The Control of Seizure-Like Activity in the Rat Hippocampal Slice

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ABSTRACT The sudden and transient hypersynchrony of neuronal firing that characterizes epileptic seizures can be considered as the transitory stabilization of metastable states present within the dynamical repertoire of a neuronal network. Using an in vitro model of recurrent spontaneous seizures in the rat horizontal hippocampal slice preparation, we present an approach to characterize the dynamics of the transition to seizure, and to use this information to control the activity and avoid the occurrence of seizure-like events. The transition from the interictal activity (between seizures) to the seizure-like event is aborted by brief (20–50 s) low-frequency (0.5 Hz) periodic forcing perturbations, applied via an extracellular stimulating electrode to the mossy fibers, the axons of the dentate neurons that synapse onto the CA3 pyramidal cells. This perturbation results in the stabilization of an interictal-like low-frequency firing pattern in the hippocampal slice. The results derived from this work shed light on the dynamics of the transition to seizure and will further the development of algorithms that can be used in automated devices to stop seizure occurrence.

INTRODUCTION

The brain has been conceptualized as a set of dynamic networks of interacting ensembles of cells, whose activity includes synchronized behavior. Characterization of the dynamical regimes that govern the transition from interictal activity to hypersynchronous ictal events (seizures) provides not only insights into the network mechanisms of collective neuronal oscillations and synchronous activity, but also raises the possibility of controlling this transition. Most of the current research in epilepsy and mechanisms of neuronal synchrony emphasizes the molecular and cellular aspects, but our understanding of the integrative functions of the brain, specifically the global collective dynamics leading to the pathological synchronization of neuronal networks, is poorly understood. The development of nonlinear time series analyses has fostered the application of these methods for the understanding of the underlying dynamics of complex biological systems (Elbert et al., 1994) and, in some cases, for the control of their activity (Christini and Collins, 1996; Garfinkel et al., 1992). Considering evidence that spontaneous interictal brain activity can be paced in vitro employing chaos control techniques (Schiff et al., 1994), as well as evidence from other systems where similar methods were used by taking advantage of the system's nonlinear dynamics (Shinbrot et al., 1993; Christini et al., 2001), we hypothesize that the transition from interictal to ictal activity can be controlled by adequately placed and timed minimal perturbations.

Nonlinear time series analysis of voltage traces from epileptic patients, as well as from animal epilepsy models, has revealed that seizure activity represents a nonlinear process with dynamics distinct from interictal states (Pijn et al., 1991; Lehnertz and Elger, 1995; Elger and Lehnertz, 1998; Lopes da Silva and Pijn, 1999). State transitions from interictal to ictal events have been inferred from the geometrical properties of the attractors reconstructed from the original voltage recordings, specifically from correlation dimension and Lyapunov exponents (Iasemidis and Sackellares, 1996; Lian et al., 2001). However, the need for stationarity and length of the recordings makes the interpretation of these values difficult (Rapp, 1994). We have recently used interpeak interval (IPI) delay plots to investigate the transition to seizure in human epilepsies (Perez Velazquez et al., 1999), using quantitative mathematical analyses that do not have data requirements as stringent as the methods mentioned above. These studies suggested that intermittency is a dynamical regime underlying human seizures, which together with other experimental and theoretical evidence, further indicates that sudden changes in physiological variables bring specific brain networks near a bifurcation point at which the transition to seizure takes place (Lopes da Silva and Pijn, 1999). Considering this information, it is conceivable to propose that the transition to seizure can be arrested by specific perturbations dictated by the known dynamics of the epileptogenic areas.

In this study, we sought to characterize the dynamics of the transition from interictal to ictal activity and to use this knowledge to control the activity thereby preventing seizure occurrence. We use an in vitro seizure-like model that is characterized by spontaneous recurrent interictal activity that develops into seizure-like events (SLEs), considered to be a model of status epilepticus (Rafiq et al., 1993, 1995). The transition from interictal to ictal activity was marked by the sudden and transient stabilization of a high-frequency hypersynchronous steady state. Brief direct electrical

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stimulation halted the transition from preictal to ictal activity, by forcing the stabilization of an interictal-like steady state, as opposed to the hypersynchronous ictal state.

MATERIALS AND METHODS

Brain slices and solutions

Male Wistar rats (18- to 25-days old) were anesthetized with halothane and decapitated. The brain was quickly dissected and maintained in ice-cold artificial cerebrospinal fluid (ACSF) for 4–5 min. Brain slices were cut according to Rafiq et al. (1993, 1995). Briefly, the dorsal cortex of each hemisphere was cut parallel to the rostral/caudal axis and glued dorsal surface down to an aluminum block, caudal end facing the blade. The block was secured at a 10–12° angle, and brain slices 400-μm thick were obtained using a vibratome. After sectioning, slices were maintained at room temperature in oxygenated ACSF for at least one hour before recording. The ACSF contained in mM: NaCl, 125; KCl, 2.5; NaH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 1.5; $NaHCO_3$, 25; glucose, 10, pH 7.4 when aerated with 95% O_2/5% CO_2. Osmolarity was 300 $\pm$ 5 mOsm. The superfusing ACSF was switched immediately after the slice was placed in the recording chamber to one containing 5 mM K^+ and 0.5 mM Mg^{2+}, to ensure the development of epileptiform activity.

Electrophysiological recordings

For data acquisition, slices were transferred to a superfusion chamber maintained at 35°C (Model PDMI-2, Medical Systems Corp.), and superfused with the solution indicated above (4 ml/minute). Electrical signals were recorded using an Axoclamp2A and/or Axopatch200B amplifier (Axon Instruments, Foster City, CA), with the lowpass filter setting at 1–3 kHz or 5 kHz respectively. Data were stored and analyzed using the PCLAMP software (Axon Instruments, Version 6.3) via a 12-bit D/A interface (Digidata 1200, Axon Instruments), or filtered at 1 kHz, digitized at 88 kHz, and stored on video tape using a digital data recorder VR-10 (Instrutech Corp., Port Washington, NY) for later playback and analysis.

Extracellular orthodromic electrical stimulation (100-μs pulse width) was delivered via a bipolar stimulating enamel-insulated Nichrome electrode, using a Grass square pulse stimulator (S88K, Astro-Med Inc., West Warwick, RI). The intensity of the stimulation was fixed for each experiment but varied between slices, adjusted to the value that evoked a field potential event recorded in the CA1 area when stimulating the mossy fibers. An extracellular recording electrode was filled with ACSF and placed in the CA1 and/or CA3 cell body layer.

Analysis methods

The methods used for the mathematical analysis of the voltage traces are detailed in Perez Velazquez et al. (1999), specifically the construction of the first-return interpeak interval scatter plots and the approximation by an inverted polynomial. Our peak detection algorithm that is used to construct a time series of IPIs is a graphical-based software written in Visual Basic (Microsoft Corp., Redmond, WA), and selects peaks based on amplitude and width criteria. These criteria depend on and are optimized for each data set (Khosravani, Carlen, and Perez Velazquez, unpublished observations). Baseline drift (DC shift) was subtracted using a windowed moving-average filter. Scatter plots of the IPI values were constructed by plotting one IPI versus the next. IPIs were measured in seconds. A first-return one-dimensional mapping function was obtained by approximating the scatter plot with a nonlinear equation. For curve fitting we use the standard nonlinear least-squares routine, the Levenberg-Marquardt method (Press et al., 1999), which minimizes a least-squares type of function through iterations. Specifically, the value of χ^2, which represents the sum of the squares of the deviations of the theoretical curve from the experimental data points, is minimized. The scatter IPI plots were then approximated by the best fit to an algebraic equation (one-dimensional map). Once obtained, the analysis of the mapping function was performed according to the classical methods in nonlinear dynamics (Guckenheimer and Holmes, 1983; Berge et al., 1984; Hoppensteadt and Izhikevich, 1997). Maple V software (Waterloo Maple Inc.) was used to solve differential and algebraic equations. The Origin software package (MICROCAL Software Inc., Northampton, MA) was used for data conversion and analysis.

RESULTS

Dynamical regimes of the spontaneous activity in the hippocampal slices

Under our recording conditions, >90% of the hippocampal slices display spontaneous interictal activity (Fig. 1), and in 47.6% (30/63) of those, this activity develops into SLEs (Fig. 2). The SLEs in these slices are characterized by high-frequency bursts (10–30 Hz) with relatively high interburst frequency (2–8 Hz) at the start, which becomes lower toward the end of the SLE (Figs. 2–4). The bursting activity suddenly disappears for a few minutes, possibly representing postictal depression, and then interictal activity resumes, which will eventually result in another SLE (see Fig. 5 for consecutive recordings).

To determine the dynamics of the transition to seizure, we use recursive or first-return plots, a time-delay embedding technique (Berge et al., 1984; Takens, 1981; Sauer, 1994), where the system's dynamics is simplified by reducing its dimension. This is achieved by constructing one-dimensional return maps (Berge et al., 1984). The time-delay

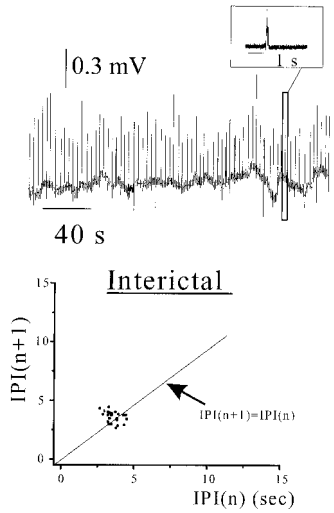

FIGURE 1 Spontaneous interictal-like activity in the hippocampal slice. The trace corresponds to a field recording in the CA1 layer, in a slice that did not manifest SLEs. The first-return interburst interval plot of spontaneous interictal activity is shown below. Note the cluster of points at ~0.3 Hz (3–4 s), indicating that the activity in this slice had a periodic low-frequency firing steady state. This periodic activity persisted throughout the recording period (~1 h).

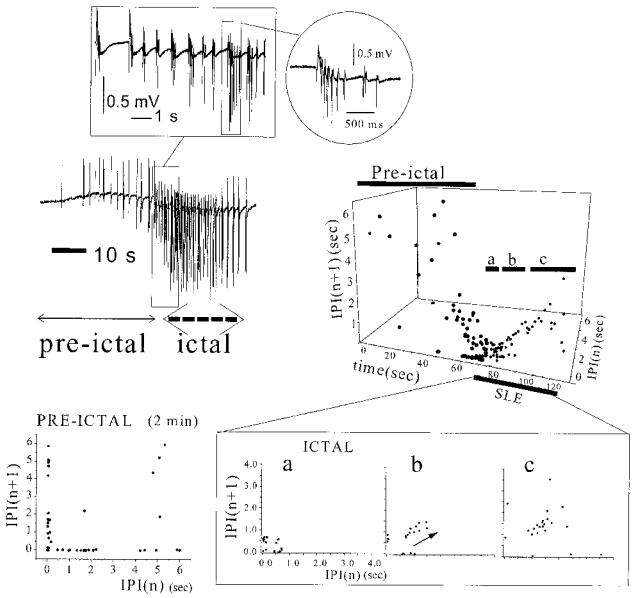

FIGURE 2 Spontaneous transition from interictal to ictal (seizure-like) activity. Field potential recording in the CA1 layer showing the transition between preictal activity and the spontaneous SLE (ictal). Insets depict details of ictal burst events. First-return IPI scatter plots of 2 min of preictal activity (*left graph*), and the SLE (*ictal*, each graph corresponds to the labeled epochs within the SLE, *a, b and c*, each ~12 s of activity). Note the progression of the IPIs on the diagonal in b and c, marked by an arrow, characteristic of intermittency. The three-dimensional scatter plot reveals the time evolution of the successive IPIs.

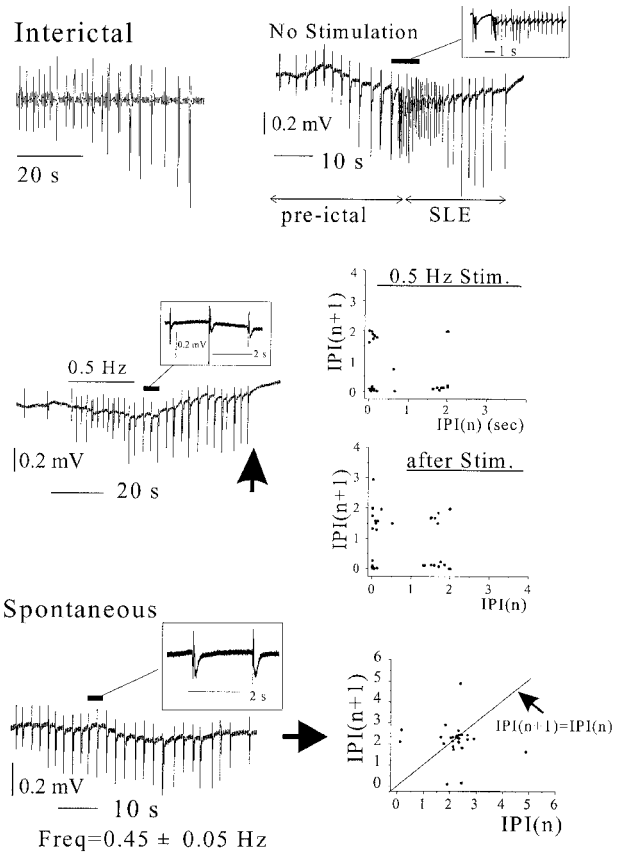

FIGURE 3 Perturbation of the spontaneous activity in hippocampal slices. Periodic forcing at 0.5 Hz stops the transition to seizure. Field potential recording in the CA1 layer displaying the interictal activity at the beginning of the recording, which lasted 6–7 min before the recurrent appearance of SLEs. Note that the interictal activity started at low frequency, 0.3–0.5 Hz, suggesting that this state could be forced to stabilize, as shown below. Right trace shows the spontaneous transition from preictal to the SLE, in the absence of control stimulation. Inset depicts the initial eight seconds of the SLE, at ~3 Hz. Middle trace shows a 20-s 0.5 Hz control stimulation applied to the mossy fibers prevents the transition to the SLE, and the activity finally ceases (*arrow* at the end). The IPI plots (*right*) show the clusters of points during periodic forcing (*upper plot*, clusters at ~2 s), and after the perturbation (*lower plot*). Notice that the activity continues at ~0.5 Hz after the 20-second perturbation. The points are not all situated on the diagonal because the peak detection algorithm detected two peaks in each field potential event (see *inset traces*), hence the sequence of intervals long–short–long (2–0.1 s). Lower trace shows 5–7 min after the low-frequency perturbation, the spontaneous activity resumed in this slice, oscillating near the forced frequency (average 0.45 Hz). This slice did not have any other spontaneous SLE during the 1-h recording period, even though these could be evoked by high-frequency trains.

embeddings extract information about the topological structure of the attractor and the underlying dynamics (Packard et al., 1980). This concept is derived from Takens' delay embedding theorem (Takens, 1981) that, briefly stated, says that the attractor reconstructed by a time-delay plot of an observable (such as the IPI in our case) is equivalent to the original multidimensional attractor that portrays the system's dynamics. In other words, the time-delay map provides us with the basic information of the underlying dynamical regimes of the original system.

We measured extracellular field potentials in the CA1 or CA3 areas and used the time interval between successive peaks as our state variable (Sauer, 1994), and constructed the first-return IPI scatter plots by plotting IPI_{n+1} versus IPI_n (Figs. 1–4 and 6), as described previously (Perez Velazquez et al., 1999). These plots can be considered as Poincare sections (Le Van Quyen et al., 1997). This method allowed for the identification of the dynamical regime involved in the transition to the SLE and facilitated the selection of an appropriate perturbation (electrical stimuli described below) to avoid the transition from interictal to seizure development. In this time-delay representation, periodic behavior appears as a fixed point (or steady state) located on the bisectrix, or identity map where $IPI_{n+1} = IPI_n$. Inspection of the interictal activity, observed in the slices that did not present spontaneous SLEs (52.4% of the slices, 33/63), reveals regular activity at low frequencies, with an average of 0.35 ± 0.15 Hz (range 0.13–0.55 Hz). This regular, periodic activity, is represented as a cluster of points near the identity map (Fig. 1), and is termed limit cycle in dynamical terminology. Although ideally only one point on the diagonal should be expected, the set (cluster) of points is due to the variability of the biological preparation as opposed to more precise theoretical simulations. The cluster of points

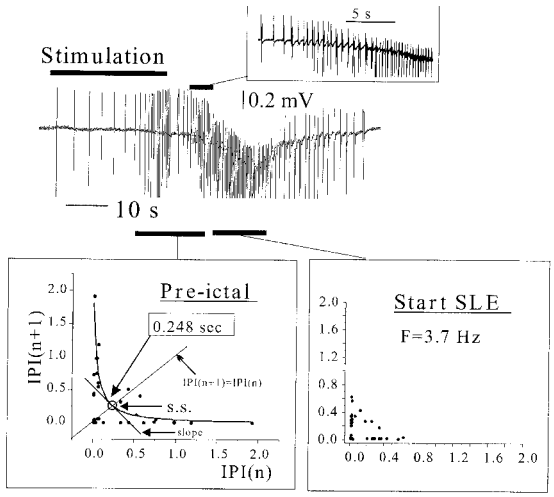

FIGURE 4 Induction of SLEs by mimicking the preictal burst dynamics. Trace shows the perturbation in a slice that did not present spontaneous SLEs. In this case, reproducing the first-return IPI scatter plot of the transition to the SLE (as seen in slices that showed spontaneous SLEs) by a 25-s extracellular stimulation to the mossy fibers (notice this time the stimulation is aperiodic as we tried to simulate the spontaneous preictal activity), the activity triggered an SLE. Note that the ictal event started after the stimulation was turned off (*inset* shows the beginning of the SLE). The preictal IPI plot (using the IPIs for the 12 s preceding the SLE, shown by the *black horizontal bar*) shows the fitting of the scatter plot to an inverted polynomial $(a + bX + cX^2)^{-1}$, where $X =$ IPI(n) and $a = 0.58$, $b = 12.3$, $c = 7.5$. The steady state (*s.s.*) is the crossing with the diagonal, at 0.248 s ($\sim$4.0 Hz), and the slope at this point is ~ -1 (-0.98), indicating a metastable (flip bifurcation) state. The start of the SLE (*right IPI plot*, initial 15 s of the SLE) shows the high-frequency activity, with average of 3.7 Hz, which can be interpreted as the transient stabilization of the preictal flip point at $\sim$4 Hz.

indicates that the activity of these slices has a stable limit cycle, also inferred from the sustained, long-term bursting activity at the specific frequency, as shown in the field potential recording of Fig. 1. However, the transition to the SLE is characterized by a more continuous plot with short–long intervals (Figs. 2 and 4), long between the bursts and short between the peaks on each burst (see inset in Fig. 2 for details of ictal events during an SLE). Multiple peaks (normally two) were also observed on the interictal bursts and hence the short–long sequence that is evident, for example, in the plots of Figs. 3 and 6, *A* and *C*. The IPIs for the preictal state preceding the SLE are distributed along an underlying L-shaped curve (Figs. 2 and 4). This distribution can be modeled by a recursive relation, that produces a return map $\mathrm{IPI}_{n+1} = f[\mathrm{IPI}_n]$, where f is the function that determines the one-dimensional map (Fig. 4) and can be considered to represent a global nonlinear model (Decroly and Goldbetter, 1987; Hoppensteadt and Izhikevich, 1997). The nature of the scatter plot corresponding to the transition from interictal activity to the SLE is suggestive of the presence of low-dimensional dynamics (Garfinkel et al., 1992; Braun et al., 1997). The obtained plot can be best approximated by a nonlinear least-squares fit of the scatter plot (Perez Velazquez et al., 1999) as described in Materials and Methods, to an inverted polynomial, $(a + bX + cX^2)^{-1}$, where $X =$ IPI, which best represents the one-dimensional mapping function f and defines the recursive relation $X_{n+1} = (a + bX_n + cX^2{}_n)^{-1}$. One-dimensional maps have been used in other systems to study the dynamical regimes (Roux, 1983; Glass et al., 1983; Decroly and Goldbeter, 1987). These maps are valuable tools because they allow for a discrete representation of the original time series that simplifies the mathematical study, in addition to the solid theory behind one-dimensional maps (Berge et al., 1984; Pomeau and Manneville, 1980; Collet and Eckmann, 1980; Guckenheimer and Holmes, 1983; Hoppensteadt and Izhikevich, 1997). The three-dimensional plots (Figs. 2 and 6) allow for an appreciation of the temporal evolution of successive IPIs.

The stability analysis of the features of this mapping function reveals, initially, the presence of a fixed point, or steady state, representing high-frequency hypersynchronous neuronal firing, the SLE. As depicted in Fig. 4, this fixed point can be determined geometrically by the intersection of the map with the bisectrix, or it can be obtained analytically from the mapping function: $X_{n+1} = (a + bX_n + cX^2{}_n)^{-1}$, solving for $X_{n+1} = X_n$, which yields $X = 0.248$ s for the parameter values (a, b, and c) that best approximated the plot, shown in the figure legend. The slope of the map at this fixed point determines its stability (Berge et al., 1984) and in this case is close to -1 (-0.98), indicating the presence of a metastable state. A fixed point with these features is termed a subharmonic or flip bifurcation. The concept of bifurcation is central in nonlinear dynamical systems theory, and can be interpreted as a change in the qualitative properties of the dynamics. This particular type of flip (subharmonic) bifurcation leads to type III intermittency or to a subharmonic cascade, depending on a condition satisfied by the first terms of the Taylor series expansion of the map. Specifically, if the expression $(d^2f/dx^2)^2/2 + (d^3f/dx^3)/3$ is less than 0, the flip is called subcritical and leads to type III intermittency (Hoppensteadt and Izhikevich, 1997). It was determined that type III intermittency is the dynamical regime underlying some human seizures (Perez Velazquez et al., 1999). For the parameter values shown in Fig. 4, the value of the above expression is <0 (-0.69), and therefore the flip bifurcation is subcritical, indicating that intermittency is the dynamical regime underlying these specific SLEs. Note also the progression of ictal IPIs on the bisectrix, in Fig. 2, which is again suggestive of intermittency (Berge et al., 1984; Hoppensteadt and Izhikevich, 1997). For other parameter values the flip may be supercritical. The main point to stress here is the presence of the bifurcation point that determines a change in the system's behavior that could be responsible for the transition to the SLE. Thus, according to our interpretation, the SLE is seen as the transient (SLEs last 30–60 s under our conditions) stabilization of the metastable flip bifurcation point.

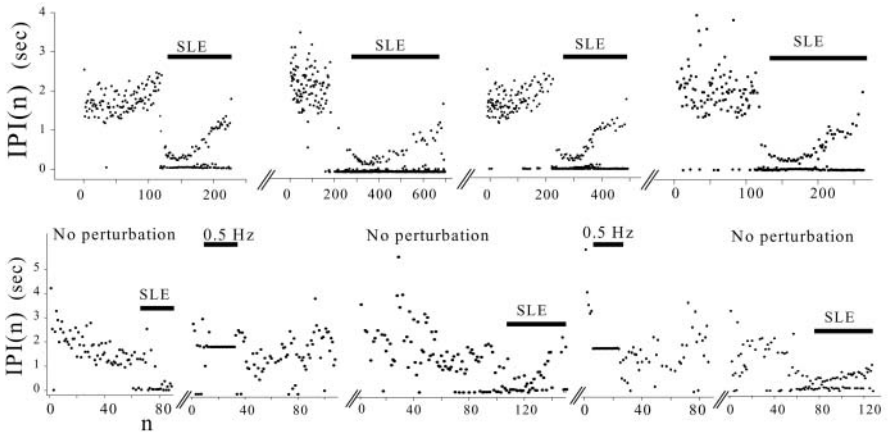

FIGURE 5 Control of the transition to the SLE by low-frequency periodic forcing. Graphs represent the IPIs versus the peak number (*n*), corresponding to the continuous spontaneous activity in a slice that had SLEs. Upper row shows four successive transitions from preictal to SLEs. Note that the IPIs become smaller at the start of the SLE and gradually increase during its progression. Lower row shows five successive recordings in another slice demonstrating that 0.5 Hz periodic forcing stimulation stops the transition to the SLE. Left plot (*No perturbation*), typical transition to the SLE, the IPIs becoming shorter. Next, stimulation at 0.5 Hz aborts the transition to the SLE. No perturbation after the previous success results in another SLE. Periodic 0.5 Hz stimulation aborts again the transition. Next, no perturbation results in another SLE.

The control of the transition to the SLE

With the preliminary knowledge, mentioned above, about the dynamics of the transition to seizure, we explored the possibility that the transition to the ictal event could be perturbed and hence prevented. In this framework, seizures, or SLEs, can be thought of representing the transient stabilization of steady states of high frequency hypersynchronous firing of large neuronal populations. Hence we hypothesized that, by stabilizing another steady state, for example low-frequency firing as occurs during interictal activity (Fig. 1), then the occurrence of the SLE could be avoided. As mentioned, an almost equal number of hippocampal slices (53%) had interictal activity that did not result in SLEs, with an average interictal bursting frequency of 0.35 $\pm$ 0.15 Hz. This strongly indicates the presence of a low-frequency interictal-like stable state (or one that could be forced to stabilize) in the activity of the hippocampal slices under these conditions. We therefore endeavored to stabilize the interictal-like steady state, using brief periodic forcing stimulation, to prevent the transition to the SLE in slices capable of spontaneous seizure-like activity. We performed an empirical study where the efficacy of several perturbation paradigms was evaluated in their ability to disrupt SLE development, by enforcing the interictal-like firing pattern. The effectiveness of the perturbations was evaluated with spatial and temporal considerations. Two different locations for the extracellular perturbations in the hippocampal circuitry were tried: mossy fibers and Schaffer collaterals. Further, the timing of the stimulation in relation to the natural progression of the spontaneous activity was investigated.

Considering all this information, we applied, in slices that had SLEs, brief (20–50 s) low-frequency (0.5 Hz) electrical stimuli to the mossy fibers, to force the interictal-like state (Fig. 3). The intensity of the extracellular stimulation was the

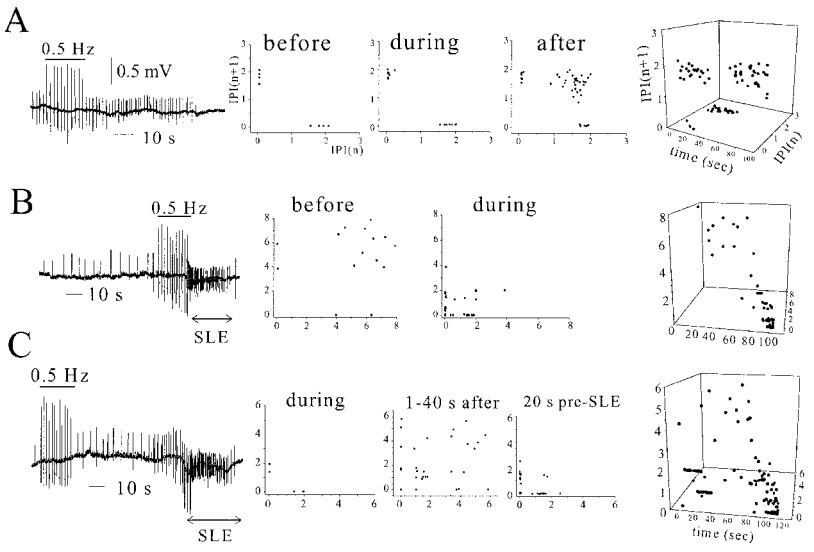

FIGURE 6 Timing of the low-frequency perturbation is crucial to stop the transition to seizure. (*A*) Field potential recording in the CA1 layer showing that low-frequency forcing (0.5 Hz) stops seizure occurrence if applied at the time when the spontaneous preictal activity is near that state, as depicted in the left-hand side IPI scatter plot (*before*), just before the perturbation. Middle plot shows forced activity during the 20-s stimulation, delivered to mossy fibers (clusters at 2 s). The activity continued after the perturbation at the forced frequency (*right plot*), with the cluster of IPIs around 2 s. The spontaneous activity ceased 50–60 s after the perturbation, as shown in the field recording on the left. The three-dimensional plot shown at the right-hand side depicts the time evolution of the IPIs. (*B*) Applying similar perturbation in the same slice when the preictal activity was irregular and not close to 0.5 Hz did not stop the occurrence of the SLE. The IPIs plots correspond to the activity before and during the stimulation. The three-dimensional graph depicts the appearance of the high-frequency activity that marks the beginning of the SLE. (*C*) Applying the 0.5 Hz perturbation (20 s, same location and intensity as in *A* and *B*) before the appearance of the preictal activity does not stop the progression toward the SLE. The activity becomes irregular right after the perturbation (*1–40 s after plot*) and develops into the more organized, higher frequencies, IPIs shown in the graph 20 s before the SLE.

minimal needed to evoke a population spike recorded in the CA1 area. The result of this perturbation is shown in Figs. 3, 5, and 6. The transition from interictal to ictal activity was aborted by a 0.5-Hz perturbation in seven of nine slices, in 68% (19/28) of the times (significantly different with a 99.9% confidence level, $p < 0.001$, as compared with unperturbed slices, χ^2-test). The success rate was lower when other frequencies were tried: 22.2% at 0.3–0.4 Hz ($p < 0.05$), and no control was achieved using less than 0.3 Hz. Similarly, higher frequencies, in the range 0.8–20 Hz, had no effect (4% success rate, 1/25, $p = 0.78$) or triggered SLEs. Random or white noise stimulation was equally ineffective (10%, 4/37, $p = 0.56$). When the stimulation/perturbation was applied to other hippocampal areas (Schaffer collaterals, entorhinal cortex), no control was ever achieved ($n > 25$). Another important variable is the intensity of the stimulation applied to the mossy fibers (range 200–800 μA), which had to be the minimal sufficient to evoke a field potential in the CA1 area, otherwise no control could be accomplished ($n = 20$). In general, the evoked synaptic responses were not attenuated by our short low-frequency stimulation: the average amplitude of the evoked response at the end of the perturbation (20–50 s) was 96.6 $\pm$ 4.7% of that measured at the start. Hence, synaptic depression may not account for the observed effects.

Successful control was also a function of the timing of the perturbation. Specifically, during the time when the spontaneous preictal activity had a frequency ~0.5 Hz (Fig. 6) control was achieved more predictably. The effect we have described can be called periodic forcing of the neuronal activity. We interpret it as the forced stabilization of a metastable state representing low-frequency interictal activity, thereby successfully avoiding the transition to the SLE which, without perturbation, occurs via the stabilization of the flip fixed point. This point is elaborated in the Discussion section. Indeed, the first-return IPI scatter plot of slices that presented interictal activity without spontaneous SLEs (Fig. 1) had similar features as those corresponding to the successful control by our perturbation shown in Figs. 3 and 6 *A*. It is important to stress that, by adequately timed perturbation, we mean that the start of the perturbation should be when the spontaneous interictal activity is close to the frequencies around 0.5 Hz, as it does not seem to be related to the timing relative to the start of the SLE. For example, in unsuccessful attempts, the timing of the start of the low-frequency forcing relative to the SLE onset had a wide range, between 12 and 110 s (average 46.5 $\pm$ 27.8 s, $n = 40$).

Although brief low-frequency forcing was able to halt the transition to the SLE, we could also trigger SLEs in slices that did not present them spontaneously, by recreating the first-return IPI plots as observed in slices with spontaneous SLEs, corresponding to the preictal activity leading to the SLE (see Fig. 2). This is demonstrated in Fig. 4, where the scatter IPI plot during and immediately after the stimulation is approximated to the mapping function mentioned above. Analysis of this one-dimensional map reveals the existence of a flip bifurcation point at ~4 Hz, as described above. The SLE starts with an interburst interval near 4 Hz. Hence, by recreating the dynamics of the transition to seizure we were able to trigger the SLE.

DISCUSSION

We have used an in vitro model of status epilepticus (Rafiq et al., 1993, 1995), to gain insight into the dynamics of the transition to seizure and used the system's own dynamics to stop seizure generation. The dynamical regimes derived from the IPI recursive, or first-return, plots, suggest that the spontaneous activity in these slices exhibits several steady states. Two of these are most prominent, one representing periodic, or limit-cycle, behavior at relatively low frequencies, during interictal activity. The other represents high-frequency hypersynchronous firing that marks the start of the SLE. Periodic forcing by short-duration electrical perturbations arrested the transition to the SLE by stabilizing the interictal-like firing pattern.

The self-sustained epileptiform activity in this in vitro model resembles human status epilepticus. Characteristic of status epilepticus is the short interval between recurrent ictal events, and the transition from simple to complex epileptiform discharges preceding the seizure onset, phenomena observed in this in vitro slice model (see also Rafiq et al., 1995). However, because the slice obviously simplifies the whole cortical-limbic neuronal circuitry involved in epileptic patients, the in vitro observations have to be interpreted with caution.

As opposed to model-based classical feedback control methods (Wiener, 1961), which require a detailed analytical model of the system under study, in model-independent chaos control techniques one studies the nonlinear dynamical structure and then uses this knowledge to develop ways of directing the system's activity toward the desired state by acting on a variable. The lack of a requirement for accurate analytical models of the systems under control has obvious advantages, because they are difficult to develop for complex biological phenomena. Model-independent chaos control methods (Ott et al., 1990) have been applied to alter the behavior of physical (Shinbrot et al., 1993) and physiological (Christini and Collins, 1996, 1997a; Christini et al., 2001; Garfinkel et al., 1992; Schiff et al., 1994; Hall et al., 1997) complex systems. Variants of these methods have been designed to stabilize flip-saddle unstable fixed points (Christini and Collins, 1997b). Our modest goal in this study was to stabilize one possible unstable (or metastable) steady state of the system, while ignoring the complex stable or unstable manifold calculations needed in other control paradigms (Ott et al., 1990). Hence, by empirical study of the effects of the perturbations near the desired steady state (in our case the frequency of bursting that is present during interictal activity) we attempted to stabilize what can be

considered a periodic orbit of the possibly complex (chaotic?) attractor for the ensemble activity.

We assume that the time series of spikes (peaks) is an expression of the process that governs network activity (Sauer, 1994). Therefore the study of IPI plots provides insights into population dynamics, as has been shown for studies of the dynamics of electroreceptor activity in fish (Braun et al., 1997), in mammalian brain (Schiff et al., 1994; Di Mascio et al., 1999), and in cardiac tissue (Christini and Collins, 1996; Garfinkel et al., 1992; Christini and Collins, 1997a). The IPI scatter plot corresponding to the transition from preictal to ictal activity has structure (e.g., is not space-filling), which is indicative of chaos or low-dimensional dynamics, as shown in other physiological systems (Garfinkel et al., 1992; Braun et al., 1997). However, determination of chaotic dynamics from time series is a controversial issue (Rapp, 1994) and was not the purpose of our study. By approximating the first-return plot to an algebraic equation, the one-dimensional map, one can obtain further quantitative insights into the dynamical regimes as these maps represent the essential dynamic properties. This is a common method that has been applied to a large number of physical, chemical (Roux, 1983), and biological systems (Glass et al., 1983; Berge et al., 1984; Perez Velazquez et al., 1999). The dissipative nature of brain activity justifies the use of one-dimensional maps. An interesting practical application of these maps has been shown recently in the control of cardiac arrhythmia in humans using an adaptive nonlinear control method (Christini et al., 2001). The dynamical characteristics are extracted from the geometry of the fixed points (i.e., steady states) in these maps, as proposed by other investigators (Kelso and Fuchs, 1995), specifically with regard to their stability and bifurcation characteristics. We find that flip, or subharmonic, bifurcations occur in human seizures (Perez Velazquez et al., 1999) and in the in vitro slice preparation shown here. Bifurcations are conceptualized as qualitative changes in the system's dynamics (Hoppensteadt and Izhikevich, 1997; Titcombe et al., 2001). Specifically, the unraveling of the possible bifurcations that take place in epileptiform activity may add fruitful insights to understand (and control) the transition from interictal to ictal activity (Lopes da Silva and Pijn, 1999).

In general, to stop seizure occurrence we must know where, how, and when to apply the perturbation. In our experiments, the location of the stimulating electrode was chosen to be the mossy fibers based on previous observations that the CA3 neurons pace the interictal firing, leading to the recruitment of more cells that bring about the SLE (Perez Velazquez and Carlen, 1999). Low-frequency forcing was selected by inspection of the activity in slices with no spontaneous SLEs (Fig. 1), suggesting the presence of an interictal-like stable state. The timing of the perturbation was inferred from the proximity to the low-frequency interictal-like firing stable state (Fig. 6). When the system is close to that steady state (or near the stable manifold for the fixed point, in dynamical language) our perturbation effectively forces the system to stabilize into that low-frequency state, aborting its transition to the hypersynchronous high-frequency seizure. Periodic forcing can link weakly coupled oscillators (Hoppensteadt and Izhikevich, 1997), and many brain areas are periodically or stochastically forced (septum-hippocampus, thalamus-cortex). In support of our observations we note that evidence exists that low-frequency electrical stimulation (1 Hz) inhibits the development of amygdala kindled seizures in rats (Weiss et al., 1995; Velisek et al., 2002), and low-frequency transcranial magnetic stimulation (0.33 Hz) also alleviates seizure disorders in human patients (Tergau et al., 1999). Also using in vitro preparations, low-frequency periodic pacing stimulation has been shown to suppress the tonic phase of SLE generation in the high-potassium seizure model (Jerger and Schiff, 1995), and in the 4-aminopyridine seizure-like model (Barbarosie and Avoli, 1997). However, it is possible that, in other seizure models, different stimulation paradigms are effective, as demonstrated in the suppression of epileptiform events by high-frequency sinusoidal fields in hippocampal slices bathed in low-calcium or in the presence of picrotoxin (Bikson et al., 2001). Adaptive electric fields have also been successfully applied to induce or ameliorate seizure-like events in the hippocampal slice (Gluckman et al., 2001).

Our electrical perturbations and the collective phenomena here reported should be reflected at the cellular level. A possible cellular mechanism that could account for the halting of the SLEs after short periodic forcing is the phenomenon of synaptic depression. However, our successful perturbations were too short (20–50 s) to induce depression of synaptic responses, for which longer times are needed, for example 1 Hz for 15 min (Chen et al., 2001). The investigation of these more specific mechanisms was not the purpose of our study.

Our results provide a framework to understand the dynamics of the transition to seizure and for the possible control of this progression and may shed light on possible dynamical mechanisms for the activity of neuronal circuits, specifically transient stabilization of metastable states (Lopes da Silva and Pijn, 1999). A set of coupled nonlinear oscillators has an infinite number of ways of performing, but within certain conditions it tends to stabilize into specific states of activity (attractor), and remains there until perturbed. Switching from one to another attractor is called a bifurcation, which requires a parametric change in the system. Our studies do not shed light into specific cellular or molecular targets altered by the perturbations, which may be involved in the transition to seizure. We submit the idea that brain activity, which could be displaying complex dynamics (e.g., chaotic) during nonseizure epochs, stabilizes transiently in specific metastable periodic orbits within the chaotic attractor, which represent interictal or ictal activity. This concept can be extended to the generation of brain rhythms. The particular sequence of orbits or transitions can

achieve intermittent stability through intrinsic mechanisms, for example population synchronization (Rafiq et al., 1993, 1995; Perez Velazquez and Carlen, 1999). The transient synchronous stabilization of unstable states is a concept that has also been inferred from experiments using sympathetic neuronal networks possessing many metastable states (Chang et al., 2000), where transient phase-locked states become stable at the population level. These metastable states in the neuronal population are achieved through linear and nonlinear interactions. These investigators propose that this metastability affords a variety of the network responses to distinct stimuli. During dynamical regimes governed by intermittency, transient stabilization of several metastable states occurs without the need of strong external stimuli. Indeed, transitions to periodic behavior (such as that found during seizures) are often realized via intermittency in chemical and physical systems (Kiss and Hudson, 2001). Hence, we propose that similar transitions via intermittency are involved in the generation and termination of seizures. The notion that electrical stimulation changes the stability of brain oscillations has also been supported in other studies where deep brain stimulation is used to treat Parkinsonian tremor; specifically, the network dynamics were found to change via a Hopf bifurcation (Titcombe et al., 2001). As opposed to equilibrium dynamics where functions (such as free energy, or entropy) can be optimized, in nonequilibrium systems (such as the brain) a fundamental concept is the stability of discrete steady states. We propose that, in some simple cases such as the brain slice preparation shown here, it is possible to take advantage of this idea and alter the stability of specific steady states. The use of nonlinear analysis is justified as it provides additional insights that classical time-frequency analyses are incapable of providing, because these are not sensitive to nonlinear temporal trends (Kantz and Schreiber, 1997). Application of linear methods to signals generated by nonlinear systems may result in spurious conclusions, such as time series that appear random when indeed determinism is present (Vandenhouten et al., 2000). In general, Fourier decomposition and similar methods are not adequate to reveal, for example, chaotic dynamics, and the exact nature of the bifurcations and stability of fixed points is much harder to grasp by looking at power spectra, for example. The simple IPI plots used here provide a more dynamic view and can be exploited to uncover specific dynamical regimes and the nature of dynamical bifurcations, by detailed analysis of the mapping function (Perez Velazquez et al., 2001).

Although it was shown several decades ago that brainstem stimulation can alter the rhythms of the cortical electroencephalogram (Moruzzi and Magoun, 1949), the possibility that seizures can be arrested by electrical stimulation has been explored in vivo only in the case of vagus nerve stimulation (Takaya et al., 1996), deep brain stimulation (Velasco et al., 1995), and recently, trigeminal nerve stimulation (Fanselow et al., 2000). In conclusion, our work indicates that direct electrical perturbation, with proper spatiotemporal application in the area where seizures are being generated, can abort the onset of SLEs. These methods, coupled to seizure-predicting algorithms (Elger and Lehnertz, 1998; Jerger et al., 2001; Litt et al., 2001), may provide a framework for the development of automated devices capable of halting the transition to seizures in patients with intractable epilepsy.

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REFERENCES

Barbarosie, M., and M. Avoli. 1997. CA3-driven hippocampal-entorhinal loop controls rather than sustains in vitro limbic seizures. *J. Neurosci.* 17:9308–9314.

Berge, P., Y. Pomeau, and C. Vidal. 1984. Order within Chaos. John Wiley & Sons, New York.

Bikson, M., J. Lian, P. J. Hahn, W. C. Stacey, C. Sciortino, and D. M. Durand. 2001. Suppression of epileptiform activity by high frequency sinusoidal fields in rat hippocampal slices. *J. Neurophysiol.* 53: 181–191.

Braun, H. A., K. Schafer, K. Voigt, R. Peters, F. Bretschneider, X. Pei, L. Wilkens, and F. Moss. 1997. Low-dimensional dynamics in sensory biology 1: thermally sensitive electroreceptors of the catfish. *J. Comput. Neurosci.* 4:335–347.

Chang, H. S., K. Staras, and M. P. Gilbey. 2000. Multiple oscillators provide metastability in rhythm generation. *J. Neurosci.* 20:5135–5143.

Chen, Y. L., C.-C. Huang, and K.-S. Hsu. 2001. Time-dependent reversal of long-term potentiation by low-frequency stimulation at the hippocampla mossy fibre-CA3 synapses. *J. Neurosci.* 21:3705–3714.

Christini, D. J., and J. J. Collins. 1996. Using chaos control and tacking to suppress a pathological nonchaotic rhythm in a cardiac model. *Phys. Rev. E.* 53:R49–R52.

Christini, D. J., and J. J. Collins. 1997a. Control of chaos in excitable physiological systems: a geometric analysis. *Chaos.* 7:544–549.

Christini, D. J., and J. J. Collins. 1997b. Real-time, adaptive, model-independent control of low-dimensional chaotic and nonchaotic dynamical systems. *IEEE Transactions on Circuits and Systems.* 44:1027–1030.

Christini, D. J., K. M. Stein, S. M. Markowitz, S. Mittal, D. J. Slotwiner, M. A. Scheiner, S. Iwai, and B. B. Lerman. 2001. Nonlinear-dynamical arrhythmia control in humans. *Proc. Natl. Acad. Sci. USA.* 98:5827–5832.

Collet, P., and J. P. Eckmann. 1980. Iterated maps on the interval as dynamical systems. Birkhauser, Basel.

Decroly, O., and A. Goldbeter. 1987. From simple to complex oscillatory behaviour: analysis of bursting in a multiply regulated biochemical system. *J. Theor. Biol.* 124:219–250.

Di Mascio, M., G. Di Giovanni, V. Di Matteo, and E. Esposito. 1999. Reduced chaos of interspike interval of midbrain dopaminergic neurons in aged rats. *Neuroscience.* 89:1003–1008.

Elbert, T., W. J. Ray, Z. J. Kowalik, J. E. Skinner, K. E. Graf, and N. Birbaumer. 1994. Chaos and physiology: deterministic chaos in excitable cell assemblies. *Physiol. Rev.* 74:1–47.

Elger, C. E., and K. Lenhertz. 1998. Seizure prediction by nonlinear time series analysis of brain electrical activity. *Eur. J. Neurosci.* 10:786–789.

Fanselow, E. E., A. P. Reid, and M. A. L. Nicolelis. 2000. Reduction of pentylenetetrazole-induced seizure activity in awake rats by seizure-triggered trigeminal nerve stimulation. *J. Neurosci.* 20:8160–8168.

Garfinkel, A., M. L. Spano, W. L. Ditto, and J. N. Weiss. 1992. Controlling cardiac chaos. *Science*. 257:1230–1235.

Glass, L., M. R. Guevara, and A. Shrier. 1983. Bifurcation and chaos in a periodically stimulated cardiac oscillator. *Physica*. 7D:89–101.

Gluckman, B. J., H. Nguyen, S. L. Weinstein, and S. J. Schiff. 2001. Adaptive electric field control of epileptic seizures. *J. Neurosci.* 21:590–600.

Guckenheimer, J., and P. Holmes. 1983. Nonlinear oscillations, dynamical systems and bifurcations of vector fields. Springer-Verlag, New York.

Hall, K., D. J. Christini, M. Tremblay, J. J. Collins, L. Glass, and J. Billette. 1997. Dynamic control of cardiac alternans. *Phys. Rev. Lett.* 78:4518–4521.

Hoppensteadt, F. C., and E. M. Izhikevich. 1997. Weakly connected neural networks. *In* Applied Mathematical Sciences, Vol 126. J. E. Marsden, L. Sirovich, and F. John, editors. Springer-Verlag, New York.

Iasemidis, L. D., and J. C. Sackellares. 1996. Chaos theory and epilepsy. *Neuroscientist*. 2:118–126.

Jerger, K. K., T. I. Netoff, J. T. Francis, T. Sauer, L. Pecora, S. L. Weinstein, and S. J. Schiff. 2001. Early seizure detection. *J. Clin. Neurophysiol.* 18:259–268.

Jerger, K. K., and S. J. Schiff. 1995. Periodic pacing an in vitro epileptic focus. *J. Neurophysiol.* 73:876–879.

Kantz, H., and T. Schreiber. 1997. Nonlinear time series analysis. Cambridge University Press, Cambridge.

Kelso, J. A. S., and A. Fuchs. 1995. Self-organizing dynamics of the human brain: critical instabilities and Silnikov chaos. *Chaos*. 5:64–69.

Kiss, I. Z., and J. L. Hudson. 2001. Phase synchronization and suppression of chaos through intermittency in forcing of an electrochemical oscillator. *Phys. Rev. E.* 64:1–8.

Lehnertz, L. D., and C. E. Elger. 1995. Spatio-temporal dynamics of the prymary epileptogenic area in temporal lobe epilepsy characterized by neural complexity loss. *Electroencephalogr. Clin. Neurophysiol.* 95:108–117.

Le Van Quyen, M., J. Martinieri, C. Adam, and F. J. Varela. 1997. Unstable periodic orbits in human epileptic activity. *Phys. Rev. E.* 56:3401–3410.

Lian, J., J. Shuai, P. Hahn, and D. M. Durand. 2001. Nonlinear dynamic properties of low calcium-induced epileptiform activity. *Brain Res.* 890:246–254.

Litt, B., R. Esteller, J. Echauz, M. D'Alessandro, R. Shor, T. Henry, P. Pennell, C. Epstein, R. Bakay, M. Dichter, and G. Vachtsevanos. 2001. Epileptic seizures may begin hours in advance of clinical onset: a report of five patients. Neuron. 30:51–64.

Lopes da Silva, F. H., and J. P. M. Pijn.1999. Epilepsy as a dynamic disease of brain systems. *Adv. Neurol.* 81:97–104.

Moruzzi, G., and H. W. Magoun. 1949. Brainstem reticular formation and activation of the EEG. *Electroencephalogr. Clin. Neurophysiol.* 1:455–473.

Ott, E., C. Grebogi, and J. A. Yorke. 1990. Controlling chaos. *Phys. Rew. Lett.* 64:1196–1199.

Packard, N. H., J. P. Crutchfield, J. D. Farmer, and R. S. Shaw. 1980. Geometry from time series. *Phys. Rev. Lett.* 45:712–716.

Perez Velazquez, J. L., and P. L. Carlen. 1999. Synchronization of GABAergic interneuronal networks in seizure-like activity in the rat entorhinal-hippocampal slice. *Eur. J. Neurosci.* 11:4110–4118.

Perez Velazquez, J. L., H. Khosravani, A. Lozano, B. L. Bardakjian, P. L. Carlen, and R. Wennberg. 1999. Type III intermittency in human partial epilepsy. *Eur. J. Neurosci.* 11:2571–2576.

Perez Velazquez, J. L., H. Khosravani, M. A. Cortez, P. L. Carlen, O. C. Snead III, and R. Wennberg. 2001. The transition in and out of the seizure: A nonlinear dynamical approach. *Epilepsia*. 42(Suppl.):116.

Pijn, J. P., J. van Neerven, A. Noest, and F. H. Lopes da Silva. 1991. Chaos or noise in EEG signals: dependence on state and brain site. *Electroencephalogr. Clin. Neurophysiol.* 79:371–381.

Pomeau, Y., and P. Manneville. 1980. Intermittent transition to turbulence in dissipative dynamical systems. *Commun. Math. Phys.* 74:189–197.

Press, W. H., S. A. Teukolsky, W. T. Vetterling, and B. P. Flannery. 1999. Numerical recipes in C. The art of scientific computing. 2nd ed. Cambridge University Press, Cambridge.

Rafiq, A., R. DeLorenzo, and D. A. Coulter. 1993. Generation and propagation of epileptiform discharges in a combined entorhinal cortex/hippocampal slice. *J. Neurophysiol.* 70:1962–1974.

Rafiq, A., Y.-F. Zhang, R. J. DeLorenzo, and D. A. Coulter. 1995. Long duration self-sustained epileptiform activity in the hippocampal-parahippocampal slice: a model of status epilepticus. *J. Neurophysiol.* 74:2028–2042.

Rapp, P. E. 1994. A guide to dynamical analysis. *Integr. Physiol. Behav. Sci.* 29:311–327.

Roux, J.-C. 1983. Experimental studies of bifurcations leading to chaos in the Belousof-Zhabotinsky reaction. *Physica.* 7D:57–68.

Sauer, T. 1994. Reconstruction of dynamical systems from interspike intervals. *Phys. Rev. Lett.* 72:3811–3814.

Schiff, S. J., K. Jerger, D. H. Duong, T. Chang, M. L. Spano, and W. L. Ditto. 1994. Controlling chaos in the brain. *Nature*. 370:615–620.

Shinbrot, T., C. Grebogi, E. Ott, and J. A. Yorke. 1993. Using small perturbations to control chaos. *Nature*. 363:410–417.

Takaya, M., W. J. Terry, and D. K. Naritoku. 1996. Vagus nerve stimulation induces a sustained anticonvulsant effect. *Epilepsia*. 37:1111–1116.

Takens, F. 1981. Detecting strange attractors in turbulence. lecture notes in math. Vol. 898. Springer-Verlag, New York.

Tergau, F., U. Naumann, W. Paulus, and B. J. Steinhoff. 1999. Low-frequency repetitive transcranial magnetic stimulation improves intractable epilepsy. *Lancet*. 353:2209–2210.

Titcombe, M. S., L. Glass, D. Guehl, and A. Beuter. 2001. Dynamics of Parkinsonian tremor during deep brain stimulation. *Chaos*. 11: 766–773.

Vandenhouten, R., M. Lamberts, P. Langhorst, and R. Grebe. 2000. Nonstationary time-series analysis applied to the investigation of brainstem system dynamics. *IEEE Trans. Biomed. Eng*. 47:729–737.

Velasco, F., M. Velasco, A. L. Velasco, I. Marquez, F. Jimenez, I. Marquez, and M. Rise. 1995. Electrical stimulation of the centromedian thalamic nucleus in control of seizures: long-term studies. *Epilepsia*. 36:63–71.

Velisek, L., J. Veliskova, and P. K. Stanton. 2002. Low-frequency stimulation of the kindling focus delays basolateral amygdala kindling in immature rats. *Neurosci. Lett*. 326:61–63.

Weiss, S. R. B., X.-L. Li, J. B. Rosen, H. Li, T. Heynen, and R. M. Post. 1995. Quenching; inhibition of development and expression of amygdala kindled seizures with low frequency stimulation. *Neuroreport*. 6:2171–2176.

Wiener, N. 1961. Cybernetics. MIT Press, Cambridge.