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Seizure entrainment with polarizing low frequency electric fields in a chronic animal epilepsy model

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Abstract

Neural activity can be modulated by applying a polarizing low frequency ($\ll 100$ Hz) electric field (PLEF). Unlike conventional pulsed stimulation, PLEF stimulation has a graded, modulatory effect on neuronal excitability, and permits the simultaneous recording of neuronal activity during stimulation suitable for continuous feedback control. We tested a prototype system that allows for simultaneous PLEF stimulation with minimal recording artifact in a chronic tetanus toxin animal model (rat) of hippocampal epilepsy with spontaneous seizures. Depth electrode local field potentials recorded during seizures revealed a characteristic pattern of field postsynaptic potentials (fPSPs). Sinusoidal voltage-controlled PLEF stimulation (0.5–25 Hz) was applied in open-loop cycles radially across the CA3 of ventral hippocampus. For stimulated seizures, fPSPs were transiently entrained with the PLEF waveform. Statistical significance of entrainment was assessed with Thomson's harmonic F-test, with 45/132 stimulated seizures in 4 animals individually demonstrating significant entrainment ($p < 0.04$). Significant entrainment for multiple presentations at the same frequency ($p < 0.01$) was observed in 3 of 4 animals in 42/64 stimulated seizures. This is the first demonstration in chronically implanted freely behaving animals of PLEF modulation of neural activity with simultaneous recording.

Keywords

Seizure control; low frequency; electric fields; modulation; temporal lobe epilepsy; multitaper

1. Introduction

The use of electric fields or currents to interact with the brain has played an important role in the development of modern neuroscience and clinical neurological practice (Brazier 1959 and 2005). In conventional clinical practice, and in the majority of animal research, stimulation is performed with short (< 1 ms) pulses at amplitudes designed to excite

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neuronal firing. But it has long been known that the application of electric fields with much longer duration or lower frequency content can have a positive or negative modulatory effect on neuronal function (Denney and Brookhart 1962; Purpura and Malliani 1966; Nakamura *et al* 1968; and Jefferys 1981). The physics of this low frequency interaction was worked out in detail in the 1980s (Chan and Nicholson 1986; Chan *et al* 1988; Tranchina and Nicholson 1986): under the influence of an electric field applied across the primary soma-dendrite axis, asymmetric neurons – especially pyramidal neurons – polarize. This polarization yields a local shift in transmembrane potential at the soma with respect to threshold for action potential initiation, and therefore a shift in excitability. The polarization is proportional in amplitude and sign to the applied field. As a tool, the polarizing low-frequency electric field (PLEF) provides a highly versatile and reversible spatially localized modulatory input to the network. Although we frequently refer to this methodology as “stimulation” in this article, we stress that the mechanism of action, unlike pulsed fields which typically activate neurons, is to modulate the neuronal threshold for excitation, up or down, in response to intrinsic inputs.

Over the last decade, PLEFs have been used to interact with and control neuronal networks both *in vitro* and *in vivo*. In brain slice experiments this has included demonstrations of epileptiform activity modulation (Gluckman *et al* 1996), stochastic resonance (Gluckman *et al* 1996b), control of seizure propagation (Richardson *et al* 2005), and adaptive feedback control of epileptiform activity (Gluckman *et al* 2001). Critical to this work was the development of techniques for simultaneous recording of neural activity during stimulation with minimal recording artifact: in this way continuous feedback could be implemented. A suitable electrode placement for axial hippocampal stimulation *in vivo* was devised based on current clinical electrode placement practice and used to demonstrate neural modulation in acutely instrumented, anesthetized animals (Richardson *et al* 2003).

The next logical step is to extend these results to modulate and control spontaneously recurring seizures in freely behaving animals. We have developed a complete prototype stimulation and recording system for use *in vivo* under chronic implant conditions designed to allow simultaneous stimulation and recording and accommodate stimulation artifact reduction.

We report here the first demonstration of seizure modulation from this prototype. We use a rodent model of chronic limbic epilepsy as a test bed for our work. Rats implanted unilaterally with a small focal injection of tetanus toxin in the hippocampus chronically develop spontaneous, generalized seizures with behavioral manifestations (Finnerty and Jefferys 2000; Jefferys and Walker 2006). We stimulated the animals with sinusoidal PLEFs under an open-loop, repetitive on-off protocol (e.g., one hour on, one hour off) (Sunderam *et al* 2006). We demonstrate that PLEF stimulation modulates neural activity during seizures.

2. Methods

2.1. Overview

The following synopsis of methods is provided so that the reader may move directly to the Results section without loss of continuity.

Spontaneous, intermittent seizures were produced following a single focal injection of tetanus toxin into the ventral hippocampus (2.2.1–2). Activity was continuously monitored using video and intracranial field potential measurements for weeks (2.2.3–4) and the seizures detected using an automated algorithm (2.3.1). A stimulation electrode implanted unilaterally in the ventral hippocampus was used to apply periods of open-loop, periodic, approximately sinusoidal PLEF stimulation at discrete frequencies below 25 Hz (2.2.5). This

applied field modified the seizure dynamics by transiently entraining or phase synchronizing the early-stage seizure spikes. In order to quantify this entrainment without ambiguity from the stimulation artifact (2.3.2), we first extracted the sequence of spike times during seizures (2.3.3), and then quantified entrainment from its point process spectrum in a moving window. In each 4 s window, a statistical test based on the multitaper method of spectral estimation was used to ascertain the presence of a significant line (delta function) at the stimulation frequency in the spike time spectrum: this was evidence of phase-locking or entrainment of spike times by the applied field (2.3.4). The fraction of seizures occurring during PLEF stimulation at each frequency with at least one window of entrained spikes was compared against that expected to occur by chance from binomial considerations to assess whether entrainment at that frequency was significant (2.3.5). This procedure was repeated separately for each of four animals.

2.2. Experimental Protocols

2.2.1. Surgery and seizure induction—All procedures were approved by the IACUC of George Mason University, where these experiments were performed. Adult male Sprague-Dawley rats ($n = 4$, 250 – 350 g) were anesthetized with 1.5 mL of a mixture of ketamine (80 mg mL⁻¹) and xylazine (12 mg mL⁻¹) per 100 g of body weight as the induction dose, followed by maintenance doses of 0.1 mL of xylazine (100 mg mL⁻¹) approximately every half hour. The animals were identified as A, B, C and D respectively.

Seizures were induced by microinjection of 1 μ L of tetanus toxin into the right ventral hippocampus (Finnerty and Jefferys 2000; Jefferys and Walker 2006). Tetanus toxin (Sigma) was mixed at a concentration of 5 ng/ μ L in 2 % bovine serum albumin (BSA) in phosphate-buffered saline (Vreugdenhil *et al* 2002). BSA is added to reduce binding of tetanus toxin to the injection cannula walls. Injection was done with a 1 μ L SGE-0001RN syringe in a UMPII syringe pump (World Precision Instruments). The syringe cannula was inserted at a rate of 1.2 mm/minute to the target position of AP = -5.15; ML = -5.35; DV = -6.1 under digitally instrumented stereotaxy (Angle-One, MyNeurolab), then left in place for 5 min to allow elastic stress in the tissue to dissipate. Injection was then performed over a period of 5 min, after which the cannula was left in place for another 5 min before withdrawal at a rate of 1.2 mm/min.

A 250 μ m diameter stimulation electrode with 3 mm exposed length was placed into either the right or left ventral hippocampus at a maximum insertion speed of 1.2 mm/min, at a trajectory angled to align the electrode within the arc of the CA3 of the ventral hippocampus with the exposed area for a right-sided stimulation electrode extending from AP = -4.1; ML = +4.96; DV = -4.6 to the end at AP = -5.15; ML = +5.35; DV = -7.6 as illustrated in figure 1. All stereotaxic coordinates are bregma-referenced in millimeters and chosen according to Paxinos and Watson (2004). An identical stimulation electrode was then placed 2 mm anterior along a parallel trajectory for stimulation current return. Left-sided stimulation electrode placement (for animals A and D) was along a trajectory mirroring the right side placement. The geometry of stimulation electrode placement in the hippocampus is similar to that commonly used for intracranial recording and evaluation for epilepsy surgery (e.g., see figure 5Ad in Schiff *et al* 2005).

The resulting current, and therefore electric field, locally expands radially outward from the electrode, and is therefore aligned with the dendrite-soma axis of the nearby CA3 pyramidal neurons as described in Richardson *et al* (2003). Although tetanus injection was always on the right side, the stimulation electrode placement was on the left side for animals A and D and on the right side for animals B and C. Bipolar recording electrode pairs were placed in the dorsal hippocampus bilaterally at positions AP = -2.5; ML = $\pm$ 2.0; DV = -3.3, and unilaterally contralateral to the stimulating electrode at AP = -3.5; ML = $\pm$ 3.0; DV = -3.3.

Additionally, skull screw electrodes (0–80 stainless steel, Plastics One) were placed over somatosensory cortex at positions AP = +1, -4; ML = ± 3.0 ; and over the occipital sinus at AP = -7, ML = 0.

2.2.2. Electrode Construction—Stimulation electrodes were fabricated from 250 μm diameter 316L stainless steel wire with polyimide insulation. The final 3 mm of the wire was uninsulated from the tip to expose a geometric area of 2.5 mm², and then an electrochemically deposited iridium oxide film (EIROF) was added using a protocol modified from Meyer *et al* (2001) as follows:

The deposition solution was prepared with iridium tetrachloride (4 mM) and oxalic acid (40mM), and adjusted to a pH of 10.3 with the addition of high molarity potassium carbonate (500 mM). Iridium oxide was electrochemically deposited onto stainless steel substrates by sweeping from 0 to 0.55 V and back at 50 mV/s for 50 cycles, followed by a dwell time of 3 s, and then by 1600 cycles of a 1 Hz square wave between 0 and 0.55 V with respect to an Ag/AgCl pellet. The sequence formed by the triangular and square waves constituted one deposition cycle, and took approximately 45 min to complete. Electrodes were subjected to 32 deposition cycles (approximately 24 h). Electrode performance (figure 2) is characterized by the charge storage capacity (CSC), which is measured as follows: the charge Q passed through an electrode at time t is computed from the cumulative integral

$Q(t) = \int_{-\infty}^t I(t') dt'$ of the current $I(t)$. The charge storage is equal to the maximum charge passed during the cycle, and given by $CSC = Q_{\max} - Q_{\min}$, where $Q_{\max} = \max_t [Q(t)]$ and $Q_{\min} = \min_t [Q(t)]$ are the peak and trough respectively of $Q(t)$ in the interval $t \in [0, T)$. If the charge storage were purely capacitive, CSC would be independent of frequency and the maximum current would increase linearly with the frequency. Here the maximum current increases by less than a factor of two with a fifteen fold increase in frequency.

Bipolar recording electrodes were formed from pairs of stainless steel wire (125 μm diameter), glued together and cut with a staggered vertical separation of 300 μm , and deposited with EIROF to lower their recording impedance.

2.2.3. Electronics and recording technique—Stimulation electronics were designed using an analog isolation amplifier, based on the National Clare Loc110 linear optocoupler, to control the potential between the pair of stimulation electrodes. Isolation ensures that only the stimulation electrodes pass charge, and that charge passage is balanced so that the net charge passed is zero at all times. Voltage programming across the electrode pairs allows us to directly limit the whole-cell potential to less than 1 V. The individual half-cell potentials at each electrode were therefore restricted to be within the water window to prevent harmful electrochemical tissue damage (Merrill *et al* 2005).

Recording electronics were designed using differential preamplifiers with high common mode rejection and low bias current in order to accommodate the large common mode signal introduced during PLEF application. Measurement ground was connected through a 10 k Ω resistor to the occipital screw electrode. Custom head-mounted electronics included preamplifiers for depth and cortical (skull screw) EEG, and unity-gain amplification for monitoring stimulation electrode potentials with respect to the occipital screw. Additionally, a two-axis microelectromechanical system (MEMS) accelerometer was incorporated into the head-mounted electronics for monitoring head tilt and motion (Sunderam *et al* 2007).

Local field potentials (LFPs) were filtered from 0.1–1000 Hz. All signals including LFPs, stimulation amplitude and current, and head acceleration were sampled with 16 bit resolution at 2000 samples per second using a multifunction acquisition board (National

Instruments, PCI-6229). In addition to a standard 12/12 hr light/dark cycle, animals were weakly illuminated with infra-red illumination. Black and white video with infra-red sensitivity was recorded at 3 frames per second. Animals were cabled and monitored starting 3–5 days post surgery. Continuous recording was maintained for up to 4 weeks post-implantation.

2.2.4. Seizure Characteristics—Within a few days of implantation, spontaneous epileptiform discharges occurred, quickly evolving into fully generalized Racine Class 5 motor seizures (Racine 1972) with a peak rate of approximately 30 per day in weeks 2–4 post-implantation.

A typical seizure from an unstimulated period is shown in figure 3 along with the measured head acceleration. Time marked is relative to seizure onset. Prior to seizure onset, both the LFP and head acceleration are relatively calm. From the video recording, we know that the animal was asleep. Note that the accelerometers are sensitive to DC acceleration, and therefore register components proportional to the gravitational acceleration along their axes. The slow DC acceleration changes observed at about time $t = 10$ s reflect a slow rearing motion indicative of seizure generalization. Following rearing, ictal behavior is characterized by convulsions. Electrographically, there is often a transient suppression of the field potential activity ($t \sim 40$ – 60 s in figure 3) followed by a recovery phase ($t \sim 60$ – 120 s) before the seizure finally ends and the animal regains normal function. This seizure evolution pattern is consistent with human (Schiff *et al* 2005) and animal data (Blume *et al* 1993; Bikson *et al* 2003; Ziburkus *et al* 2006).

Finnerty and Jefferys (2000) have described for the tetanus toxin model of temporal lobe epilepsy a stereotypical seizure progression through five stages prior to seizure generalization. This staging was based on the electrographic character of the seizure discharges measured from bipolar hippocampal depth electrodes similar to the ones used here. Subunits of these discharges resemble interictal spikes which they refer to as “field postsynaptic potential” (fPSP) units. Each fPSP unit consists of one or more population spikes riding on the envelope of a local field potential fluctuation of 100–250 ms duration. The seizure is characterized by an organized and progressive increase in fPSP rate to 9–16 Hz and bilateral synchronization of fPSP timing that then leads to generalization (Finnerty and Jefferys 2000). Details of fPSP spikes at seizure onset, during the progression to generalization, and after generalization are shown in the expanded traces of figure 3. For convenience, we refer to these units as fPSP spikes and mark the time of each unit (criteria and procedure for detection are specified later in Data Analysis Procedure) with a filled circle (red). Note that fPSP morphology as well as the sign or polarity of the maximal peak evolves through the seizure.

2.2.5. Stimulation protocol—After a baseline recording period, open-loop, voltage-controlled (0.5–0.8 V peak) sinusoidal stimulation (0.5–20 Hz) was applied to investigate its effect on seizure activity. The stimulation protocol was designed to contain alternating on and off periods of duration 15, 30 or 60 min each: for instance, a repeating sequence of 3, 0, 15, 0, 7, 0, 11, 0 Hz stimulation for one hour each (0 Hz indicates no stimulation). The choice of frequency range applied is justified in the Discussion section.

Measured rms (root mean squared) applied current levels during epochs containing seizures were in the 40–65 μ A range. A first order estimate of the electric field from these electrodes at the CA3 cell body layer from this current level, based on a finite line source in an isotropic conductive medium with conductivity of 200 Ω cm, is of order 16–20 mV/mm (Richardson *et al* 2003). This is the same order of magnitude as peak endogenous fields measured during sharp waves (Buzsaki 1986)

The applied field in the tissue by Ohm's law is proportional to the local current density, and therefore the applied current. Under voltage-controlled stimulation, the time course of the applied current is not precisely sinusoidal due to the electrochemically mediated charge exchange. Additionally, the CSC is frequency-dependent, so that the maximal current only slowly increases over the range of frequencies reported here. These effects are illustrated from the current-vs-voltage curves shown for 1, 9, and 15 Hz stimulation of animal B in figure 2.

2.2.6. Histology—Microscopy of the stimulated hippocampus was performed to locate the implanted stimulation electrodes relative to hippocampal structures. Animals A, B and C were sacrificed after completion of recording, and their brains removed and frozen for histological examination. Brains were sectioned horizontally on a cryotome at a thickness of 16 microns, and then stained with a standard Nissl stain. Animal D was transcardially perfused and fixed with paraformaldehyde, and sent for commercial sectioning and staining (FD Neurotechnologies, Inc.).

2.3. Data Analysis Procedure

Figure 4 is a flow chart of the data analysis procedure. It has the following main steps, detailed below: identification of seizures, reduction of stimulation artifact, fPSP spike detection and reduction to point process, point spectral estimation and spectral line identification, and statistical analysis.

2.3.1. Seizure detection and verification—Specific data files and/or channels were excluded from analysis if they were found to contain any of the following problems: persistent clipping artifacts indicative of voltage amplitude saturation, high level of line noise or other signal artifacts, or stimulation artifact that could not be satisfactorily reduced. The procedure used for artifact reduction is described below.

Seizures were detected from depth LFP recordings as departures of the relative power in the 5–25 Hz frequency band above a threshold value set at a ratio of 3 times the baseline power. Detections separated by less than 15 s were considered part of the same event. Only events of duration greater than 10 s were analyzed. Seizure segments were verified by an expert (SLW) through complete visual review of the raw data. Seizures were grouped for analysis by stimulation frequency. Seizure onset times were determined with one second resolution which is sufficient for the analysis presented here.

2.3.2. Stimulus artifact reduction—Under an applied electric field, even with ideal electrodes and recording electronics with infinite common-mode rejection, we expect an additive recording potential artifact equal to the line integral of the electric field between the recording sites. If we assume a time-invariant tissue impedance, stimulation electrode geometry, and linear recording electrode response, then this additive term $E(t)$ should be a linearly filtered version of the applied current $I(t)$: $E(t) = A * I(t)$, where $*$ denotes convolution. Ideally, elimination of this artifact requires compensating for the frequency-dependent amplitude and phase of this transfer function A (Chernyy *et al* 2008). For the purposes of this paper, where only periodic stimuli were applied, we approximate this transfer function A for each seizure by a proportionality constant α with zero phase lag: hence, $E(t) = \alpha I(t)$, where α is assumed constant over the interval of interest. We then extract α and the neural signal $N(t)$ from the measured potential signal $X(t) = N(t) + \alpha I(t)$ by convolving the signals $X(t)$ and $I(t)$. If we assume that $N(t)$ and $I(t)$ are uncorrelated, then $\alpha = \langle X(t)I(t) \rangle / \langle I^2(t) \rangle$, where $\langle \cdot \rangle$ indicates time average. Finally, the artifact-free LFP signal is estimated as $\hat{N}(t) = X(t) - \alpha I(t)$.

The artifact transfer coefficient α was estimated independently for each recording channel from a 15 s baseline prior to each seizure. The spike time series was extracted for a 150 s LFP segment starting 30 s prior to each seizure.

An example of data before and after reduction of 15 Hz stimulation artifact using the above procedure is shown in figure 5. Note that the fPSP spikes are much “sharper” (have higher frequency content) than the stimulation artifact, and are easily distinguished after artifact reduction. Note also that some small component of the artifact remains, primarily because we did not allow for any phase lag. More complete artifact reduction might be achieved with a more sophisticated linear filter model and fitting method (Chernyy *et al* 2008).

2.3.3. fPSP spike detection and reduction to point process—The fPSP spikes have well-defined sharp peaks and are therefore composed of characteristic frequencies much higher than the imposed stimulation artifact. Therefore, these peak times can typically be extracted accurately despite residual stimulation artifact. We define the fPSP spike times as follows: Local extrema of the LFP time series are detected as the zero-crossings of the artifact-reduced time series gradient (first difference). The gradient of the extrema sequence is computed as $|\Delta Z_k / \Delta t_k|$, where t_k and Z_k are the spike times and amplitudes, respectively, Δ is the backward difference operator ($\Delta x_k = x_k - x_{k-1}$), and $|\cdot|$ denotes the absolute value. Extrema with absolute value and gradient exceeding thresholds preset globally for each channel and animal are retained as genuine fPSP spikes. We constrain the *absolute* value and gradient since fPSP spikes can switch polarity during the course of a seizure. Additionally, a minimum inter-spike interval of 50 ms is imposed by retaining only the largest spike in a sliding window of length 100 ms. Separate fPSP spike time series were extracted for each seizure and each depth LFP channel.

2.3.4. Point spectral estimation and spectral line identification—Entrainment of the fPSP spikes by PLEF stimulation yields sharp lines in the frequency spectrum of the spike time series: these lines or peaks occur at the integer multiples of the stimulation frequency. We follow the multitaper spectral estimation techniques and the implementation of Thomson’s harmonic F-test for determination of sharp spectral lines as described for point process data by Jarvis and Mitra (2001). The multitaper spectral estimates significantly reduce the bias and variance of the spectral estimate by providing multiple spectral estimates from the same time window. This is achieved by multiplying the measured data by a series of K orthogonal discrete prolate spheroidal sequence (DPSS) or Slepian tapers, and then averaging their spectra. Likewise, Thomson (1982) demonstrated that the signal-to-noise ratio (SNR)—defined by the height of a peak in a spectral estimate, computed from the first moment of the tapered Fourier estimates, divided by the background, computed from the second moment of the residuals— would be distributed as $F_{2,2(K-1)}$ *under the null hypothesis that there is no spectral peak*. $F_{2,2(K-1)}$ is the distribution of the ratio of two χ^2 -distributed random variables with 2 and $2(K-1)$ degrees of freedom, respectively. Therefore, to test for a peak at a given frequency, we test that the observed SNR sits outside the $F_{2,2(K-1)}$ distribution with confidence set by critical value p .

Since some of the seizures were subjected to a periodically varying field at fixed frequency, the presence of a significant line at this frequency in the point spectrum proves that there is entrainment: i.e., the fPSP spikes are phase-locked with the stimulation frequency and neural activity is modulated by the applied electric field. This assertion is made with the caveat that unstimulated seizures will have a significant peak at the same frequency only by chance with probability p per window. We also use this expected false detection rate for the unstimulated seizures as a statistical control.

Spectra were computed using the Chronux (www.chronux.org) toolbox (version 1.5) in Matlab (The Mathworks, Inc.). For each seizure, we computed the multitaper point spectrum (time-bandwidth product of $TW = 4$ and $K = 7$ tapers; spectral concentration $W = 1$ Hz) and tested consecutive non-overlapped windows of $T = 4$ s duration for a significant line (critical p value of 0.001 or 0.1 %) at the stimulation frequency. Note that for a window of size $T = 4$ s the frequency resolution of the estimated spectrum is $1/T$ or 0.25 Hz. The use of DPSS tapers limits spectral leakage to nearby Fourier frequency bins so that, for $TW = 4$, the achieved spectral concentration W is 1 Hz. Control seizures were tested at all applied stimulation frequencies for comparison. The number of windows that showed significant entrainment, N_w , from a total of 37 for 150 s long segments, was counted for each seizure and taken as a measure of the extent or duration of entrainment. This test was separately applied to each fPSP spike time series from each depth electrode for each seizure.

2.3.5. Assessment of effect—For each group of N_{sz} seizures stimulated at frequency f , the number of seizures entrained N_{ent} (i.e., each having the number of entrained windows N_w greater than zero) was counted. This was compared with the number expected by chance (binomial probability) from a set of N_{sz} seizures: if the measured N_{ent} exceeded the 99 % confidence limit c , entrainment at f was considered significant.

The confidence limit on N_{ent} is fixed using binomial considerations: Each 4 s window of fPSP times has a probability $p = 0.001$ of a significant line component at frequency f , the chosen criterion for entrainment. The probability that a seizure will have a number of entrained windows $N_w > 0$ by random chance is:

$P_+ = P(N_w > 0) = 1 - P(N_w = 0) = 1 - \binom{37}{0} p^0 (1-p)^{37}$, where each seizure has a total of 37 windows tested. The binomial probability that exactly r seizures out of a set N_{sz} will have

$N_w > 0$ is then $P(r) = \binom{N_{sz}}{r} P_+^r (1 - P_+)^{N_{sz}-r}$. The 99 % confidence limit c on N_{ent} is

computed by finding the smallest integer value of r such that $\sum_0^r P(r) \geq 0.99$. This, ultimately, is the assumed confidence limit to be exceeded in order to determine that a significant level of entrainment was observed in the set of seizures that occurred during stimulation at a particular frequency. Error testing of these methods will be discussed in the results section.

This study was designed to test for neural entrainment during seizure by open-loop sinusoidal PLEFs, but not to assess possible effects on seizure frequency or seizure duration. Assessment of seizure frequency or duration effects requires statistical comparisons of seizure counts or durations in multiple stimulation on-off periods with stimulation at different frequencies. The sample numbers achieved in this study did not achieve the minimum required statistical power β (i.e., the probability of avoiding a false negative error) of 0.8 (Faul et al. 2007). Therefore, we do not discuss effects on seizure frequency or duration further.

3. Results

3.1. Overview

During PLEF application we observed that the fPSP spikes become transiently entrained or phase-locked to the stimulation. This effect is illustrated for a one-second period during a seizure in figure 6. Here we show both the depth LFP recording and the current, which was applied at 15 Hz. It is clear that the identified fPSP spikes align closely with the peak in the applied current. As we describe below, we observe quantitatively similar responses in 3 of 4

animals at many frequencies (table 1). Figures showing raw traces and statistical evidence of entrainment in seizures from all four animals are provided in Supplement A.

A Fourier spectrum (not shown) of the LFP of the recording in figure 6 would have a sharp peak at 15 Hz. But, because our stimulation adds a recording artifact proportional to the applied field, spectral analysis of the recorded neural signal would be contaminated despite efforts at unbiased artifact reduction. Therefore, as described in the Methods section, we adopted an analysis based on the extracted fPSP spike *times*. Because the spikes themselves have very sharp features (high frequency components), their extracted times are relatively insensitive to the artifact. In section 3.2 we quantify entrainment as an observed significant spectral line component at the applied frequency (Thomson's harmonic F test) in finite windows of spike times during seizures: furthermore, entrainment is deemed significant *only* if the proportion of stimulated seizures with one or more 4 s windows of significant entrainment at that frequency is greater than that expected from chance alone. In section 3.3 we examine the electrode placement as determined by histology and correlate it with the observed entrainment. In 3.4 we examine the possible influence of residual field artifact on the determination of significant entrainment: this is done by adding simulated artifact to control seizures and quantitatively comparing any spurious entrainment with that observed in stimulated seizures with residual artifact. Finally in section 3.5, we demonstrate conclusively that the F distribution of signal-to-noise ratio (SNR) assumed by Thomson's harmonic F test under the null hypothesis of no entrainment is valid in our experiments by comparing against empirically observed distributions.

3.2. Quantification of Entrainment

Examples of analyzed seizures from animal D, as recorded from a depth electrode ipsilateral to the stimulation electrode, are shown in figure 7. The left column is from an unstimulated seizure, and the right from a seizure stimulated with a 15 Hz applied field. The data from figure 6 comes from this same seizure, starting at time $t = 16$ s (indicated by the arrow in figure 7G). In the upper panel for each is the spectrogram based on 4 s windows extracted from the fPSP spike-time series. Spectrograms are normalized by the spike rate and coded in decibels (dB), so that a Poisson spike series would appear flat with value 0 dB. Additionally, the spectrograms are featureless or undefined in value for windows with fewer than two spikes. Below the spectrograms are plots of the average spike rate (panels B and F), again measured in 4 s windows.

No obvious sharp features or lines are observed in the unstimulated seizure data (left side), and Thomson's F-statistic for the spectral component at 15 Hz, plotted in panel C, never exceeds the 99.9 % confidence level (gray line). In contrast, there is a prominent line that transiently appears at 15 Hz in the spectrogram of the stimulated data (right hand column) along with its 30 Hz harmonic. Only the main peak at 15 Hz exceeds the 99.9 % F-statistic confidence level, and does so for three consecutive four-second windows, and again for a single window later in the seizure. (The F-statistic for the second harmonic peaks at approximately a 99.4 % single-window confidence level.)

In the bottom panels (figure 7D, H) we plot the number of windows N_w above the 99.9% confidence level at each frequency throughout the seizure with a resolution of 0.25 Hz. There are 37 windows per seizure, with each window having a probability $\alpha = 0.1$ % (based on the assumed 99.9 % confidence level) of being above the critical value by chance. Therefore, the expected probability at any given frequency of observing at least one window above the criterion, as computed from binomial statistics, is approximately $P = 4$ %. This expected false detection rate is slightly overestimated because nearly half the 37 windows analyzed per seizure have fewer than two detected fPSP spikes and therefore have flat or undefined spectra. A better estimate, based on 20 windows yields a false detection rate of

approximately 2 %. For the unstimulated seizure (figure 7D), we observe 3 out of 140 possible frequencies tested from 0.25 to 35 Hz have $N_w = 1$, consistent with the expected false detection rate. From similar binomial considerations, the probability of observing $N_w = 1, 2, 3$ or 4 from a single seizure by chance is approximately 0.04, 10^{-3} , 10^{-5} or 10^{-7} respectively. Therefore the observation for the stimulated seizure shown in figure 7H is highly unlikely to be a false detection.

Summary statistics for the above channel of animal D are presented in figure 8. We have defined a particular window as having significant entrainment if the F-statistic is greater than the 99.9 % confidence limit. Here, we define an entrained *seizure* as one with at least one significant window of entrainment at the *stimulation frequency*. The number of seizures stimulated at each frequency is plotted in figure 8A, and the number of those entrained plotted in figure 8B. Based on the above binomial considerations, seizure entrainment is therefore determined with an expected 2–4 % error (false detection) probability depending on the number of 4 s windows containing two or more fPSP spikes. The asterisks in figure 8B indicate the 99 % significance boundary for the number of seizures entrained based on a 4 % error probability per seizure and the number of seizures stimulated. Therefore, statistically significant entrainment was observed at frequencies 3, 11, 14, 15 and 20 Hz. In figure 8C, we display with box plots the distributions of N_w for the entrained seizures. (Box plots are designed to highlight the first and third quartile of the distribution about the median, along with outliers.) Statistical significance of these observations is further supported with the observation that many of the entrained seizures had two or more entrained windows, each with probability less than 0.1 % of being observed from random data.

Similar significant entrainment was observed for three of four animals studied when stimulated with this protocol. Detailed entrainment statistics for each rat and recording location are reported in table 1. Examples of entrainment for each animal and each frequency are shown in the supplemental figures (S1–S11). Considering only frequencies at which the proportion of stimulated seizures entrained was significant at the 99% confidence level, 12/12 seizures were entrained for animal A (at 15 Hz), none for B, 9/29 for C (at 11 and 15 Hz), and 21/23 for D (at 3, 11, 14, 15 and 20 Hz): hence entrainment was significant for a total of 42/64 seizures stimulated at 3, 11, 14, 15 and 20 Hz. We note that the details of the fPSP spike trains vary as a function of position, as the epileptiform activity is rarely completely synchronized in activity. Still, significant fPSP spike entrainment (mean duration ~12s, $p < 0.01$) during seizures was observed at locations *both* ipsilateral and contralateral to stimulation for a total of 36 of 64 seizures at several frequencies (3, 11, 14, 15, 20 Hz) in the three animals (A, C and D) that showed some significant entrainment. In 55 % of windows with entrainment, entrainment was observed in all measured channels, with spikes occurring in phase across channels. At frequencies associated with significant entrainment, almost all stimulated seizures (33/35 at 3, 11, 14, 15 and 20 Hz) were entrained for animals A and D, while about 30 % (9/29 at 11 and 15 Hz) of stimulated seizures were entrained for animal C.

Significant entrainment overall at a particular frequency (marked in table 1 with an asterisk) was determined from the proportion N_{ent}/N_{sz} with a 99 % confidence limit with respect to chance. But the number of stimulated seizures N_{sz} , and therefore the statistical power of the results, varied by frequency. A post hoc estimate of the power of the test of a sample proportion N_{ent}/N_{sz} against zero (Faul *et al* 2007; G*Power3) yields a power of 80 % or better for all observations of $N_{ent} \geq 2$. In the cases where either $N_{ent} = 1$ or $N_{sz} = 1$ we do not have enough statistical power to draw a conclusion.

Statistical Control—In contrast, the entrainment statistics for the unstimulated seizures were consistent with those expected from chance. For example, for animal D and the depth channel ipsilateral to the stimulation electrode, we analyzed 44 unstimulated seizures.

If we consider entrainment at the $N_f = 11$ frequencies from figure 8 at which this animal was stimulated, none of these unstimulated seizures were observed to have two or more entrained windows, and 9 were observed to have exactly one entrained window (3 at 1 Hz, 2 each at 3 and 15 Hz, and 1 each at 9 and 18 Hz). This per window error rate $N_{ent}/(N_f N_{sz} N_w) = 9/(11 \times 44 \times 20) \sim 0.001$ matches the specified 0.1 % critical p value used in our F statistic for detecting spectral lines. Note that we have used the estimate of $N_w = 20$ windows on average per seizure with two or more spikes in each window. This data also matches the corrected 2 % per seizure expected error rate: $9/(44 \times 11) = 1.8$ %. These results for the statistical control (unstimulated) seizures were consistent for all the animals.

3.3 Electrode Placement

The trajectory for the stimulation electrodes was chosen such that the exposed 3 mm end of the electrode would be aligned within the arc of the CA3 through a large fraction of the ventral hippocampus, as illustrated in figure 1. Unfortunately, the detailed anatomy varies from animal to animal which results in variations in actual placement. Horizontal slices approximately midway in the exposed electrode are shown in figure 9 for animals A, B and C. As seen, the placement for animals A and C were within the arc of the CA1 or CA3. The placement for animal B seemed to be on or just outside the arc of the CA3. The histology from animal D, which had the most reliable entrainment, was uninterpretable, either due to mishandling or misalignment in histological sectioning or due to anomalous anatomical features, so the location of the electrode tract could not be clearly determined by histology.

Of the three animals for which we have interpretable histology, significant entrainment was observed in both A and C; while animal B showed no significant entrainment (see table 1). The lack of significant entrainment in animal B is consistent with the histological finding of poor electrode placement (figure 6), in which the working electrode was located just outside the CA rather than within it. As noted in Richardson *et al* (2003), proper geometrical placement is critical for the use of PLEF stimulation.

3.4. Artifact reduction quantification

The artifact reduction method used in this analysis was imperfect, and therefore left some residual stimulation artifact in the signal. Although we asserted that the methodology used here –i.e., extracting fPSP spike times, then looking for sharp lines in the Fourier spectra of the event times – should be insensitive to the residual artifact, we demonstrate that here with the following steps: First we estimated the amplitude of the residual artifact in the stimulated data after reduction. Second, we iteratively added sinusoidal artifact to recordings from unstimulated seizures and repeated the spike extraction and entrainment analysis to estimate N_w as a function of simulated artifact amplitude. The results are summarized in figure 10 for seizures from animal D with simulated 15 Hz artifact.

An estimate of the residual artifact comes from assuming that the pre-seizure neural activity has a variance independent of the stimulation, and that the signal variance from stimulated pre-seizure periods is a mixture of the residual artifact and this uncorrelated neural signal. We computed the pre-seizure signal variance for 10 s windows starting 30 s prior to seizure onset for both unstimulated data and stimulated but artifact-reduced data. If we assume that the residual artifact is uncorrelated with the ongoing neural activity and that both are zero mean processes, then the variance values should add as $VAR(Neural + residualArtifact) = VAR(Neural) + VAR(residualArtifact)$. If we assume the artifact to be sinusoidal, then its

peak amplitude a is estimated as $\sqrt{2\text{VAR}(\text{residualArtifact})}$, because $\text{VAR}(a \sin(t)) = a^2/2$. We present in the box plot in the upper left of figure 10A, the distribution of estimated residual artifact amplitudes from the seven seizures in this animal stimulated at 15 Hz, the maximum of which is about 0.23 mV. In comparison, typical fPSP spikes during seizures have peak amplitudes of the order 1–2 mV.

For each of the 44 unstimulated seizures, we then added a sinusoidal artifact with amplitudes ranging from 0 to 0.5 mV in 0.05 mV steps, extracted the fPSP spike times, and quantified entrainment at 15 Hz as before. As expected, we did find some additional putatively entrained windows, the number of which increased as we increased the simulated artifact amplitude. The fraction of these seizures having $N_w \geq n = \{1, 2, 3\}$ is shown in the (blue, green, and red) curves in figure 10A as a function of simulated artifact amplitude. In figure 10B, we compare these probabilities $P(N_w \geq n)$ measured at amplitude 0.5 mV, or roughly twice our estimated *maximum* residual artifact value during stimulation, to the probability estimated from the 7 stimulated seizures, all of which had N_w between 1 and 3. The probability of observing significant entrainment for unstimulated seizures, even with artificially added artifact at twice the maximum estimated level seen for stimulated seizures, is far lower than for the stimulated seizures according to a Kolmogorov-Smirnov test ($p \sim 0.000001$). We conclude that the stimulation artifact does not explain the observed significant entrainment for stimulated seizures.

3.5. Evaluation of the SNR distribution assumed by Thomson's F test

An assumption of the analysis performed above was that the multitaper estimate of the SNR of a spectral line, in the absence of a true harmonic component, would be distributed as $F_{2,2(K-1)}$. The underlying assumptions invoke finite measurements of a noise background that is smooth in amplitude over the discrete frequency bin width defined by the finite time-window. We then detect a spectral line by observing an unusually high SNR, defined as being well out into the right tail of the $F_{2,2(K-1)}$ distribution.

As a final probe of our analysis, we investigated the experimentally observed distribution of SNRs for our spike time series to see if they agree with the ideal $F_{2,2(K-1)}$ distribution. Results are shown in figure 11 for seizures in animal D that were either not stimulated or stimulated at 15 Hz (same data as figures 7–8). In the analysis, SNR is computed for each time window at each frequency from 2 to 20 Hz. In 11a and 11b we show the probability density function (PDF: i.e., the relative probability that the value of a random variable, in this case SNR, will lie in a specified interval) and cumulative density function (CDF: i.e., the probability that SNR will be less than or equal to a specified value) averaged over all frequencies for unstimulated seizures (blue line); averaged over all frequencies except the stimulation frequency for stimulated seizures (black line), and averaged over stimulated seizures at the stimulation frequency (green line). Comparison of these distributions on the basis of the PDF or CDF helps us determine whether the observed SNR is consistent with a chance observation or with the presence of a true harmonic at the stimulation frequency. Note that in 11b, the CDF of SNRs for stimulated seizures not at the stimulation frequency (black line) overlaps with and is nearly indistinguishable from that of the unstimulated seizures (blue line). The SNR distributions for all frequencies but the stimulated frequency for stimulated seizures are consistent with the theoretical distribution $F_{2,2(K-1)}$ (red line). In our analysis, the analytical F distribution is used to generate the critical SNR for a given confidence bound, here set at 99.9 % for a single window. If we use the unstimulated CDFs as an empirically generated distribution, the same critical SNR yields an even greater confidence bound of 99.93 %.

We further investigated whether there were systematic frequency-dependent differences between the observed SNR distributions and $F_{2,2(K-1)}$. In panels c and d of figure 11, we

plot the SNR distributions for each frequency for both the unstimulated (c) and stimulated (d) seizures. There do not seem to be any systematic differences for either as a function of frequency, except at the stimulation frequency for stimulated seizures, when compared to the $F_{2,2(K-1)}$ distribution.

4. Discussion and conclusion

We have demonstrated the ability to interact with seizure activity using periodic PLEF stimulation (figure 6; additional figures in Supplement A). This effect was initially observed (Sunderam *et al* 2006) as transient peaks at the stimulation frequency in the LFP spectrum during seizures. However, the PLEF adds a differential mode artifact to the recording: although we try to eliminate this artifact either in hardware or software using spectrally estimated transfer functions, there may be a residual periodic component (figure 5) that contaminates the spectral peaks observed due to neural entrainment. By estimating spectra from the fPSP spike time series instead of the continuous LFP signal (figure 7), we have presented here an analysis that is robust against such errors (figure 10). Furthermore, the statistical methodology devised to test for entrainment gives results that are highly consistent with what is expected from stimulated as well as unstimulated seizures (figure 11). The point process analytical framework would be directly applicable to microelectrode recordings of unit activity during seizures as well.

These results are the first demonstration of PLEF stimulation in chronically implanted, freely behaving animals. The goal of PLEF stimulation is fundamentally different from pulsed stimulation as currently used in deep brain stimulators, which is typically employed in a manner that induces action potentials (APs), most easily initiated on axonal fibers (Merrill *et al* 2005). Though there are many successes that utilize activating pulse stimulation, it is limited in that the output is graded only in the number of fibers on which APs are initiated. Hence, if one considers the stimulation in terms of information input to the network, the resulting input is distributed across all parts of the brain to which the induced APs propagate, which is not well localized by the stimulation electrode geometry or placement. Additionally, the recording artifact from pulsed stimulation is typically very large and unreliably distorted. Therefore, recordings during pulse trains from areas near the stimulation electrodes are rarely investigated. This limits the use of feedback in neural control to responsive stimulation, defined as stimulation in response to neural measurements made only prior to or time-separated from stimulation pulse trains.

Polarizing stimulation (PLEF) provides alternatives to some of these limitations. The direct effect on the neurons is effectively a local modulation of excitability, with the change in excitability being linearly proportional to the applied field both in sign and amplitude (Jefferys 1995; Gluckman *et al* 1996; Bikson *et al* 2004). The response is local in the sense that the geometry of the stimulation electrodes determines the geometry of the applied field, and the neurons that have asymmetric distributions of primary dendrites, such as the pyramidal neurons, are modulated to the extent that they are aligned with the field. And finally, as we and others have demonstrated in slice experiments (Gluckman *et al* 1996 and 2001; Lian *et al* 2004), and now under chronic implant conditions, if the instrumentation is suitably implemented, high fidelity recordings can be made during stimulation.

The stimulation electrode placement was chosen both for its ability to polarize the pyramidal neurons of the CA3, but also because the implant trajectory and placement mimics that used for intracranial EEG measurements during evaluation for epilepsy surgery. This choice of geometry offers a direct translational path to clinical implementation of seizure control using PLEF stimulation with stereotactic electrode placement.

The basic elements for the prototype PLEF stimulation and recording system include a low-frequency isolated stimulation subsystem, a high input impedance explicitly differential recording subsystem, and a differential stimulation artifact reduction subsystem. Safety was of critical importance when designing the stimulation system. Charge passage at electrodes is mediated by a combination of capacitive charging and electrochemical reactions. We took great care to be able to avoid generating damaging electrochemical reaction products. We did this using two methods: first, we used an isolated stimulation subsystem to prevent common mode charge leakage between the stimulation pairs and the animal. Second, we limited the maximal whole-cell potential between electrodes to maintain each half cell well within the water hydrolysis window (Merrill *et al* 2005).

We boosted the charge-passing capacity of the stimulation electrodes using a thin film coating of iridium oxide. This coating allows for reversible Faradaic reactions because the major reaction products remain attached to the surface. What is exchanged with the surrounding electrolyte is a combination of water, which is in great abundance, and H^+ . Unfortunately, because the protons are not as abundant as water molecules, the reactions are mass-transport limited in tissue. Therefore we find the charge-passing performance of these electrodes is lower in tissue than in phosphate-buffered saline in the dish. Additionally, as shown in figure 2, the maximal attained current in these experiments does not vary appreciably over a factor of 15 in applied frequency. These observations are consistent with those of Cogan *et al* (2007).

It is possible that with more efficient charge passage, the transient entrainment observed in the early stage of seizures may be enhanced to alter or abort seizure initiation or generalization. We are currently experimenting with different thin film coatings to enhance the charge-passing capacity of our stimulation electrodes at low frequencies.

As with other electrical stimulation, PLEF stimulation changes the potential at the recording electrodes both by adding a common mode signal, defined by the potential difference between the average of the recording electrode positions and the recording ground, and a potential difference defined by the line integral of the applied field between a recording electrode pair. Our target field magnitude is a few tens of millivolts per millimeter. Therefore the common mode signal can be quite large—tens to hundreds of millivolts—depending on electrode configuration and distance. We therefore designed the recording preamplifiers to have very high input impedance and common mode rejection. High impedance minimizes the current the recording electrodes draw and therefore how much their interfaces become charged in response to the common mode part of the applied field. This type of charging would inherently amplify the differences between electrodes and therefore increase common mode leakage. The differential component remaining should, under the same assumptions that yield Helmholtz's reciprocity theorem and lead-field models, be a linearly filtered version of the total current applied between the stimulation electrodes. In this work we used the simplest linear estimate, with zero phase delay, which was sufficient for the monochromatic stimulation applied. Using a more sophisticated filtering method, we find the artifact may be better removed with a frequency-dependent amplitude and phase delay (Chernyy *et al* 2008), which can then be implemented to remove artifact from broadband stimulation either digitally or in hardware.

Interestingly, when the fPSP spikes ipsilateral to the stimulation electrode were entrained by stimulation, they were almost always entrained at the more distant electrodes in the contralateral hippocampus as well. This effect is to be expected. Finnerty and Jefferys (2000), in their characterization of the tetanus toxin model of temporal lobe epilepsy, noted that the seizure progression prior to generalization consists of prolonged trains of fPSP units which occur at 1–3 Hz at seizure onset, evolve into 8–15 Hz polyspikes that quickly evolve

further into separated bursts of 10–20 Hz polyspikes, and are then followed by a stage of continuous 9–16 Hz spike activity which consistently precedes generalization. It was our target to interact with and entrain this 9–16 Hz activity by driving it with periodic stimulation, especially near the upper end of this range. This 9–16 Hz activity is typically synchronized across hippocampi when unstimulated. It is therefore not surprising that when we observed entrainment, it was usually observed at all hippocampal electrode sites.

Having demonstrated successful interaction and modification of spontaneous seizures, we envision adapting PLEF stimulation for use in continuous negative feedback to “cancel” undesirable neural activity in seizure-conducive brain states, or to desynchronize bilateral activity during seizure evolution prior to generalization. There is now some evidence from both clinical and laboratory experiments of a cascade of events leading up to electrographic seizure onset (Litt and Lehnertz 2002). Although such evidence has over the last decade or so energized a major effort to develop seizure prediction systems, consistent success in that field has been elusive (Mormann *et al* 2007). PLEF stimulation could prove to be a flexible tool for redirecting the dynamics that lead up to seizures.

Additionally, PLEF stimulation may be a useful and minimally (cognitively) intrusive perturbative tool for establishing brain state, especially for detecting precursors of seizure. Preliminary evidence of such an application of PLEF stimulation was reported by Richardson *et al* (2003). Specifically, in an acutely instrumented anesthetized animal, when one hippocampus was modulated using PLEF stimulation, the characteristic response to the stimulation in the contralateral hippocampus reliably changed in amplitude within the 15 s prior to seizure onset (see figure 4 of Richardson *et al* 2003). Similar probing has been performed using paired-pulse paradigms to monitor long-term changes in excitatory and inhibitory connections between brain regions both to assess reorganization during the kindling process (Zhao *et al* 1991; Kamphuis *et al* 1992; Stringer 1993) as well as ictiogenesis during systemic application of kainic acid (Ikeda-Douglas *et al* 1998). Pulsed stimuli have long been used to map epileptogenic regions in human patients (Valentin *et al* 2002 and 2005). More recently, Kalitzin *et al* (2005) have followed this idea by tracking the responses to pulse trains applied to the hippocampus in epilepsy patients during preoperative recording sessions to assess brain state and, with limited success, predict seizure onset. In commentary on this work, Osorio *et al* (2005), urged caution in the use of pulsed stimuli for this purpose, since they may have time and state dependent responses such as evoking (seizure) after-discharges, may induce short or long term changes in synaptic or neuronal responses, and could be of high enough intensity to transiently alter the natural (baseline) state of the epileptic network. The use of PLEF stimulation as an input might offer a more flexible and subtle probe of system dynamics.

The use of PLEF stimulation as a mode of information transmission to brain may have utility beyond seizure prediction and control. We have demonstrated similar modulation of activity in cortical structures *in vitro* (Richardson *et al* 2005) in the context of controlling activity propagation. We are now also investigating PLEF stimulation as a mode for directly modulating sensory cortices for prosthetic input and modulating hippocampal rhythms during navigation. Lastly, whether PLEF can be applied to decrease seizure frequency or duration, with or without closed-loop feedback, remains an open question warranting further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Illustration of stimulation electrode placement and resulting electric field orientation in the hippocampus

A stimulation electrode is targeted to align along the dorsal-ventral axis of the ventral hippocampus centered within the arc of the CA3. The electrode is exposed over 3 mm of its length from the tip, and in its local field produces a radially expanding current. The field is therefore locally aligned with the main dendrite-soma axis of the CA3 pyramidal cells. Tetanus toxin is focally injected at a point 1.5 mm above the tip of the stimulation electrode and along the same trajectory.

Figure 2. Current vs Voltage (I–V) curves during stimulation

Stimulation was performed by controlling the whole-cell potential, defined as the potential between the two stimulation electrodes in the tissue. For this figure, current was averaged over 5 cycles of stimulation from animal B. The maximum current grows much more slowly than frequency for these electrodes. The measured current output (μA) is plotted against voltage applied (mV) between the stimulation and reference electrode for sinusoidal stimulation at 1, 9 and 15 Hz. The charge storage capacity (CSC, μC) was estimated by computing the cumulative integral of the current waveform over one averaged cycle at each frequency and subtracting the minimum from the maximum value of the result. The peak applied voltage was 800 mV.

Figure 3. Typical chronically recorded spontaneous seizure from the tetanus toxin model of temporal lobe epilepsy

A 150 s trace recorded from a bipolar hippocampal depth electrode in the left dorsal CA3 of animal D, starting 30 s before seizure initiation. The bottom traces are two axes of acceleration measured from the animal's head by DC sensitive MEMS accelerometers. Seizure generalization is typically accompanied by a rearing motion, which is reflected by slow DC changes in acceleration along the accelerometer axis (marked by *). Field postsynaptic potential (fPSP) spikes at different points in seizure evolution are shown at an expanded scale in the upper traces. Large dots mark the dominant positive or negative peak within a moving 100 ms window used to define fPSP spike times. In the top center panel, some negative spikes of relatively small fPSPs are marked because they were the only features that satisfied both the amplitude and gradient constraints for spike detection.

Figure 4. Flow chart of the data analysis procedure

There are three major steps. 1. Preprocessing: This includes seizure detection and verification, followed by stimulus artifact reduction, and then fPSP spike detection and reduction to point process. 2. Testing for Entrainment: The data is then broken into 4 s windows, within which we estimate the point spectrum and apply the F-test for lines at the drive frequency f_{stim} . 3. Quantifying entrainment: For each seizure we quantify N_w = number of windows with significant entrainment ($p < 0.001$). For each channel recorded and each frequency of stimulation applied, we further count N_{sz} = number of seizures detected and N_{ent} = number of seizures entrained (i.e., with $N_w > 0$).

Figure 5. Demonstration of stimulation artifact reduction

Shown are 2 s traces from animal D of depth LFP recording near the stimulation electrode during an applied 15 Hz sinusoidal field. The upper trace is prior to artifact reduction. The lower trace (green) is after removal of an amount of signal proportional to the applied current. Note that some residual artifact remains.

Figure 6. Hippocampal seizure activity entrained by polarizing low frequency electric field (PLEF) stimulation

Shown is a 1 s segment of hippocampal depth LFP, after stimulation artifact has been reduced (upper trace) along with the 15 Hz periodic current used to create the polarizing electrical field. Extracted fPSP spike times are marked by red dots, and appear to be synchronized with the stimulation. Trace taken from animal D, stimulated with peak current of about 90 μA , approximately 16 s into this particular seizure.

Figure 7. Examples of analyzed seizures with and without PLEF stimulation

Both seizures were taken from the same animal, D, as recorded from a depth electrode in the same hippocampus as the stimulation. Analysis was done for 150 s starting 30 s prior to seizure onset. The seizure on the left (A–D) was unstimulated, and the one on the right (E–H) was stimulated at 15 Hz. Analysis was done in 4 s non-overlapping windows. Shown are (A, E) spectrograms computed from the spike-time series, (B, F) average spike rate R_s per 4 s window, (C, G) Thomson's F-statistic normalized by a critical value ($p = 0.1\%$) at 15 Hz, and (D, H) number of entrained windows N_w for each frequency (0.25 Hz resolution) for the seizures. No obvious persistent spectral lines are observed from the unstimulated seizure, and the observed N_w values are consistent with chance. The stimulated seizure has a significant line at 15 Hz that persists for 4 windows, which is highly unlikely ($p \sim 10^{-7}$) from chance. Note that the data from Figure 6 is from the same seizure, starting at time $t = 16$ s, and marked by the arrow.

Figure 8. Seizure entrainment statistics

Results are shown here for one animal (D) determined by analysis of left dorsal hippocampal EEG ipsilateral to the stimulation site. (Upper) Histogram of N_{sz} = the total number of seizures stimulated at each frequency f . (Center) Number of N_{ent} seizures entrained by the stimulation at each frequency f . Seizures were classified as being entrained if the number of 4 s windows N_w with significant spectral lines at the stimulation frequency $N_w \geq 1$. The 99 % confidence limit for the proportion of seizures with $N_w \geq 1$ from binomial considerations is marked with an asterisk for each frequency. By this criterion, there were significantly large numbers of seizures entrained at 3, 11, 14, 15 and 20 Hz. (Lower) Box plot representation of the observed distribution of N_w at each stimulation frequency. The boxplot is a standard way of representing the distribution of a sample as a box, with the lower quartile, median and upper quartile (i.e., 25th, 50th and 75th percentile, respectively) of the distribution as the horizontal edges, and whiskers that extend from the edges up to 1.5 times the interquartile distance or up to the most extreme value in the sample, whichever is smaller. If there are values beyond the whiskers, they are called outliers and shown as dots. In some instances, as seen here at 3 and 20 Hz, the quartiles of the distribution may coincide with one another giving an asymmetric box plot; this is especially likely with very small samples of integer values. Note that the probability of any one seizure having $N_w \geq 2$ is approximately 0.06 %.

Figure 9. Stimulation electrode position from histological sections stained with a Nissl stain
Shown are horizontal slices approximately midway along the exposed electrode for animals A, B, and C. Arrows indicate tracts made by insertion of working electrode (WE) and counter-electrode (CE). Sections from animal D were not interpretable, nor could the electrode tracts be identified. The scale bar is 450 μ m in length.

Figure 10. Assessment of the impact of residual PLEF artifact on analysis of entrainment
 (A) Box plot (top left) of estimated residual artifact amplitude from the stimulated seizures of animal D ($n = 7$), estimated from the difference in root mean square (RMS) signal amplitude in the half minute just prior to seizure onset, compared to unstimulated seizures ($n = 44$) in the same period. Fraction of seizures P with entrained windows $N_w \geq n = \{1, 2, 3\}$ marked by ‘□’, ‘x’ and ‘o’ respectively, for unstimulated seizures ($n = 44$) with artificially added sinusoidal artifact as a function of artifact amplitude. (B) Probabilities of $N_w \geq n$ from stimulated seizures (closed circles) and from unstimulated seizures (dashes) with added artifact of 0.5 mV or approximately twice the estimated maximum residual artifact amplitude.

Figure 11. SNR statistics underlying the harmonic F-Test

Shown are the experimentally observed distributions for the SNR computed from the ratio of first and second moments of the tapered spectral estimates for spike time series from animal D for both unstimulated and 15 Hz stimulated seizures. (a) Probability density function (PDF) and (b) cumulative density function (CDF) for SNR averaged over all frequencies for unstimulated seizures (blue line); averaged over all frequencies except the stimulation frequency (15 Hz) for stimulated seizures (black line); at 15 Hz for stimulated seizures (green line); and for $F_{2,2(K-1)}$ (red line). The pale blue vertical bar indicates the theoretical 99.9 % confidence level. (c,d) CDFs for individual frequencies averaged over (c) unstimulated and (d) 15 Hz stimulated seizures. Empirical CDFs for all cases are consistent with the assumed null hypothesis distribution except that for the stimulation frequency of stimulated seizures.

Table 1

Entrainment statistics^a

Stim location	Stimulation Frequency f (Hz)															Any $f > 0$
	0	0.5	1	3	5	7	9	10	11	14	15	17	18	20		
Rat A	Nsz	75	16								12					28
	Ts	85	23								15					
L-Ant #	Nent	3									12*					15
	Nw		1.3±0.3								4.3±0.5					
R-Ant	Nent	2									11*					13
	Nw		1.0±0.0								4.7±0.6					
Rat B	Nsz	56		2	7	1	4		12		5					31
	Ts	108		3	22	1	7		22		6					
L-Ant	Nent		0	0	0	0	0		1		2					3
	Nw								1.0±0.0		1.0±0.0					
R-Ant #	Nent		0	0	0	0	1		0		2					3
	Nw						1.0±0.0				1.0±0.0					
Rat C	Nsz	63		4		7			11		18					40
	Ts	428		19		18			8		26					
L-Ant	Nent		0	0	1	1			3*		5*					9
	Nw				2.0±0.0				5.0±0.6		2.8±0.8					
R-Ant #	Nent		0	0	0	0			3*		6*					9
	Nw								1.0±0.0		1.5±0.3					
L-Post	Nent		0	0	0	0			2		5*					7
	Nw								2.5±2		2.4±0.7					
Rat D	Nsz	44		1	5	1	1	2	6	2	7	4	1	3		33
	Ts	333		5	17	15	3	4	11	4	15	35	4	4		
L-Ant #	Nent		0	3*	0	0	0	0	5*	2*	7*	0	0	3*		17
	Nw			1.7±0.7					2.2±0.2	1.0±0.0	2.0±0.3			2.0±1.0		
R-Ant	Nent		0	1	0	0	0	0	6*	2*	7*	1	0	1		18
	Nw			2.0±0.0					2.3±0.3	1.0±0.0	2.7±0.5	1.0±0.0		4.0±0.0		

Stim location		Stimulation Frequency f (Hz)														
		0	0.5	1	3	5	7	9	10	11	14	15	17	18	20	Any $f > 0$
R-Post	N_{ent}			0	1		0	0	0	6*	1	7*	1	0	2*	18
	N_w				1 $\pm$ 0.0					2 $\pm$ 0.3	1 $\pm$ 0.0	3.1 $\pm$ 0.7	1 $\pm$ 0.0		2 $\pm$ 1.0	

^aFor each animal and recording location (L = Left, R = Right, Ant = Anterior, Post = Posterior,

[#] = Ipsilateral to stimulation electrodes), the following are tabulated: the number of seizures N_{Sz} that occurred during stimulation, which was applied for a total duration T_s (hours) at each frequency f ; the number of these seizures that were entrained N_{ent} ; and the number of windows N_w (mean $\pm$ standard error) per entrained seizure (seizures with $N_w > 0$) that showed significant entrainment. T_s at 0 Hz refers to all stimulation off periods. The duration T_s and timing of PLEF stimulation at different non-zero frequencies were manipulated on an ad hoc basis (see 2.2.5); in addition, the seizure rate was highly nonstationary over the course of the experiment. The apparent seizure rate for PLEF stimulation at a particular frequency therefore does not reflect on its ability either to induce or suppress seizures in these experiments.

* Indicates that N_{ent} is a greater proportion of N_{Sz} than expected from chance ($p < 0.01$).