FULL-LENGTH ORIGINAL RESEARCH

Rapid brief feedback intracerebral stimulation based on real-time desynchronization detection preceding seizures stops the generation of convulsive paroxysms

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Epilepsia, 56(8):1227–1238, 2015
doi: 10.1111/epi.13064

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Summary

Objective: **To investigate the abortion of seizure generation using "minimal" intervention in hippocampi using two rat models of human temporal lobe epilepsy.**

Methods: **The recording or stimulation electrodes were implanted into both hippocampi (CA1 area). Using the kainic acid (chronic: experiment duration 24 days) and the 4-aminopyridine (acute: experiment duration 2 h) models of paroxysms in rats, a real-time feedback stimulation paradigm was implemented, which triggered a short periodic electrical stimulus (5 Hz for 5 s) upon detecting a seizure precursor. Our seizure precursor detection algorithm relied on the monitoring of the real-time phase synchronization analysis, and detected/anticipated electrographic seizures as early as a few seconds to a few minutes before the behavioral and electrographic seizure onset, with a very low false-positive rate of the detection.**

Results: **The baseline mean seizure frequencies were 5.39 seizures per day (chronic) and 13.2 seizures per hour (acute). The phase synchrony analysis detected 88% (434 of 494) of seizures with a mean false alarm of 0.67 per day (chronic) and 83% (86 of 104) of seizures with a mean false alarm of 0.47 per hour (acute). The feedback stimulation reduced the seizure frequencies to 0.41 seizures per day (chronic) and 2.4 seizures per hour (acute). Overall, the feedback stimulation paradigm reduced seizure frequency by a minimum of 80% to a maximum of 100% in 10 rats, with 83% of the animals rendered seizure-free.**

Significance: **This approach represents a simple and efficient manner for stopping seizure development. Because of the short on-demand stimuli, few or no associated side effects are expected in clinical application in patients with epilepsy. Abnormal synchrony patterns are common features in epilepsy and other neurologic and psychiatric syndromes; therefore, this type of feedback stimulation paradigm could be a novel therapeutic modality for use in various neurologic and psychiatric disorders.**

KEY WORDS: **Feedback stimulation, Phase synchrony, Epilepsy, Electrical stimulation, In vivo.**

The somewhat limited success in the pharmacologic treatment of epileptic syndromes has aroused an increasing interest in the possibility of stopping seizures with brief direct electrical intracerebral stimulation. Support for the possible success of electrical perturbations in preventing seizures is based on the assumption that if the dynamics of the abnormal synchrony that characterizes paroxysms is perturbed by stimulations, then the ictus may not appear, or will be forced to stop if already initiated. Thus, the implementation of "minimal" (short duration, low frequencies

Accepted May 22, 2015; Early View publication June 29, 2015.

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Key Points

- Phase desynchronization in between two hippocampi was a reliable seizure precursor (sensitivity = 88%, specificity = 86%, and false alarm = 0.67 per day).
- A rapid brief feedback stimulation at the desynchronization was an effective method for seizure abortion (80–100% seizure-frequency reduction).
- At seizure formation, abnormal oscillation disrupted theta wave synchronization in between the hippocampi (71% of the desynchronizations in theta band).
- A burst (5 s) of low frequency stimulation (5 Hz) at the seizure formation restored the theta wave synchronization between the hippocampi.
- The feedback stimulation method demonstrated higher seizure-frequency reduction (~90%) than the open-loop stimulation (~25%).

and intensities) perturbations to stop the transition from the preictal activity to the ictal, convulsive event by a precisely timed brief stimulation has been a sort of "dynamic dream" in this field,[1] which has been suggested in recent reviews.[2–6] Contrary to the current deep brain or vagus nerve stimulation paradigms that use intermittent (continuous) stimulation, we sought to stimulate when a paroxysm was about to occur, using an on-demand feedback stimulation paradigm based on real-time analysis of brain signals that detects a precursor of paroxysms (Fig. 1A), and implements a brief (5 s) stimuli to stop the transition to the ictal event. Scores of studies have appeared in the last decade that address these two related matters of anticipation/detection and control of seizures; however, the implementation of a reliable and efficient method incorporating these two aspects in vivo has not been as successful as the execution of each one separately. Some advances have been made recently in rodents using relatively complex methods such as optogenetic stimulation, and in patients using deep brain stimulation.[4,5,7,8]

Building on past studies that have indicated that precisely timed low-frequency electrical pulses (0.5 Hz) can stop the transition to paroxysms in vitro,[9] and other observations of reduction of paroxysms by low-frequency stimulation in vivo in rodents (1 Hz[10], 5 Hz[11]) and in patients (0.5 Hz[12], 1 Hz[13], 5 Hz[14]), we chose to briefly (5 s) stimulate in the time window preceding a possible paroxysm with low frequency (5 Hz) periodic pulses employing rat models of seizures considered to reproduce features of human intractable temporal lobe epilepsy.[15] Generally, an abnormal oscillation originates from an epileptogenic zone (often in hippocampus in temporal lobe epilepsy), which may disrupt theta wave (and others) synchronization with the other hippocampus.[16] Over time, this focal oscillation spreads and often propagates contralaterally and develops a paroxysmal discharge.[17] A feedback stimulator could disrupt the local epileptic oscillation and abort the seizure development.[9]

The results indicate a great efficiency of our feedback stimulation technique in preventing the generation of convulsive paroxysms in rats that exhibited spontaneous and recurrent paroxysm weeks after treatment with kainate, which results in the appearance of chronic spontaneous seizures during the life span of the animal. Because of the sparseness of these paroxysms in the chronic seizure model, an acute seizure model was also used to assess the seizure frequency suppression efficacy. There are different substances that promote paroxysmal activity in rats, our choice of kainic acid (KA) and 4-aminopyridine (4-AP) was based on the known reliability of these compounds in the generation of seizures[18] and not on the specific reproduction of epileptic syndromes in patients.

The presented method demonstrates that the relative simplicity of the computation of a seizure precursor detection with low false-alarm rate, and a brief low frequency on-demand electric stimuli can effectively abort seizure generation. Therefore, this technique has the potential to be used clinically in patients and will most likely have no associated side effects.

Materials and Methods

Terminology

Two rodent models of seizures were used in this study. All convulsive motor seizures (475) were recorded behaviorally with video monitoring, whereas 84% (399 of 475) of the convulsive and all nonconvulsive seizures (199) were recorded electrographically using commercial and/or custom-made low-noise amplifiers. These events we term "*paroxysms*": any abnormal electrographic activities (duration ≥10 s) associated with relatively high frequency and amplitude of spikes (Fig. 1). When no apparent behavioral alterations were observed at the time of an electrographic paroxysm, the term "*nonconvulsive paroxysm*" is used, whereas we use the expression "*convulsive paroxysm*" if an abnormal behavior was observed concomitant with abnormal electrographic recording. The "*paroxysm onset*" was defined as the time when the amplitude of the electrographic recording in the paroxysm becomes greater than twice the standard deviation of the baseline activity. The "*early paroxysm detection time*" was the time between the detection of the seizure precursor and the paroxysm onset. The "*preictal period*" was defined 1 min before the paroxysm onset, and the "*interictal period*" was the time between the end of one paroxysm and the preictal of the next. The convulsive paroxysms were defined according to the Racine scale (class III to class IV), [19] whereas the nonconvulsive paroxysms were class I or class II seizures.

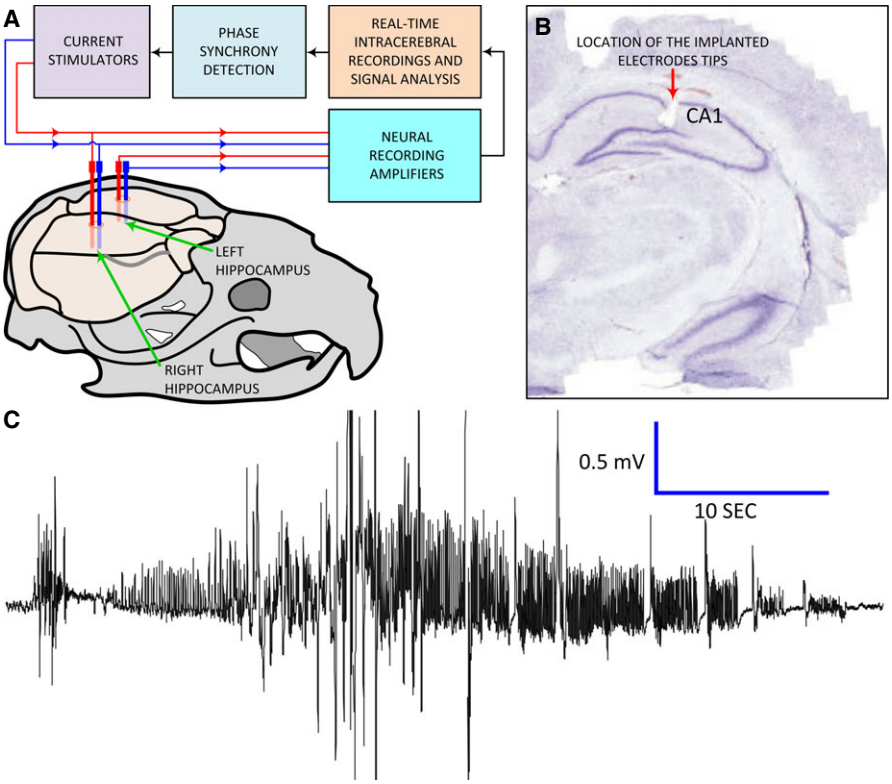

Figure 1.
(**A**) General scheme of our methodology. (**B**) A cresyl-violet stained brain section showing the location of the implanted electrode tips. (**C**) Representative example of a spontaneous paroxysm in the chronic condition (>2 months after kainate injection).
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Animals

Fifty-eight male Wistar rats (275–400 g) were used in these experiments. Of these, 39 rats were not used for the experiments due to the following reasons: six rats died during induction of status epilepticus; 12 rats lost their electrode headcaps; 19 rats did not show spontaneous seizures in the hours or weeks after KA or 4-AP injection; and two rats had the equivalent of sudden unexpected death in epilepsy (SUDEP). Manipulations of the rats were performed according to the protocols approved (protocol no. 1000008867) by the Animal Care Committee of the Hospital for Sick Children (Toronto, Canada) according to the Canadian Guidelines for Animal Care.

Electrode implantation

Rats were anesthetized with isoflurane and oxygen, and placed in a stereotaxic frame. Two burr holes were drilled in the skull overlying the right and left temporal lobes (Fig. 1A). For the chronic experiments, two bipolar electrodes (MS303, Plastics One, Roanoke, VA, U.S.A.) were bilaterally implanted chronically into CA1 regions of both hippocampi using the stereotaxic micromanipulator. For the acute condition, a bipolar electrode with a microcannula (C333-001, Plastics One) was implanted similarly into the right CA1 region for drug infusion and another bipolar electrode was implanted into the left CA1 region. The electrodes were fixed to the skull using dental acrylic. The coordinates of electrode implantation were: bregma −4.3, midline ±3.0, depth 3.1.[20] Histologic examination (cryostat sections of paraformaldehyde-fixed brains) of the rat brains at the end of the experiments confirmed the location of the electrode tips (Fig. 1B).

Seizure induction

Two well-characterized rodent seizure models were used to reproduce some features of human temporal lobe epilepsy.[18] The seizure induction procedures of the two models are described below.

Chronic condition: Kainic acid (KA, 13 mg/kg dissolved in saline) was injected intraperitoneally into 46 Wistar rats to induce temporal lobe paroxysms. One month to 2 months after the injection, recurrent spontaneous convulsive and nonconvulsive paroxysms developed in 11 rats, which were used for the chronic condition experiments.

Acute condition: 4-AP (300–500 nmol, 6–8 μl) was injected in 12 rats through an implanted cannula into the right hippocampus. Following the injection, eight rats had spontaneous recurrent electrographic seizures for at least 2 h and were used in the experiments.

In vivo intracerebral and behavioral recordings

The electrode implantation procedure often reduced chronic seizure frequency in the couple of days following the surgery, but the experimental phases described below did not start until a return to the baseline of at least two convulsive paroxysms per day. The intracerebral recordings of the rats took place in electrically screened acrylic glass chambers using a commercial recording system (Axon Instruments, Foster City, CA, U.S.A.) and a programmable feedback stimulator (our prior work described Bagheri et al.[21]). The recordings were acquired at 625 Hz and 10 kHz using the commercial system and our feedback stimulator, respectively, for 24 h a day, 7 days a week in the chronic experiments, and for 4 h in the acute experiments. Our feedback stimulator is a custom-made device, which has 256 recording channels, 64 stimulation channels, and a build-in signal processor.[21] The amplifier in each recording channel has a mid-band gain programmable from 54 dB to 72 dB, programmable bandwidth of 0.1 Hz to 5 kHz with 7.99 μV_{rms} input-referred noise. As noted earlier, the behavior of the animals was recorded, simultaneously with the electrical recordings, using video cameras.

Real-time signal processing

The intracerebral bipolar recordings from both hippocampi were recorded by the feedback stimulator and sent to a computer for real-time phase synchrony analysis using a Matlab-based custom-made algorithms. Initially, the recordings were band-pass filtered with cutoff frequencies of $F \pm 2$ Hz, where F is a central frequency. The phase synchrony was investigated at central frequency of 8 Hz and the averaged phase synchrony index values throughout a 1 s time window. The phase synchrony index is defined as $R = |\langle e^{i\Delta\theta} \rangle|$ where $\Delta\theta$ is the phase difference between the two hippocampal recordings.[21–23]

Feedback electrical stimulation

The feedback electrical stimulation consisted of a burst of square-wave current pulses bipolar biphasic current pulses of 150 μA, pulse width 100 μs, frequency 5 Hz, and duration 5 s triggered by the real-time synchrony analysis on response to the seizure precursor detection. The stimulation was delivered to the right or left hippocampus (depending on seizure initiation). The stimulation current was chosen according to safety considerations,[24] which was three times lower than the maximum deliverable charge per phase.[11,21]

Experimental details

All rats with seizures were divided randomly into two groups: (1) *nonstimulation group* and (2) *stimulation group*. In the nonstimulation group (five rats in the chronic experiment and four in the acute experiment), seizures were monitored and marked, and the seizure frequency per hour was determined. The stimulation group went through four experimental phases for the evaluation of the efficacy of the feedback stimulation: *phase I*, no stimulation; *phase II*, feedback stimulation; *phase III*, no stimulation; and *phase IV*, open-loop stimulation. Each of the phases was 6 days long. In phase I, seizures were monitored and marked; subsequently, in phase-II, the feedback stimulator was turned on to trigger the electrical stimulation upon an electrographic seizure precursor detection. The number of feedback stimulations per day in the stimulation group was quantified and used in phase IV. Then, in phase III, the feedback stimulation was turned off. Next, in phase IV, an open-loop stimulation paradigm was implemented using the same average number of stimulations per day as in phase II, but in a periodic manner (equal intervals), thus not associated with the detection of the seizure precursor. This phase served as a control for the specificity of the feedback stimulation. During these four phases, all paroxysms were monitored, classified, and the frequency per day determined.

Statistical analysis

The following statistical measures were used to assess the paroxysm detection performance.

True positives (TP): the number of paroxysms that followed the detection of the putative seizure precursor—a decrease in the phase synchronization index.

False positives (FP) or false alarm: when a paroxysm does not follow the detection of the seizure precursor.

True negatives: nonparoxysmal activity correctly identified as nonparoxysmal.

False negatives (FN): the paroxysms that occurred without the detection of the seizure precursor.

Sensitivity: the ratio of the number of TP to the total number of TP and FN.

Specificity: the ratio of the number of true negatives to the total number of TN and FP.

Statistical tests were performed in Matlab using the Statistics Toolbox. Results are expressed as mean ± standard deviation (STD). Statistical significance of differences in synchrony indices and spectral power during interictal, preictal and paroxysm periods, and paroxysm rate differences between baseline, open-loop stimulation, and closed-loop stimulation periods, were evaluated using a one-way repeated-measure analysis of variance (ANOVA). A Fisher's least significant difference (LSD) test was used to determine the significance between the various group means. The level of significance was set to $p < 0.05$.

Results

A total of 598 paroxysms were recorded electrographically in 19 rats. A representative electrographic example of a paroxysm in the chronic condition a few weeks after KA injection is shown in Figure 1C (also Fig. 2A). Our strategy was to implement the feedback stimulation upon a seizure

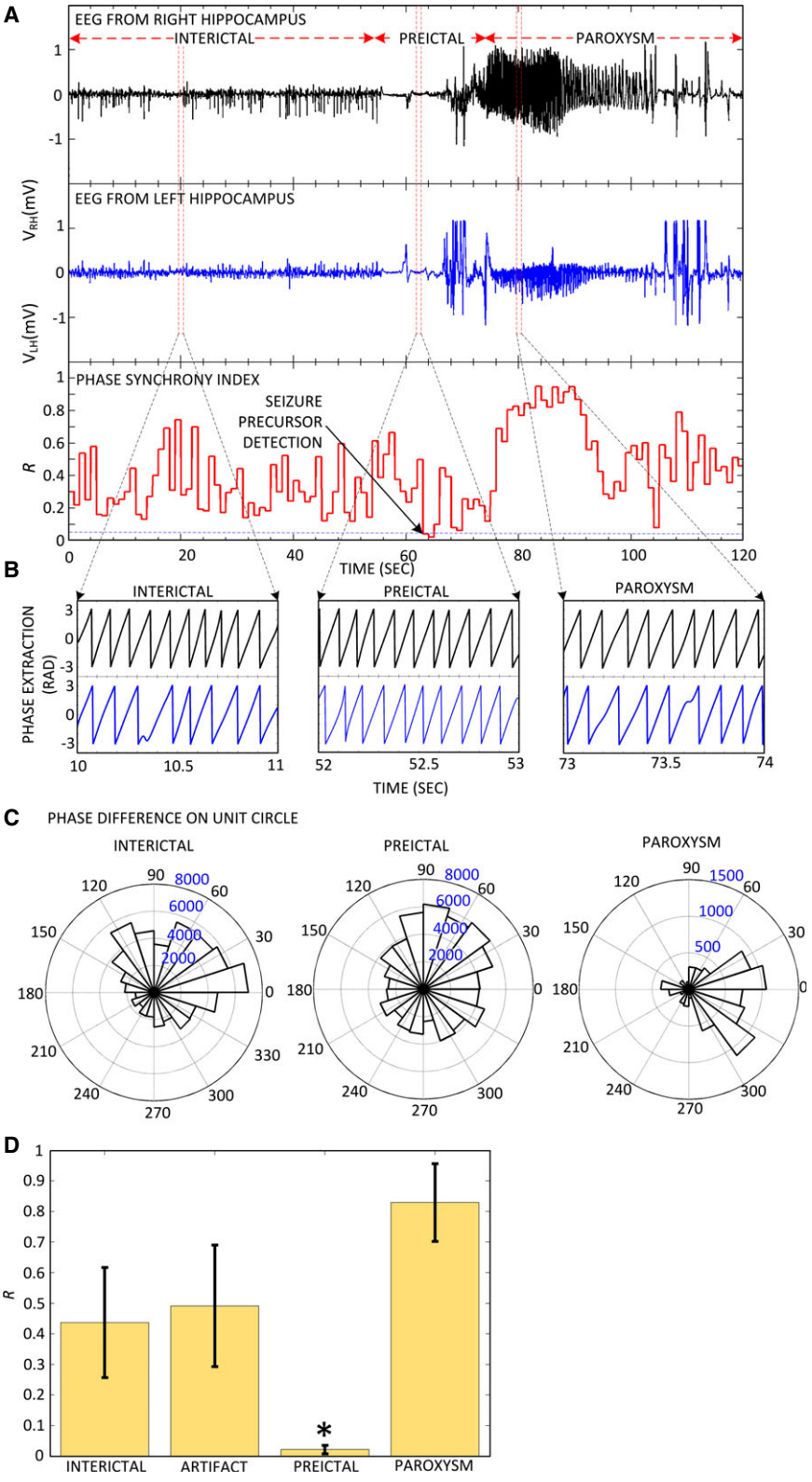


Figure 2.
Seizure precursor detection performance in the chronic condition. (**A**) An example of the precursor detection (a value of the R synchrony index, evaluated at central frequency of 8 Hz, below the threshold of 0.05 marked by the solid arrow) for a rat of the nonstimulation group. Upper traces are the recordings from both hippocampi showing the paroxysm approximately from the time period 70–100 s; the lower graph is the time evolution of the R index. The high synchrony (R) value is noticed during most of the paroxysm. (**B**) Phase (wrapped) extraction from interictal, preictal, and ictal periods in both hippocampal signals, that were used to compute the synchrony index (see formula for R in Methods); the typical sawtooth shape (extracted phase) can be seen in all three segments, indicating that the algorithm was able to extract the phases regardless of signal amplitude (note that the signals during the paroxysm have higher amplitudes). (**C**) The phase differences on the unit circle, where it can be seen the more dispersed distribution of angles during the preictal state, which translates into a lower R index. (**D**) Average of the evolution of the phase synchrony index R between two hemispheres during interictal, artifact, preictal, and paroxysmal periods. The magnitude of R drops significantly during the preictal period (*$p < 0.05$ for preictal vs interictal, artifact or paroxysm periods). "Artifact" denotes the times when the rat was moving (the moving artifacts were marked using the video recordings).

precursor detection based on changes in synchrony between the two hippocampal signals; therefore, the phase synchrony index (R) was initially assessed to determine the detection performance.

Seizure precursor detection performance

Figure 2A illustrates the recordings from the right and left hippocampus and the evolution of the R index in the third row. Its value fluctuates between 0.2 to 0.7 during

the interictal period; however, it drops below 0.1 during the preictal period, and gradually increases subsequently above 0.8 during the paroxysm. The phase desynchronization ($R < 0.1$) was observed before the paroxysm onset in all rats ($n = 19$) in both the chronic and acute conditions. For this reason, we refer to this drop in synchrony as the *seizure precursor* in the rest of the article. The paroxysm detection threshold (Fig. 2A) was consistent for the all animals; therefore, no optimization was required. This threshold was used in the feedback system to trigger a stimulation. Figure 2B illustrates the phase extraction from the signals and Figure 2C shows the phase differences on the unit circle. Figure 2D shows the average of the synchrony index R during the different periods and the R value during the preictal period is significantly lower ($p < 0.05$).

In the chronic condition, the paroxysm detection performance was evaluated online and offline (reevaluated) in the nonstimulation group and during the no-stimulation phases of the stimulation group. The detection performance is illustrated in Figure 3A–C. The phase synchronization analysis detected 95% (351 of 369) of the convulsive paroxysms and 66% (83 of 125) of the nonconvulsive paroxysms, with 0.67 ± 0.59 false alarms per day. The overall sensitivity and specificity of the detection were 88% and 86%, respectively. The average early paroxysm detection time was 53.64 ± 13.64 s.

In the acute condition, the paroxysm detection performance was similarly evaluated online and offline in the nonstimulation group, and the results are shown in Figure 3D–F. The phase synchronization analysis detected 90% (27 of 30) of the convulsive paroxysms and 79% (59 of 74) of nonconvulsive paroxysms. The overall sensitivity and specificity of the detection were 84% and 94%, respectively; with 0.47 ± 0.20 false alarms per hour. The average early paroxysm detection time was 21.50 ± 11.50 s.

Seizure suppression by feedback stimulation

In the chronic condition, the seizure frequency in the stimulation group was ~5.39 paroxysms per day in phase I (no-stimulation baseline). In phase II, the feedback stimulation delivered to the hippocampus after the detection of the precursor (Fig. 4A) resulted in five of the six rats in the stimulation group becoming convulsive paroxysm free, and a 93% reduction of seizure frequency in the sixth rat. Following the feedback stimulation phase, the rats underwent a no-stimulation phase III, which resulted in the average seizure rate going back to that of phase I (Fig. 4C,E). In phase IV, an open-loop stimulation was delivered. The open loop stimulation reduced the paroxysm frequencies found in phase I and phase III by 32% and 19%, respectively (Fig. 4C,E). Thus, we conclude that our feedback stimulation significantly reduced paroxysmal activity due to the precise timing of stimulation triggered by the seizure precursor detector.

In the acute condition, the average number of paroxysms in the nonstimulation group was quantified in 30 min periods (Fig. 5). The stimulation group, which received the feedback stimulation right after the detection of the seizure precursor, had a reduced 81% paroxysm frequency (Fig. 5) compared to the nonstimulation group ($p < 0.05$).

Possible mechanisms

Some possible reasons for the pronounced decrease in synchrony a few seconds before the ictus and the grounds for the great efficiency of our feedback stimulation in preventing generation of paroxysms were assessed.

Possible mechanisms for the seizure precursor. The decrease in synchrony prior to a paroxysm (Fig. 2A) may be caused by the different activities in the localized neighboring cells.[2,25] If each neighborhood starts to fire spikes at different frequencies (yet synchronously within that neighborhood), then it is conceivable that two relatively distant regions will have different neighborhoods firing at distinct frequencies thus the decrease in phase locking between the two hippocampal signals is expected. This scenario is supported by the spectral analysis shown in Figure 6A. Power levels in different frequency bands were changing almost simultaneously in the right and left signals during the interictal and ictal periods; however, during the preictal period, there were apparent divergences in power between both recordings, and this occurred in 87% (520 of 598) of the preictal stages evaluated. In particular, the θ band power had a sharper difference between the two signals. Thus, as a consequence of this divergence in oscillatory power, a phase desynchronization between both hippocampi is expected to occur at the preictal period prior to the paroxysm onset (as shown in Figures 2A and 4A). The divergence in power was seen as well in other frequency bands; however, it was more abundant and pronounced in the θ band: it occurred in 71% (370 of 520) with >50% increase from baseline at θ, 56% (292 of 520) with >23% increase from baseline at β, 35% (183 of 520) with >52% increase from baseline at α, 48% (250 of 520) with >52% increase from baseline at δ, whereas the γ band was relatively unaffected (4%, 21 of 520). The power differences at all bands between both recordings were not statistically different during the paroxysmal period (Fig. 6A).

Effects of the feedback stimulation. To investigate possible reasons of the success of our feedback stimulation, similar power spectral analysis after the stimuli was carried out, and as well, we assessed the temporal evolution of the phase synchrony index after the stimuli as compared to the synchrony when no stimulation was implemented (and thus paroxysms developed). Figure 6B indicates that the net effect after the stimulation was a decrease in power at low frequencies with no apparent changes at higher bands (β and γ). The decreased power

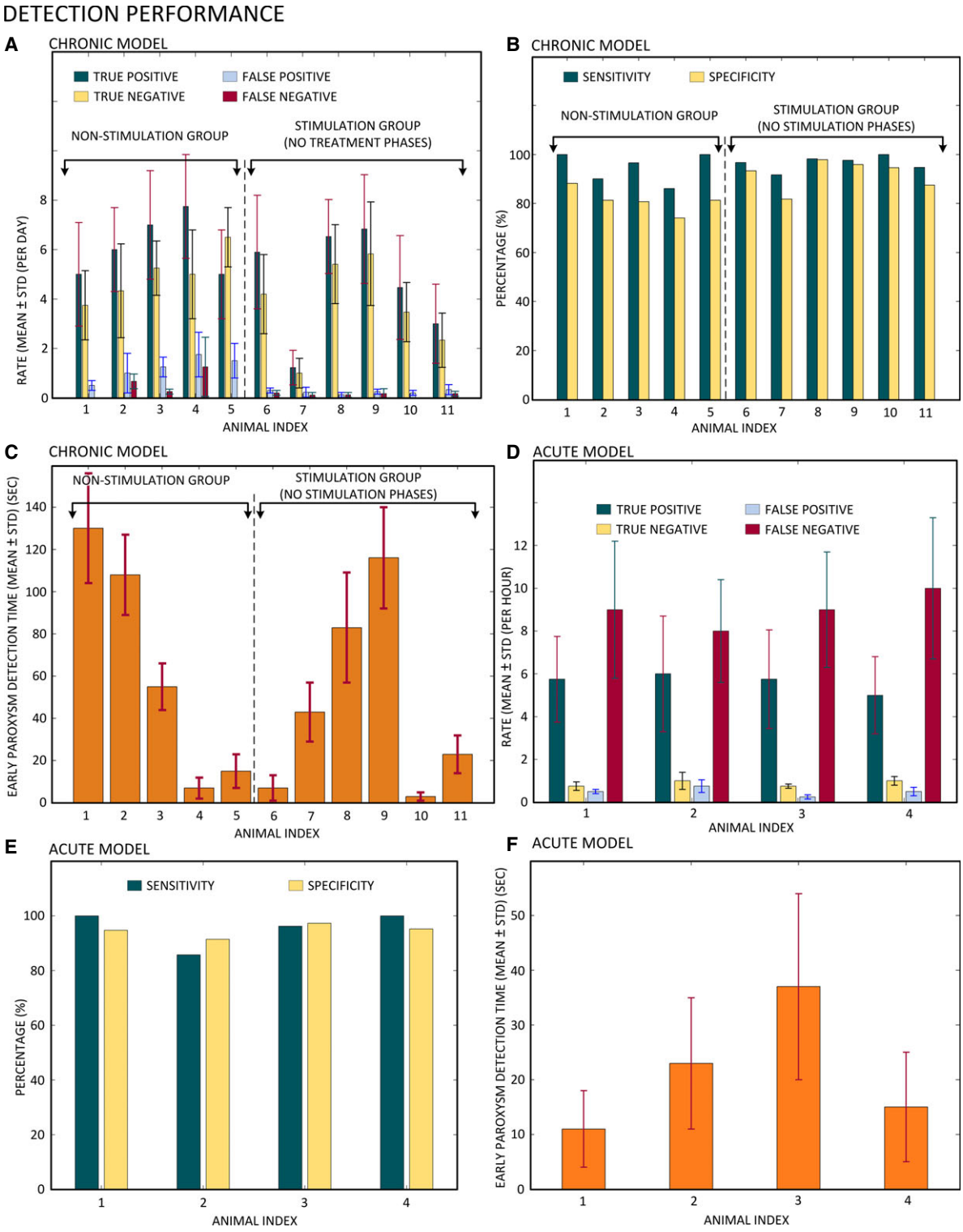
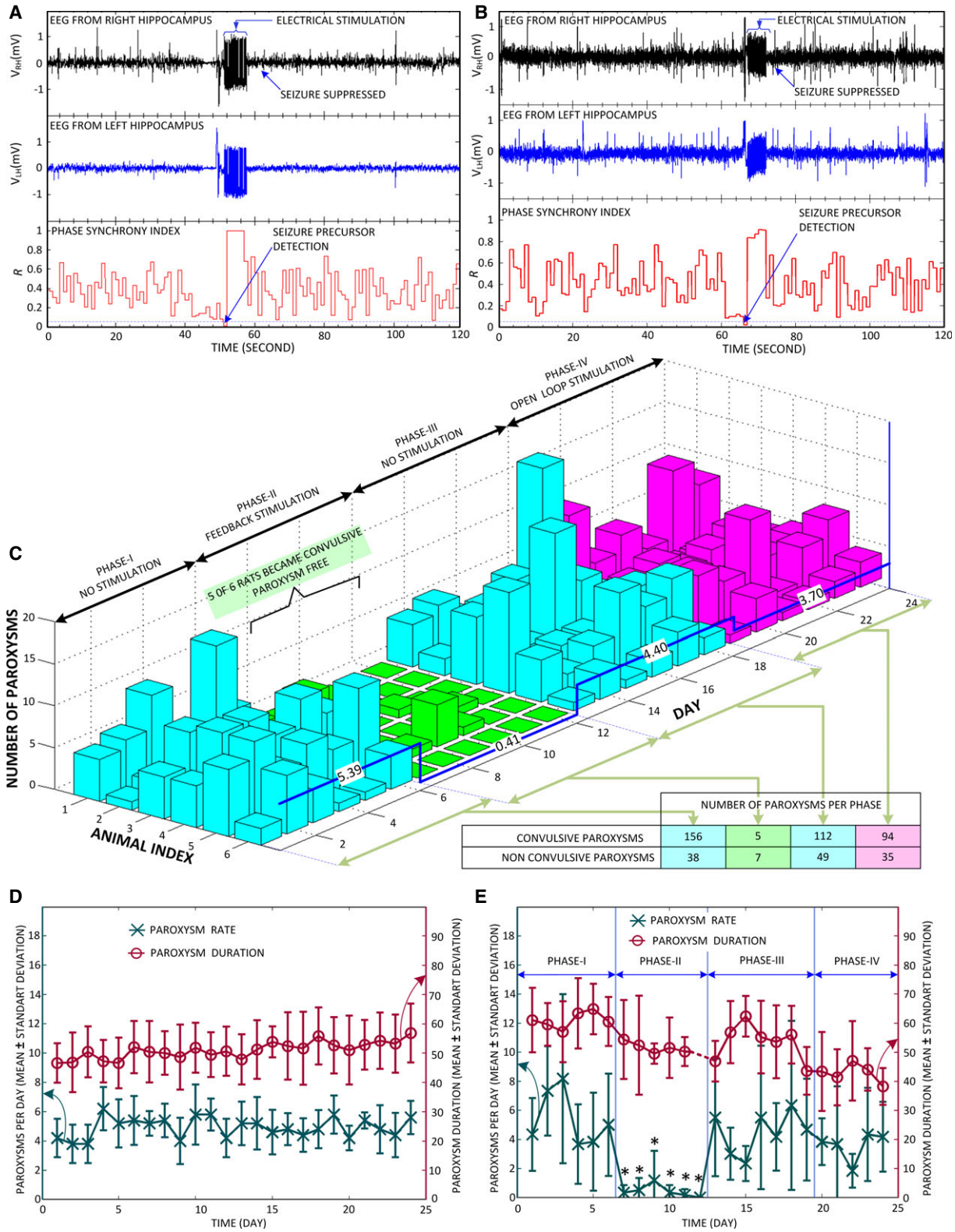


Figure 3.

Detection performance of the seizure precursor in the chronic and acute conditions. (**A**, **B**) Seizure-onset detection performance based on 494 seizures from the nonstimulation group (five rats) and no-stimulation periods of the stimulation group (six rats). See Materials and Methods for the terminology and statistical measurement used. (**C**) Early paroxysm detection times for both groups: the time when the minimum in the phase synchrony index was detected before seizure onset. (**D**, **E**) Seizure-onset detection performance based on 104 paroxysms from the nonstimulation group in the acute condition (four rats). (**F**) The time before seizure onset when the minimum of the R synchrony index was detected.



relative to that during the normal development of the paroxysm was 56%, 80%, and 40% of the δ, θ, and α bands, respectively. The power remained at the reduced levels for 2–3 s following the feedback stimuli and later gradually recovered back to the interictal level. Regarding the temporal evolution of the phase synchrony index), the

Figure 4.
Representative examples of seizure suppression by the feedback electrical stimulation for a rat in the stimulation group of chronic (**A**) and acute (**B**) models. After the R index falls below the predefined threshold (denoted by the discontinuous line in third row), the feedback system triggers the stimuli and no paroxysmal event follows, and there is no increase in the synchrony characteristic of seizures (see Fig. 2A, third row). The high value of the synchrony index (*R* in third rows of **A** and **B**) was due to the 5 s stimulation artifact. (**C**) Seizure suppression results in the chronic condition. During the 6 days of the no-stimulation phase I, ~5.39 seizures per day on average were observed. The stimulation phase II reduced seizure rate to 0.41, and afterwards, another no-stimulation phase (phase III) yielded ~4.40 seizures per day. The final phase, open-loop stimulation (see Results for details), presented on average 3.70 seizures per day. The table/inset indicates number of convulsive and nonconvulsive paroxysms. Statistical analysis of paroxysm frequency (black line) and paroxysm duration (red line) of the chronic experiment in nonstimulation (**D**) and stimulation (**E**) groups. The feedback stimulation phase had reduced 91.68% and 89% paroxysm frequencies compared to the nonstimulation group and no-stimulation phases of stimulation group, respectively (*$p < 0.05$ for feedback stimulation vs. no-stimulation phases and nonstimulation group).

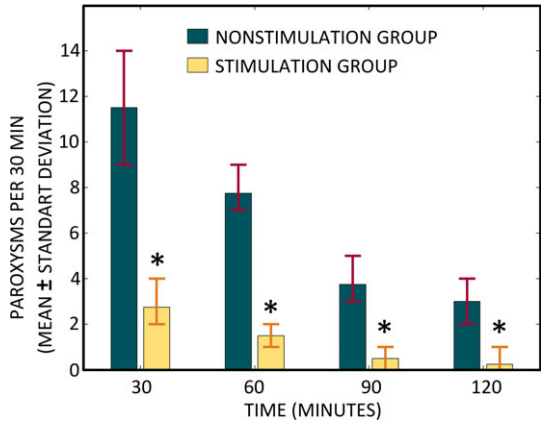


Figure 5.
Seizure suppression results in the acute condition. The stimulation group had a reduced 89% paroxysm frequency compared to the nonstimulation group (*$p < 0.05$ for stimulation vs nonstimulation groups).

typical increase in synchrony during the paroxysm was observed (Fig. 2A), but after a successful stimulation (in that there was no paroxysmal discharge right after) (Fig. 4A,B), the synchrony values did not change significantly from those values during the baseline, interictal periods. Hence, the combined results of the spectral and the synchrony analyses indicate that the stimulus upon the detection of the desynchronization just before a possible seizure effectively prevents the progress of the abnormal pattern of synchrony (mainly at δ, θ, and α bands) between the two hippocampi that develops into a paroxysm.

Discussion

This study presents a relatively simple and efficient approach for stopping the development of paroxysms based on a feedback stimulation at low frequencies for a few seconds after the detection of a seizure precursor, which effectively abolished all convulsive paroxysms in five of six rats treated with KA, and reduced by 89% paroxysm frequency after 4-AP administration. In total, the paradigm reduced seizure frequency by a minimum of 85% to a maximum of 100%, with 83% of the animals rendered seizure-free.

Efforts to alter epileptiform activity by direct electrical stimulation has had a long history.[26,27] After dynamic system theory was used to develop the framework of viewing epilepsy as a "dynamical disease,"[28,29] the seizure prediction/detection/anticipation and control has been investigated with unparalleled enthusiasm. Over the Past decade, many seizure prediction/detection algorithms have been proposed to anticipate seizures.[30–32] However, these algorithms were tested offline using high performance computers and the detection sensitivities ranged from 62% to 98%. Moreover, their detection delays *after* the seizure onset were 5–15 s, which obviously is too late to be used as a signal for perturbations that may abort the paroxysm before it starts. The performance of these algorithms may be improved by increasing computational power;[33] in such cases, the heavy computational load may slow down the close loop system and may be not suitable for real-time applications. The presented study used a relatively simple seizure precursor detection method, which had been shown previously in other studies on synchronization in epileptiform activity.[25,34,35] This method can be implemented in real time in a computer or a microchip.[21,36] The advantage of using phase synchrony as a precursor is that the detection performance is less affected by changes in the recordings over time. The time span of the experiments was at least 24 days for each animal, and a decrease in the amplitude of the hippocampal recordings was sometimes noted (a deterioration of chronic in vivo recordings over long periods is a common occurrence); however, the efficiency of the seizure precursor detection did not change over time, since our method relies on the phase of the oscillations and not on signal amplitudes. We propose that the phase synchrony analysis could be a generic precursor of paroxysmal events, and we have detected the phase desynchrony as well in patients with different syndromes.[22]

The stimulation paradigms currently used in patients are either continuous (or intermittent, such as vagus nerve stimulation) or responsive (such as responsive neurostimu-

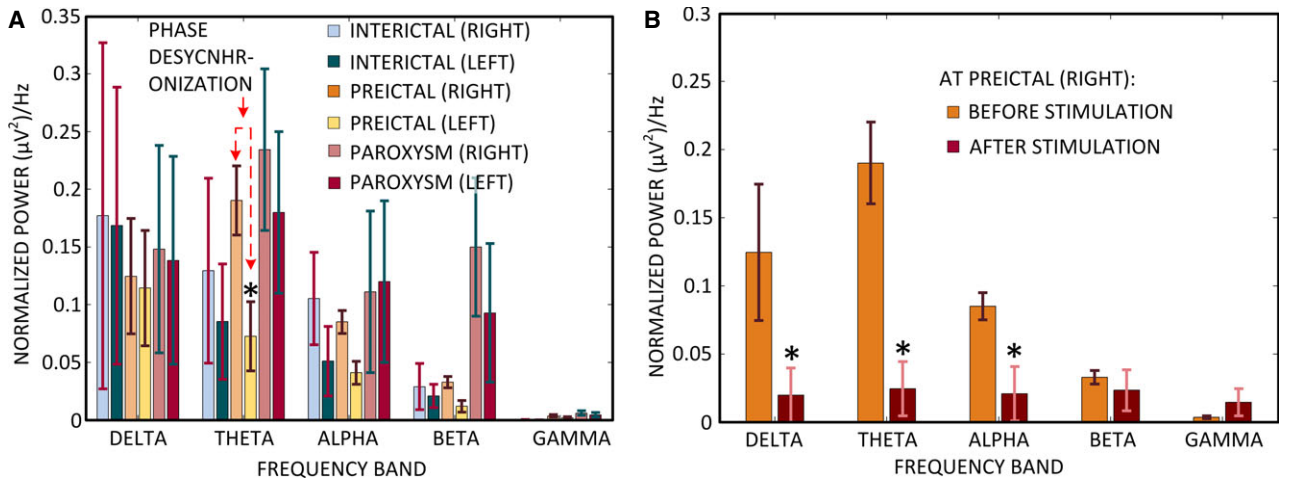


Figure 6.

(**A**) Mean power spectra (±STD) of the intracerebral recordings from the right hippocampus and left hippocampus during interictal, preictal, and paroxysm periods. There was a statistically significant divergence in power between both hemisphere signals during the preictal period at the theta band indicated in the figure (*$p < 0.05$ for right vs. left sides), whereas during the interictal and ictal times, the power fluctuates in a coordinated manner. These phenomena will be reflected in a decrease in phase synchrony during the preictal time. (**B**) Mean power spectrum (±STD) analysis before and after feedback stimulation during the preictal time. The power in the lower frequency bands diminishes as compared to that just before the stimulation, more prominently at theta frequencies (80% reduction in theta power; *$p < 0.05$ for "after" vs. "before" stimulation).

lation device) stimulation.[5,37,38] The optimal paradigm for the latter methods requires the anticipation of when a seizure may appear, to stimulate before the paroxysm materializes so that, the stimulation perturbs the transition to the seizure. This was our main purpose in this study. This paradigm derives from neurophysiologic and theoretical perspectives. Neurophysiologically, it is conceivable that an early relatively mild stimulation, before the full paroxysm develops, can perturb the incipient abnormal synchrony patterns that will later result in the full-blown seizure, whereas after the seizure has started stimulations of greater strengths may be required due to the robust high synchrony normally associated with seizures. In fact, in our experiments, we observed that the stimulation during this late time, when the high synchrony and the electrographic activity was already fully developed, did not abort the paroxysms. Hence, considering that the stimuli were efficient aborting the generation of paroxysms when triggered before seizure onset, it is tempting to conclude that the early abnormal patterns of synchrony before the full-blown paroxysm can be more easily perturbed than the high and robust synchrony during ictal events. Sometimes our method was unable to stop seizure occurrence due to an undetected precursor, but not to a failed stimulation; which underscores the great efficacy of our stimuli at arresting paroxysm onset when properly timed. On more theoretical grounds, it is well-known that a dynamic system close to a bifurcation point is more sensitive to perturbations. Because seizures have been considered dynamic bifurcations, or phase transitions,[22,38,39] relatively low strength inputs on approaching the critical point will be most able to alter the brain's activity, whereas after the bifurcation point has been crossed, the inputs to perturb the dynamics will need to be more intense.

A variety of stimuli/perturbations have been reported to halt in vivo paroxysmal activity, even including psychological methods.[40] Perhaps the reports in vivo more closely associated with our experiments are those that used proportional feedback using real EEG recordings as template for the intermittent (2 min on–2 min off) stimulation,[41] and the hippocampal stimulation at several frequencies in the KA model in rats that resulted not in suppression of seizures but rather in their shortening.[11] In the latter study, the frequency of the stimulation was found to be the most crucial parameter to shorten the seizure duration and, as in our study, the best response was obtained with low frequencies, 5 Hz in particular. In other studies, high frequency (130 Hz) stimulation, rather than low, has been reported to reduce seizure (>50% reduction in seizure frequency in about one third of rats with chronic seizures using a 1 min stimulation train), and the successful seizure control was associated with desynchronization of the brain signals, which parallels our observation of the temporal evolution of the phase synchrony after our stimuli that remained at baseline levels and not increasing as it does during the paroxysms.[42] Hence, basically, the stimuli perturb/abort the higher-than-normal synchrony that develops into paroxysms. Stimulation at 100 Hz has been reported to reduce certain aspects of seizures induced by 4-AP, in that the quasi-periodic activities, but not the high frequency spiking, were abolished.[43] High frequency stimuli (13–250 Hz) were effective as well for

seizure termination in another rodent model of seizures.[44] Other forms of nonperiodic stimuli have been used. For example, 21% of seizures were annihilated by single DC pulses[45]; and increase of generalized tonic–clonic threshold and desynchronization of abnormal seizure state formation were demonstrated using 4 Hz nonperiodic electrical stimulation.[46–48]

Recently, optogenetics method reported a 29.6% reduction in convulsive seizures after KA injection.[4] One advantage of optogenetic techniques is that a more precise stimulation can be achieved as it depends on where the responsive cells are located; however, these techniques rely on nontrivial genetic manipulations. Our results, using a simpler methodology that resulted in better seizure control (80–100%), indicate that there may not be the need to implement costly and difficult techniques. Our stimulation paradigm, a brief (5 s) low frequency train, was based on previous studies that indicated that precisely timed low frequency electrical pulses can stop the transition to paroxysms in vitro,[9] and other observations of reduction of paroxysms by low-frequency stimulation in vivo in rodents[10,49,50] and in patients.[12,13]

In conclusion, we propose that the notion of detecting abnormal patterns of synchrony in brain signals and implementing a feedback perturbation to alter/stop them and normalize the cellular collective activity can be used in the treatment of other syndromes. This, however, will most likely require a patient-specific approach, or personalized medicine, as it is known that the spatiotemporal characteristics of synchrony patterns are unique for each patient.[51]

Disclosure of Conflict of Interest

Roman Genov and Jose L. P. Velazquez have received support from the Natural Sciences and Engineering Research Council of Canada (NSERC); Jose L. P. Velazquez has received support from the Ontario Brain Institute (OBI); and the remaining authors have no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

1. Perez Velazquez JL, Wennberg R. Metastability of brain states and the many routes to seizures: numerous causes, same result. *Recent Res Dev Biophys* 2004;3:25–59.
2. Medeiros DD, Moraes MF. Focus on desynchronization rather than excitability: a new strategy for intraencephalic electrical stimulation. *Epilepsy Behav* 2014;38C:32–36.
3. Gionfriddo MR, Greenberg AJ, Wahegaonkar AL, et al. Pathways of translation: deep brain stimulation. *Clin Transl Sci* 2013;6:497–501.
4. Jiruska P, Powell AD, Deans JK, et al. Effects of direct brain stimulation depend on seizure dynamics. *Epilepsia* 2010;51:93–97.
5. Lockman J, Fisher RS. Therapeutic brain stimulation for epilepsy. *Neurol Clin* 2009;27:1031–1040.
6. Perez Velazquez JL. Brain, behaviour and mathematics: are we using the right approaches? *Physica D* 2005;212:161–182.
7. Sun FT, Morrell MJ. The RNS System: responsive cortical stimulation for the treatment of refractory partial epilepsy. *Expert Rev Med Devices* 2014;11:563–572.
8. Krook-Magnuson E, Armstrong C, Oijala M, et al. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nat Commun* 2013;4:1376.
9. Khosravani H, Carlen PL, Velazquez JL. The control of seizure-like activity in the rat hippocampal slice. *Biophys J* 2003;84:687–695.
10. Weiss SR, Li XL, Rosen JB, et al. Quenching: inhibition of development and expression of amygdala kindled seizures with low frequency stimulation. *NeuroReport* 1995;6:2171–2176.
11. Rajdev P, Ward M, Irazoqui P. Effect of stimulus parameters in the treatment of seizures by electrical stimulation in the kainate animal model. *Int J Neural Syst* 2011;21:151–162.
12. Tergau F, Naumann U, Paulus W, et al. Low-frequency repetitive transcranial magnetic stimulation improves intractable epilepsy. *Lancet* 1999;353:2209.
13. Child ND, Stead M, Wirrell EC, et al. Chronic subthreshold subdural cortical stimulation for the treatment of focal epilepsy originating from eloquent cortex. *Epilepsia* 2014;55:e18–e21.
14. Koubeissi MZ, Kahriman E, Syed TU, et al. Low-frequency electrical stimulation of a fiber tract in temporal lobe epilepsy. *Ann Neurol* 2013;74:223–231.
15. Hellier JL, Dudek FE. Chemoconvulsant model of chronic spontaneous seizures. *Curr Protoc Neurosci* 2005; Suppl. 31: Chapter 9:Unit 9.19.
16. Colom LV, Garcia-Hernandez A, Castaneda MT, et al. Septo-hippocampal networks in chronically epileptic rats: potential antiepileptic effects of theta rhythm generation. *J Neurophysiol* 2006;95:3645–3653.
17. Lieb JP, Engel J Jr, Babb TL. Interhemispheric propagation time of human hippocampal seizures. I. Relationship to surgical outcome. *Epilepsia* 1986;27:286–293.
18. Cramer C, Stagnitto ML, Knowles MA, et al. Kainic acid and 4-aminopyridine seizure models in mice: evaluation of efficacy of antiepileptic agents and calcium antagonists. *Life Sci* 1994;54:271–275.
19. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972;32:281–294.
20. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 7th, editor. New York: Academic Press; 2013.
21. Bagheri A, Gabran SR, Salam MT, et al. Massively-parallel neuromonitoring and neurostimulation rodent headset with nanotextured flexible microelectrodes. *IEEE Trans Biomed Circuits Syst* 2013;7:601–609.
22. Perez Velazquez JL, Garcia Dominguez L, Nenadovic V, et al. Experimental observation of increased fluctuations in an order parameter before epochs of extended brain synchronization. *J Biol Phys* 2011;37:141–152.
23. Mormanna F, Lehnertza K, Davidb P, et al. Mean phase coherence as a measure for phase synchronization and its application to the EEG of epilepsy patients. *Physica D* 2000;144:358–369.
24. Shannon RV. A model of safe levels for electrical stimulation. *IEEE Trans Biomed Eng* 1992;39:424–426.
25. Le Van Quyen M, Navarro V, Martinerie J, et al. Toward a neurodynamical understanding of ictogenesis. *Epilepsia* 2003;44(Suppl. 12):30–43.
26. Cooke PM, Snider RS. Some cerebellar influences on electrically-induced cerebral seizures. *Epilepsia* 1955;C4:19–28.
27. Cooper R, Winter AL, Winter AL, et al. Comparison of subcortical, cortical and scalp activity using chronically indwelling electrodes in man. *Electroencephalogr Clin Neurophysiol* 1965;18:217–228.
28. Milton J, Black D. Dynamic diseases in neurology and psychiatry. *Chaos* 1995;5:8–13.
29. Lopes da Silva F, Blanes W, Kalitzin SN, et al. Epilepsies as dynamical diseases of brain systems: basic models of the transition between normal and epileptic activity. *Epilepsia* 2003;44:72–83.
30. Liang SF, Chen YC, Wang YL, et al. A hierarchical approach for online temporal lobe seizure detection in long-term intracranial EEG recordings. *J Neural Eng* 2013;10:045004.
31. Schad A, Schindler K, Schelter B, et al. Application of a multivariate seizure detection and prediction method to non-invasive and intracranial long-term EEG recordings. *Clin Neurophysiol* 2008;119:197–211.
32. Aarabi A, Fazel-Rezai R, Aghakhani Y. A fuzzy rule-based system for epileptic seizure detection in intracranial EEG. *Clin Neurophysiol* 2009;120:1648–1657.

33. Chua KC, Chandran V, Acharya UR, et al. Application of higher order spectra to identify epileptic EEG. *J Med Syst* 2011;35:1563–1571.
34. Lachaux JP, Rodriguez E, Martinerie J, et al. Measuring phase synchrony in brain signals. *Hum Brain Mapp* 1999;8:194–208.
35. Rosenblum MG, Pikovsky AS, Kurths J. Phase synchronization of chaotic oscillators. *Phys Rev Lett* 1996;76:1804–1807.
36. Abdelhalim K, Jafari H, Kokarovtseva L, et al. 64-channel UWB wireless neural vector analyzer SOC with a closed-loop phase synchrony-triggered neurostimulator. *IEEE J Solid-State Circuits* 2013;48:2494–2510.
37. White HS, Wolf HH, Woodhead JH, et al. The National Institute of Health anticonvulsant drug development program: screening for efficacy. In French J, Leppik I, Dichter M (Eds) *Antiepileptic drug development. Advances in neurology*. Philadelphia, PA: Lippincott-Raven, 1998:29–39.
38. Pérez Velázquez JL, Frantseva MV. *The brain–behavior continuum-the subtle transition between sanity and insanity*. Singapore: World Scientific Publishing; 2011.
39. Breakspear M, Roberts JA, Terry JR, et al. A unifying explanation of primary generalized seizures through nonlinear brain modeling and bifurcation analysis. *Cereb Cortex* 2006;16:1296–1313.
40. Schmid-Schonbein C. Improvement of seizure control by psychological methods in patients with intractable epilepsies. *Seizure* 1998;7:261–270.
41. Colpan ME, Li Y, Dwyer J, et al. Proportional feedback stimulation for seizure control in rats. *Epilepsia* 2007;48:1594–1603.
42. Good LB, Sabesan S, Marsh ST, et al. Control of synchronization of brain dynamics leads to control of epileptic seizures in rodents. *Int J Neural Syst* 2009;19:173–196.
43. Ching J, Khan S, White P, et al. Long-term effectiveness and tolerability of vagal nerve stimulation in adults with intractable epilepsy: a retrospective analysis of 100 patients. *Br J Neurosurg* 2013;27:228–234.
44. Cymerblit-Sabba A, Schiller M, Schiller Y. Termination of chemoconvulsant-induced seizures by synchronous and asynchronous electrical stimulation of the hippocampus in-vivo. *Brain Stimul* 2013;6:727–736.
45. Osorio I, Frei MG. Seizure abatement with single dc pulses: is phase resetting at play? *Int J Neural Syst* 2009;19:149–156.
46. Medeiros Dde C, Cota VR, Vilela MR, et al. Anatomically dependent anticonvulsant properties of temporally-coded electrical stimulation. *Epilepsy Behav* 2012;23:294–297.
47. Mesquita MB, Medeiros Dde C, Cota VR, et al. Distinct temporal patterns of electrical stimulation influence neural recruitment during PTZ infusion: an fMRI study. *Prog Biophys Mol Biol* 2011;105:109–118.
48. Cota VR, Medeiros Dde C, Vilela MR, et al. Distinct patterns of electrical stimulation of the basolateral amygdala influence pentylenetetrazole seizure outcome. *Epilepsy Behav* 2009;14(Suppl. 1):26–31.
49. Toprani S, Durand DM. Long-lasting hyperpolarization underlies seizure reduction by low frequency deep brain electrical stimulation. *J Physiol* 2013;591(Pt 22):5765–5790.
50. Rashid S, Pho G, Czigler M, et al. Low frequency stimulation of ventral hippocampal commissures reduces seizures in a rat model of chronic temporal lobe epilepsy. *Epilepsia* 2012;53:147–156.
51. Schevon CA, Cappell J, Emerson R, et al. Cortical abnormalities in epilepsy revealed by local EEG synchrony. *NeuroImage* 2007;35:140–148.