically during epochs of stimulation train delivery were significant for both the left ($F = 3.45$, $p = 0.019$) and right ($F = 31.3$, $p = 2E-14$) hippocampus. Post-hoc testing showed that only the cosine of time of day was significant for the left ($t = -2.65$, $p = 0.009$) and right ($t = 9.4$, $p = 2E\text{-}15$). Although LFP energy on the onset side was consistently suppressed during stimulation throughout the experiment, seizures occurred at baseline rates (9 seizures total, 4.5 seizures/week; Fig. 5C). Circadian fluctuations in LFP energy peaked later in the day than with continuous stimulation, however, circadian patterns in clinical seizure occurrence appeared to be unchanged (black asterisks in Fig. 5B & D).

Since line-length aggregates a broad range of frequency components into a single measure of signal energy, we subsequently conducted a Fourier-based analysis to elucidate whether individual frequency bands were responsible for the circadian patterns in overall LFP energy observed in regression modeling. Briefly, identical post-stimulation pulse time segments used for LL analysis were epoched, template subtracted, and transformed into the frequency domain using an FFT. Each frequency component from recording segment averaged FFTs were subjected to a circular-to-linear correlation with time of day. Significantly correlated frequency bands were assessed at a critical value of 0.05 using the cluster-based approach described in the Materials and methods section. This analysis revealed a frequency band from 3 to 20 Hz in the left hippocampus and 6–48 Hz in the right hippocampus that varied significantly with time of day (black traces in Fig. 6). Applying the same FFT analysis to identically processed unstimulated recordings (previously utilized for comparison purposes in the *Chronic experiment 1* subsection) did not identify any significant circadian relationships (gray traces in Fig. 6). Paralleling the findings for LL-based analyses, this result suggests that the observed circadian patterns are specific to the stimulated condition.

4. Discussion

The current study utilized a novel simultaneous video-intracranial EEG recording/implantable wireless telemetry platform to conduct chronic seizure and stimulation monitoring in a freely behaving NHP with idiopathic epilepsy. Spontaneous seizure monitoring revealed a focal onset, secondarily generalizing tonic-clonic seizure type, consistent with a right-sided onset where right hippocampal ictal activity preceded left hippocampal ictal activity. After characterizing normative ictal activity, the effect of LFS on hippocampal LFPs was explored. A series of acute stimulation experiments indicated that unilateral stimulation at higher pulse-width and current amplitude settings could transiently reduce the energy of ongoing field activity in the ipsilateral hippocampus. Because this suppressive effect was found to be increasingly potent with increasing stimulation duration, approximately two weeks of continuous LFS using optimized settings was delivered to the seizure onset side hippocampus while its effect on seizure rate and LFP activity was monitored. LFP suppression by continuous, focally applied LFS diminished in efficacy over longer time periods, varied with circadian rhythms, and ultimately did not reduce seizure rate. Chronically delivering LFS using ON-OFF cycling, instead of continuous settings, maintained LFP suppression during stimulation but similarly did not prevent seizures.

Semiologic and electrographic characterization of spontaneous seizures in primates is exceedingly rare in the literature and, to our knowledge, this study represents the first time such chronic monitoring has been used to describe idiopathic epilepsy in an NHP. Previous work by our group (Lipski et al., 2015) used right hippocampal LFP power-based seizure detection, without concurrent video, to record seizures in this animal. Our current findings that video-confirmed seizures occur at the same rate, time of day, and with identical electrographic correlates as previously described, validates the prior electrographic seizure detector and demonstrates stability of this animal's seizures and implanted clinical electrodes are over a multiyear period. These data continue to suggest that the seizure onset zone involves the right mesial temporal lobe, as all of these seizures are invariably recorded first in the right hippocampus before eventual spread to the left. Likewise, these seizures bear striking similarity to seizures in humans with MTLE and primate models of MTLE (Chen et al., 2013; Perez-Mendes et al., 2011).

We chose to investigate the effects of low-frequency stimulation on hippocampal activity because it remains a largely underexplored treatment paradigm despite its potential to reduce battery strain and extend implant lifespan. In contrast, HFS has been explored in numerous human studies, however, many of these reports are limited by small sample size and study design (summarized in Han et al., 2014). A recent Cochrane review found that, of the few randomized controlled trials specifically investigating hippocampal HFS (McLachlan et al., 2010; Tellez-Zenteno et al., 2006; Velasco et al., 2007), HFS conferred a pooled mean reduction in seizure frequency of 28.1% over sham but no subjects achieved seizure freedom (Sprengers et al., 2014). Reports of LFS in humans are sparse and underpowered, but have so far demonstrated a reduction of interictal epileptiform activity (Chkhenkeli et al., 2004) without a significant seizure prophylactic effect (Boëx et al., 2007).

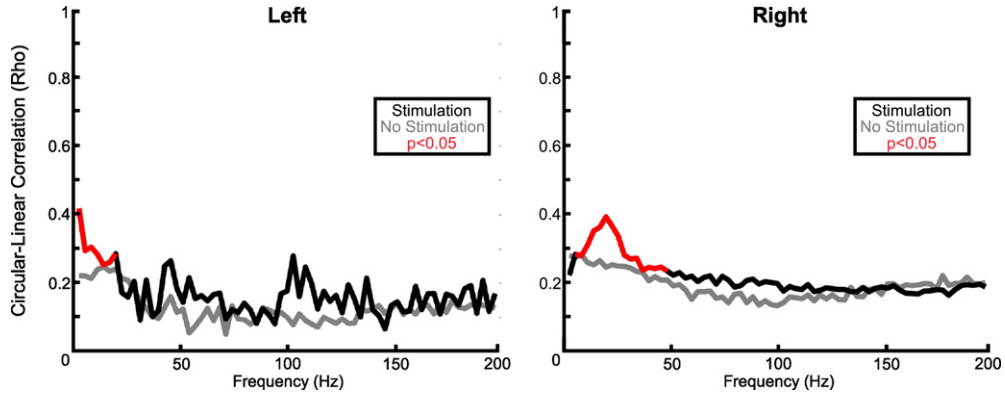

Fig. 6. Circadian variations in frequency. The strength of correlation between time of day and the frequency amplitude are plotted for bilateral hippocampal LFPs. Data from chronic experiment 2 (cycling stimulation) is plotted in black and data from unstimulated experiments in gray. Frequencies meeting statistical criteria for significant circadian variation in amplitude are highlighted in red.

This discrepancy has been investigated in rodent studies which suggest that seizure prevention manifests only after long-term LFS, theoretically by preventing kindling and increasing seizure threshold through gradual depression of neuronal excitability (Albensi et al., 2004; Han et al., 2014; Kile et al., 2010; Schrader et al., 2006; Zhang et al., 2009). The dependence of LFS on gradual increases in the seizure threshold seems to stand in contrast to the acutely suppressive effects of HFS which may be profound enough to abort ongoing seizure activity. This distinction suggests that these two frequency paradigms of stimulation may be best utilized in synergistic fashion such that chronically delivered LFS may progressively decreases the likelihood of seizure occurrence whereas targeted HFS may terminate any epileptiform activity escaping these preventative measures.

Our findings indicate that both pulse-width and current amplitude contribute to the ability of hippocampal LFS to reduce LFP energy. Although a mechanistic understanding of these effects is outside the scope of the current set of experiments, previous work in rodent models provides evidence for GABAergic neurotransmission playing a critical role in post-stimulation suppression. In vitro recordings in bilaterally connected hippocampal slices suggest that each pulse of LFS delivered to white matter tracts evokes a large, coordinated population burst which then leads to a period of reduced population firing mediated by slow after-hyperpolarization and GABA-B currents but not GABA-A currents, long-term depression, or depolarization (Toprani and Durand, 2013; Toprani et al., 2013). Corroborating the importance of GABAergic systems, other groups have also found similar post-burst suppression of population firing with direct optical LFS of interneurons (Ladas et al., 2015). The relative effect of these inputs may critically depend on brain state, however, as the inhibitory effects of GABA-mediated currents appears to be reversed during seizures (Ellender et al., 2014). Furthermore, anti-epileptic drugs commonly used in clinical settings may modify the impact of stimulation and even augment its effects (Asgari et al., 2016).

We were able to identify LFS parameters capable of modulating hippocampal LFP energy acutely, and in some instances, chronically. Interestingly, the optimal LFS parameters identified in this study were similar to those used in clinical trials. Specifically, 150 μs pulse width used here is strikingly similar to that often used in the RNS device (160 μs; Heck et al., 2014). Additionally, our empirically identified 4 mA current amplitude setting is comparable to that used in both the RNS and SANTE trials (Fisher et al., 2010; Heck et al., 2014). Although we used a frequency of stimulation approximately two orders of magnitude below that implemented in both of these trials, we also found that intermittent rather than continuous stimulation was important for maintaining the suppressive effects of stimulation.

Although cycling LFS was able to reliably reduce LFP energy chronically, it did not ultimately prevent seizures. There exist many possible explanations for this lack of efficacy and testing these hypotheses is a major focus of our future work. One straightforward explanation lies in the relative ON and OFF times of cycling. For example, it is possible that seizures escape from the suppressive control of stimulation during OFF times and thus shortening this period as much as possible, while still preventing habituation seen with continuous stimulation, would be more effective. Alternatively, the abrupt initiation of stimulation during each ON cycle may evoke ictal activity itself (Toprani and Durand, 2013), however, no ictal activity was elicited during any acute stimulation testing which would argue against this cause. Regardless, this potential ictogenic source could be ameliorated by gradually increasing stimulation amplitude at the beginning of each pulse train. Suboptimal spatial extent of stimulation is another potential category of explanation. Increasing the volume of tissue being stimulated has improved outcomes in some studies (Boëx et al., 2011) and could be accomplished here by switching from highly focal, unilateral bipolar stimulation to quadripolar or even bilateral stimulation. A more complex intervention could involve implantation of additional electrodes in different brain regions, such as the anterior nucleus of the thalamus. The Activa RC + S is capable of accepting two more 4-contact electrodes than we are currently utilizing, and bilateral hippocampal and anterior thalamic electrodes would offer improved ability to evaluate ongoing brain state and modulate activity within limbic circuits (Afshar et al., 2012; Freestone et al., 2013; Stypulkowski et al., 2013, 2014; Van Gompel et al., 2015).

Irrespective of the reason for the lack of observed therapeutic benefit with LFS, this negative finding suggests that relatively simplistic indices of neural activity such as line-length do not necessarily predict the seizure suppressive effects of a given stimulation paradigm. Indeed, there is a tremendous richness of information contained within broadband LFP signals not captured by aggregate energy measures alone. This idea is supported by Fourier analysis of data from chronic experiment 2 which revealed that the circadian patterns in line-length observed during cycled stimulation were driven by modulation of particular frequencies ranging from the theta band through the alpha, beta, and even low gamma bands. In contrast, it is possible that stimulation therapies which modulate very high frequency activity, particularly above the canonical gamma band, may be more effective in suppressing seizures as this type of activity has been strongly associated with ictogenesis (Bragin et al., 2010; Jacobs et al., 2010; Kondylis et al., 2014; Staba et al., 2007). More sophisticated analytic techniques such as sliding-window spectral analyses or even functional connectivity measures may better disentangle physiologic states of interest, but further work is needed to identify electrographic biomarkers that predict stimulation efficacy.

This study employed the latest generation of a rechargeable, sensing-enabled neurostimulator capable of wireless data streaming.

Technologic advancements in these implantable devices stand to have considerable impact on both neuroscience research and clinical care. Only recently, with the addition of recording capabilities, has it become possible to obtain chronic brain recordings in freely behaving animals and humans over multiyear periods. As evidenced by our present finding of a circadian rhythmicity in the neural response to stimulation, these long-term recordings can reveal features of neurodynamics existing on large temporal scales to which the neuroscience community was previously blind. Specific to epilepsy, recording the acute effects of stimulation on neuronal population activity may provide a short-latency feedback invaluable in the rapid, patient specific optimization of antiepileptic stimulation. There remains a considerable gap in knowledge with regard to the mechanisms by which neurostimulation prevents and/or aborts seizures and, consequently, what stimulation parameter combinations are most effective in providing therapeutic benefit. Indeed, further research exploring what features of the LFP are predictive of the seizure modulating effects of stimulation is warranted.

5. Conclusions

This work demonstrates that low-frequency hippocampal stimulation can transiently suppress synchronous population activity locally. Stimulation-induced suppression is highly dependent on both pulse-width and current amplitude, with lower levels of either rendering stimulation ineffective. Additionally, low-frequency stimulation is appropriate for cycling, but not continuous, applications. Finally, recent advancements in implantable neurostimulator technology, including recording and closed-loop capabilities, allow for previously impossible long-term neurophysiologic monitoring that will further our understanding of the nature of epilepsy, the effects of neurostimulation, and the effect of both on neurocognitive processes.

Acknowledgements

The authors thank Scott Stanslaski, Robert Devine, David Linde, Paul Stypulkowski, and Jon Giftakis (Medtronic, Inc.) for device and engineering support. TAW and EDK were trainees in the Physician Scientist Training Program (PSTP) at the University of Pittsburgh School of Medicine. RMR received research grant funding from Medtronic, Inc. The conception, design, execution, and analysis of experiments, as well as the preparation of and decision to publish this manuscript, were made independent of any funding organization.

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