Unstable Periodic Orbits in Human Epileptic Hippocampal Slices

Pen-Ning Yu, *IEEE Student Member*, Min-Chi Hsiao, Dong Song, *IEEE Member*,
Charles Y. Liu, Christi N. Heck, David Millett, and Theodore W. Berger, *IEEE Fellow*

***Abstract*— Inter-ictal activity is studied in hippocampal slices resected from patients with epilepsy using local field potential recording. Inter-ictal activity in the dentate gyrus (DG) is induced by high-potassium (8 mM), low-magnesium (0.25 mM) aCSF with additional 100 μM 4-aminopyridine(4-AP). The dynamics of the inter-ictal activity is investigated by developing the first return map with inter-pulse intervals. Unstable periodic orbits (UPOs) are detected in the hippocampal slice at the DG area according to both the topological recurrence method and the periodic orbit transform method. Surrogate analysis suggests the presence of UPOs in hippocampal slices from patients with epilepsy. This finding also suggests that inter-ictal activity is a chaotic system and will allow us to apply chaos control techniques to manipulate inter-ictal activity.**

I. INTRODUCTION

Epilepsy is one of the most common neurological disorders; about 3% of all people living to the age of 60 will be diagnosed with epilepsy. So far, patients with epilepsy are treated mostly with anticonvulsant medication and surgical resection. As an alternative treatment, electrical brain stimulation therapies have attempted to control seizures without resection. Responsive neurostimulation (RNS) stimulates the seizure focus in response to seizure activity detected by EEG [1]. Although the preliminary results of brain stimulation application are promising, the underlying mechanism is still unclear. One potential approach to uncovering the underlying mechanism is to explore the dynamics of inter-ictal activity. The identification of unstable periodic orbits (UPOs) in inter-ictal activity gives us insight into the local nonlinear dynamics in the regions surrounding the UPOs [2]. Furthermore, the full dynamics could be approximated by assembling the local dynamics of each UPO. This approximated dynamics also makes short-term predictions possible [3].

UPOs have been found in rat hippocampal slices and EEG recordings from patients with epilepsy [3], [4]. It would thus be of interest to learn whether UPOs are present on hippocampal slices from patients with epilepsy. Human hippocampal slices provide us with an excellent platform to explore the dynamics of interictal activity. They have the advantages associated with *in vitro* techniques, such as low noise. Also the pattern of inter-ictal activity recorded from the hippocampal slices resembles intracranial EEG recordings [5]. In addition, this platform allows for various control theories to be tested after the validation of UPOs presence. The presence of UPOs not only characterizes the nonlinear dynamics of inter-ictal activity but also offers the hope of controlling inter-ictal activity [6].

II. METHODS

A. In vitro preparation for inter-ictal activity

Hippocampal slices were prepared from epileptic patients suffering from intractable mesial temporal lobe epilepsy. Post-surgical tissue was sectioned into slices of 500 μm with a vibratome in a cold ($4\,^{\circ}\mathrm{C}$) and oxygenated sucrose solution. Slices were then maintained at $30\,^{\circ}\mathrm{C}$ artificial cerebral spinal fluid (aCSF), equilibrated with a 5% CO_2 / 95% O_2 mixture. Inter-ictal activity was induced by high-potassium (8 mM), low-magnesium (0.25 mM) aCSF with additional 100 μM 4-aminopyridine (4-AP) and a temperature increase to $34\,^{\circ}\mathrm{C}$ in an interface recording chamber. Both the MEA60 system (Multi Channel Systems, Germany) and the interface recording system (Hass top) were used to collect the electrophysiological data. See [7] for details of the preparation and the MEA system. An aCSF-filled glass recording micropipette was positioned at the granule cell layer of DG, as shown in Fig. 1.

The 4-AP model facilitates the investigation of the characteristics of epileptiform activity. 4-AP is a potassium channel blocker. Bathing rat slices with 50uM 4-AP results in spontaneous ictal activity [8]. 100 M 4-AP aCSF is able to induce inter-ictal activity at the dentate gyrus (DG), CA1 and the subiculum in hippocampal slices from patients with epilepsy [7]. The *in vitro* model facilitates the recording of inter-ictal activity and the investigation of dynamics of inter-ictal activity, i.e. the identification of UPOs.

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Pen-Ning Yu is with the Department of Biomedical Engineering, Center for Neural Engineering, University of Southern California, CA 90089 USA (e-mail:penning@usc.edu)

Min-Chi Hsiao is with the Department of Biomedical Engineering, Center for Neural Engineering, University of Southern California, Los Angeles, CA 90089 USA (e-mail:mhsiao@usc.edu)

Dong Song is with the Department of Biomedical Engineering, Center for Neural Engineering, University of Southern California, Los Angeles, CA 90089 USA (e-mail:dsong@usc.edu)

Charles Y. Liu is with the Department of Neurological Surgery, Adult Comprehensive Epilepsy Program, and Center for Neurological Restoration at the USC University Hospital, and LAC-USC Medical Center, Los Angeles, CA 90033 USA, and Rancho Los Amigos National Rehabilitation Center, Downey, CA 90242 USA (e-mail:cliu@med.usc.edu)

Christi N. Heck is with the Department of Neurology, Adult Comprehensive Epilepsy Program , and Center for Neurological Restoration at the USC University Hospital, Los Angeles, CA 90033 USA (e-mail:check@med.usc.edu)

David Millett is with the Department of Neurology, USC University Hospital, Los Angeles, CA 90033 USA, and Rancho Los Amigos National Rehabilitation Center, Downey, CA 90242. USA. (e-mail:millett@med.usc.edu)

Theodore W. Berger is with the Department of Biomedical Engineering, Center for Neural Engineering, and the Program in Neuroscience, University of Southern California, Los Angeles, CA 90089 USA. (e-mail:berger@usc.edu)

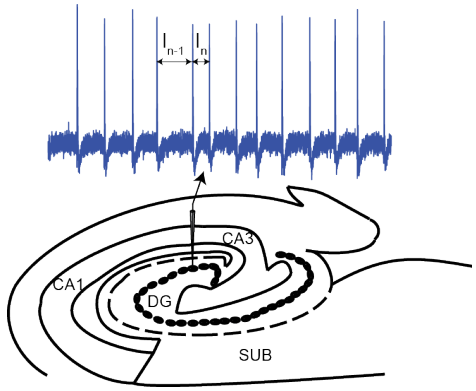

Fig. 1. Schematic diagram of human hippocampal slice. A glass micropipette at DG recorded a typical inter-ictal activity. The inter-pulse intervals are defined as I_{n-1} and I_n.

B. First Return Map

A K-dimensional system has k states and the system can be reconstructed from inter-pulse intervals, i.e. I_n vs I_{n-1} vs ... I_{n-k} [9]. However, the dimension of the inter-ictal activity is unknown. In order to simplify the analyses of dynamics, the K-dimensional system is projected onto a 2-dimensional map, i.e. first return map, as shown in Fig. 2. Although the overall structure of the dynamics might be lost, the stable and unstable manifolds will still be present. Fig. 3 shows the first return map reconstructed from the inter-pulse intervals of inter-ictal activity. This schematic corresponds to the first return map in Fig. 2. Furthermore, the evidence of UPOs appears as the presence of unstable fixed points in the first return map.

C. Locating UPOs

The topological recurrence method was used to locate the encounters with UPO in the data. This method detects UPOs by searching for the occurrence of the pattern that is indicative of an encounter with a UPO [10]. The pattern of an encounter is defined by the following criteria: (1) three approaching points on the first return map whose orthogonal

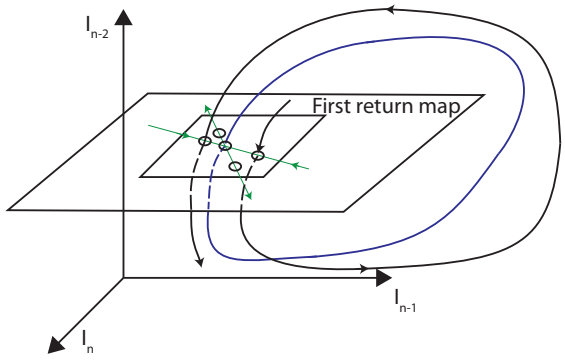

Fig. 2. The k-dimensional system projected onto the first return map. (k is 3 in this example.) The blue curve is a saddle-type UPO. The green lines are the stable and unstable manifolds. A 3-dimensional trajectory intersects the first return map at a sequence of 5 points; only the first-2-point part of the trajectory is shown.

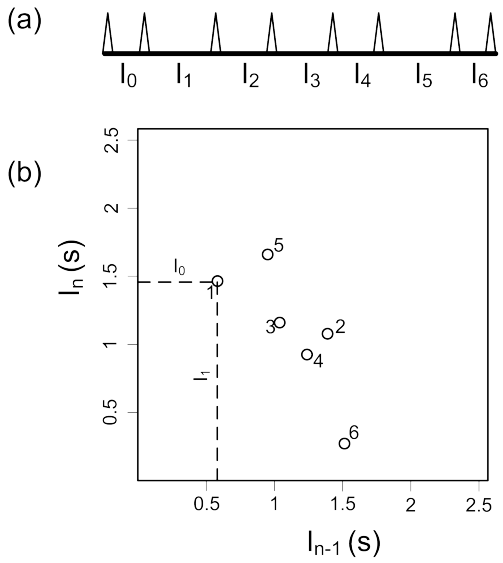

Fig. 3. First return map. (a) A schematic diagram of seven inter-ictal bursts. (b) The first return map formed by the inter-pulse interval versus the previous interval from data in (a), e.g. point 1 formed by I_0 and I_1.

distances to the line of identity decrease sequentially, followed by three departing points with sequentially increasing distances. (2) A straight line (linear least square) fitting to the three approaching points has a slope (eigenvalue) in the range of $0 \geq m_s > -1$ along the stable manifold. Straight line fits to the three departing points with a slope in the range of $-1 > m_{us} > -\infty$ along the unstable manifold. (3)The orthogonal distance from the intersection of two straight lines to the line of identity is smaller than half the mean of the distances of five points. The last approaching point and the first departing point are shared, so that only five points on the first return map are used.

The periodic orbit transform method has also been used to extract the UPOs from the inter-ictal activity. The unstable fixed points can be located as the peaks in the histogram diagram after transformation [11]. This method utilizes the transformation of the intervals of inter-ictal activity:

$$\hat{I_n} = [I_{n+1} - s_n(k)I_n]/[1 - s_n(k)], \quad (1)$$

where

$$s_n(k) = (I_{n+2} - I_{n+1})/(I_{n+1} - I_n) + k(I_{n+1} - I_n) \quad (2)$$

This transformation is based on the fact that the local region around a fixed point I^* can be modeled as a linear map. After applying transformation of Equations (1) and (2), the transformed data $\hat{I}_n$ will be concentrated in the vicinity near the fixed point I^* and shows peaks in the histogram diagram as long as the original data I_n, I_{n+1} and I_{n+2} are around the linear region. Data outside the linear region will be scattered randomly across the histogram after the transformation. In equation (2), the term $k = \kappa R$ is introduced, where R is a uniformly distributed random number in the range [-1,1] and is the magnitude of the randomization. By averaging 100 times of transformation with different k, the term k can

eliminate the singularities due to the denominators in equation (1) and the first term in equation (2). Thus the histogram diagram will show peaks of singularity from unstable fixed points but not singularity from the transformation itself.

D. Surrogate Data

In an effort to exclude the occurrence of the UPO encounters from pure noise, the number of encounters was statistically tested by surrogate data. The shuffling surrogate data were generated by randomly reordering the time series of original data [12]. The time interval distribution of the surrogate is identical to the original data, but other than that, the surrogate is simply white noise. Thus if the number of encounters from the original data is significantly larger than the surrogate data, the null hypothesis that the original data is white noise can be rejected, thereby suggesting the presence of UPOs.

III. RESULTS

After the administration of high-potassium (8 mM), low-magnesium (0.25 mM) aCSF with additional 100 μM 4-aminopyridine (4-AP) to the hippocampal slice, the inter-ictal activity can often be induced within half an hour. Fig. 4 shows a typical inter-ictal activity recorded at DG from a hippocampal slice. The inter-pulse interval was defined by the interval between the peaks of the inter-ictal activity.

A sequence of 265 inter-pulse intervals recorded at DG is shown in Fig. 5. Two sets of sequential points around the UPO were identified by the topological recurrence method defined in the method section. Fig. 6 shows the first return map plotted by the data shown in Fig. 5. The same two sets of sequential points from Fig. 5 are plotted and numbered in Fig. 6. The stable and unstable manifolds were derived by the fitting (linear least square) of a sequence of 5 points, 3 points converging toward the unstable fixed point and 3 points diverging away from the unstable fixed point, as shown in Fig. 6.

The number of encounters of original data and surrogate data were compared. A statistical measure is defined by

$$K = \frac{N - \overline{N}_s}{\sigma_s}, \tag{3}$$

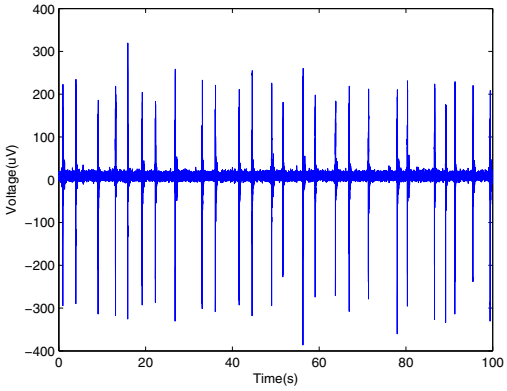

Fig. 4. 100 second local field potential recording of inter-ictal activity in DG from MEA 60 recording system.

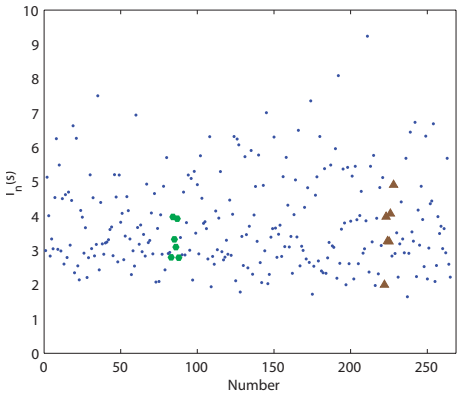

Fig. 5. Inter-pulse intervals of inter-ictal activity. 265 inter-ictal bursts were recorded in 1000 seconds. The green hexagons and brown triangles represent two sets of sequential points in the region around the UPO.

where K is the statistical significance, N is the number of encounters found in the original data, $\overline{N}_s$ is the mean number of encounters found in the surrogates , and σ_s is the standard deviation of $\overline{N}_s$. Assuming Gaussian statistics, $K > 2$ indicates with a probability of 95% that the encounters in the original data are not due to random chance.

Twenty surrogate data were generated. In the case of Fig. 4, a test shows $N = 6$, $\overline{N}_s = 2.65$ and $\sigma_s = 1.6 \Rightarrow K = 2.09$. This result strengthens the evidence for the presence of UPOs in the inter-ictal activity and excludes the null hypothesis that the converging and diverging pattern on the first return map is simply white noise.

The periodic orbit transform method has also been applied to detect the UPOs. The histogram of transformed original data is shown in Fig. 7(a). The two peaks of transformed

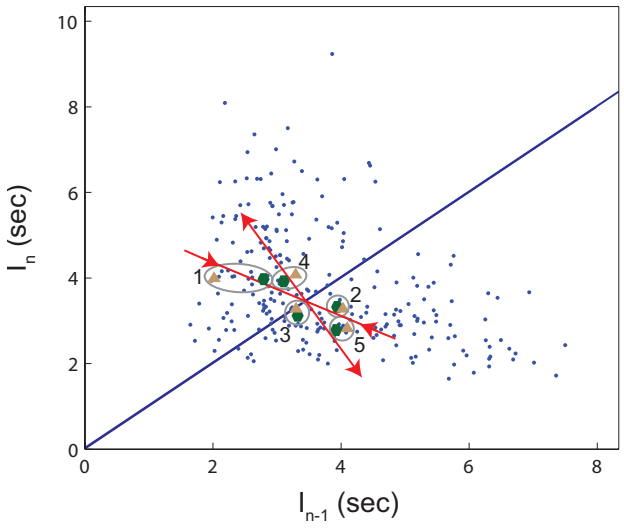

Fig. 6. First return map of inter-ictal activity. The green hexagons and brown triangles represent two sets of sequential points. Each set has 5 sequential points, including 3 points converging along the stable manifold and 3 points diverging along the unstable manifold. The unstable fixed point at the value of 3.5 sec is located at the intersection of unstable and stable manifolds. The slopes (eigenvalue) of stable manifold and unstable are -0.45 and -1.28 respectively.

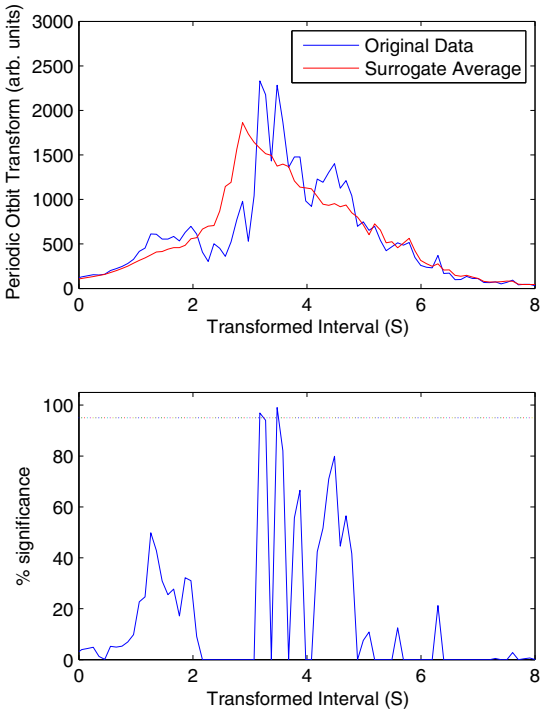

Fig. 7. Periodic orbit transformation of inter-ictal activity. (a) Histogram plot of the transformed original data and average of 100 transformed surrogate data. Each surrogate was averaged with 200 different randomization values of k, where $k = 2R, R \in [-1, 1]$. (b) Significance of unstable fixed point. Peaks at 3.2 and 3.5 s indicate unstable fixed points with 95% confident level.

original data show the unstable fixed points, i.e. UPO positions, at 3.2 and 3.5 seconds.

As in the case of the topological recurrence method, the transformed surrogates were adopted to assess the statistical significance. The comparison of the transformed original data with the transform of the surrogate data at each bin of the histogram was derived by:

$$K = \frac{N_{\hat{I}} - \overline{N}_{\hat{I}_s}}{\sigma_{\hat{I}_s}}, \tag{4}$$

where K is the statistical significance at each bin, $N_{\hat{I}}$ is the number of transformed original data at each bin, $\overline{N}_{\hat{I}_s}$ is the mean number of transformed surrogates at each bin, and $\sigma_{\hat{I}_s}$ is the maximum standard deviation of the surrogates.

A value of K above 2 at each bin indicates the location of a UPO with a statistical confidence greater than 95% assuming Gaussian statistics. The K value at each bin was transformed to a percentage of significance by the error function as shown in Fig. 7(b). Two peaks are the UPO position of 3.2 and 3.5 seconds. The UPO position at 3.5 seconds corresponds to the one shown in Fig. 6. This consistency in UPO detection between the periodic orbit transform method and the topological recurrence method strengthens the evidence for the presence of UPOs in the inter-ictal activity.

IV. DISCUSSION AND CONCLUSION

This study shows the presence of UPOs in resected hippocampal slices from patients with epilepsy. Both the topological recurrence method and the periodic orbit transform method consistently detected the UPOs from the time intervals of inter-ictal activity. The results are also consistent with earlier studies conducted on rat hippocampal slices and EEG recordings from patients with epilepsy [3], [4]. The hippocampal slice from patients with epilepsy, however, provides us with an excellent balance between *in vitro* model and the specimen from patients. The recording signal is less contaminated by artifacts and neural signals from other brain regions than the EEG signal while the inter-ictal activity from these specimens resembles epileptiform spikes seen with intracranial EEG recordings [5]. Thus this platform better enable us to explore the dynamics of epilepsy.

A chaotic attractor can be described by a framework of UPOs. The presence of UPOs in the inter-ictal activity implies the presence of determinism and suggests that the inter-ictal activity is chaotic. Furthermore, the presence of UPOs paves the way for the chaos control technique to manipulate inter-ictal activity, since UPOs are the regions around which chaos control can be applied. Following the validation of UPO presence in the *in vitro* model, the chaos control technique will be applied to hippocampal slices from patients with epilepsy. We will test the effect of the chaos control technique and we hope that our findings can contribute to the understanding of brain electrical stimulation in seizure suppression.

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