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Oscillator-Based Postictal Stimuli Prolong the Intervals Between *in vitro* Mg-free Seizure Episodes in Hippocampal Slice Preparation

Wu Chen, and Alan W.L. Chiu, *Member, IEEE*

Abstract— **The ability to accurately anticipate and suppress seizures is an important endeavor that has tremendous impact on improving the quality of lives for epileptic patients. Previous simulation studies have suggested that electrical stimulation strategy utilizing the intrinsic high complexity dynamics of the biological system may be more effective in reducing the duration of seizure like activities in the computer model. In this paper, we evaluate this on an *in vitro* rat hippocampal slice magnesium-free model in which seizure-like activities usually occur 8 to 10 times every 10 min. Simulated postictal field potential data generated by an oscillator-based hippocampal network model was applied to the CA1 region of rat hippocampal slices through a multi-electrode array (MEA) system. It was found to temporary suppress and delay the onset of future seizures for 6 min at most. The average inter-seizure time was found to be significantly prolonged after postictal stimulation, when compared to the negative control trials. This result suggests that neural signal-based stimulation related to resetting may be suitable for seizure control in the clinical environment.**

I. Introduction

Epilepsy is a common neurological disorder characterized by recurrent electrophysiological activities, known as seizures. Seizures can affect muscle movements, sensations, behaviors, emotions, consciousness, or a combination of these factors. It is one of the most widely studied diseases with researches ranging from the epileptogenesis, nonlinear network dynamics, electrographical signal feature extraction, seizure anticipation, to feedback control.

The objective of this work is to evaluate and develop feedback control strategies to suppress or reduce the occurrence of seizures. Seizure anticipation algorithms [1] in conjunction with responsive stimulation feedback control strategies [2][3] for the suppression of seizures has been in the forefront of seizure research for many years. The mechanism of this kind of feedback is to maintain the normal activity of the neuronal networks when state transitions into seizures are detected. Many kinds of stimulation strategies have been proposed and tested, such as controlled pulse stimulation [4], and high-frequency stimulation [5]. Most recently, the use of the high complexity dynamics of the biological interictal data as a responsive stimulator has shown great promise in a computer simulation study [2].

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W. Chen is with the Biomedical Engineering Program, Louisiana Tech University, Ruston, LA 71270 USA.

A.W.L. Chiu is with the Biomedical Engineering Program, Louisiana Tech University, Ruston, LA 71270 USA, (Phone: 318-257-5231; Fax: 318-257-4000; E-mail: alanchiu@latech.edu).

It has been suggested that the intermittent, recurrent nature of seizures is not a random process, and that seizures appear to serve as a dynamical resetting mechanism of the brain [6]. The postictal dis-entrainment appears to be an important phenomenon in understanding seizure suppression and control [6]. Therefore, in this paper, postictal field signals generated by an oscillator-based cognitive rhythm generator (CRG) model were used as a responsive stimulator to determine if the occurrence of seizure episodes can be reduced and that the inter-seizure time can be prolonged.

A magnesium-free *in vitro* rat hippocampal slice model was used to generate spontaneous recurrent seizure-like events (SLEs) [7]. Negative control trials, without external stimulation, were setup to obtain the baseline seizure frequency and inter-seizure times. In the stimulation trials, the oscillator-based postictal model data was applied through a multi-electrode array (MEA) recording system when the ictal onsets were detected by an increase in signal spiking frequency and a decrease in signal complexity. Our preliminary results showed that the oscillator-based model output stimulation was able to reduce the amount and frequency of seizure-like activities, and may have potential application in the therapy for epilepsy.

II. Method

A. In-vitro Magnesium-Free Hippocampal Slice Model

The experimental procedures used in this study were approved by the Louisiana Tech University Institutional Animal Care and Use Committee. Male Sprague Dawley rats were anesthetized with CO_2 and sacrificed at 4-6 weeks of age. Their brains were rapidly dissected and placed into ice-cold (~1℃), oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF) solution for 5 min. The aCSF solution contains (in mM): 123 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 2 $MgSO_4$, 24 $NaHCO_3$, 1.2 NaH_2PO_4 and 25 glucose (pH 7.4). The hippocampus was dissected, and 300 μm transverse slices were sectioned and transferred immediately into a storing chamber with ice-cold, oxygenated aCSF solution and maintained at room temperature for 1 hour. The Mg-free aCSF solution, consisted of the same aCSF recipe without the $MgSO_4$, was applied after the slice has been stabilized and transfer to the multi-electrode array (MEA) (Multi Channel Systems, Reutlingen, Germany). Electric field potential was obtained near the pyramidal neurons in CA1 region of the hippocampal slices (see Fig. 1). The data was then lowpass anti-aliasing filtered at 400Hz and sampled at 1 kHz for further analysis.

B. Modified Cognitive Rhythm Generator Model

For the simulation of electric field potential of the Mg-free *in vitro* hippocampal slice, a modified cognitive rhythm

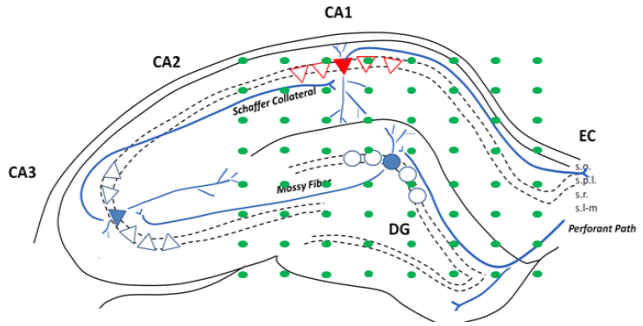

Figure 1. Hippocampal slice orientation is shown on the multi-electrode array. The data is recorded from those electrode near the s.p.l. layer of the CA1 region.

generator (CRG) model was used [1][8]. The CRG model utilized the experimentally determined input-output kernel functions to modulate the unit and network excitability. The main parameters in the kernel function, representing the mode decay constant, modulatory gain, and receptor level have been carefully studied [8] to reproduce the different dynamics that exists in manifestation of seizure episodes in the *in vitro* hippocampal slice preparation. Subpopulations of the hippocampal CA1 region can be represented using networks of four reciprocally connected model units. The state variables of each unit can then be mapped nonlinearly to generate the transmembrane potentials based on the action potential waveforms previously recorded. In order to estimate the field recording signal from the model, each neural unit signal was twice differentiated and then added together, as shown in Fig. 2.

C. Field Stimulation

Spontaneously generated SLEs can be terminated by the process of neural resetting. It is therefore, in our hypothesis, to evaluate whether intrinsic characteristics of neural activities may hold a possible key to SLE suppression strategy. Furthermore, recent study on a computer simulated seizure model indicated that model based electrical stimulation is helpful to seizure control [2]. Here, it is also natural to speculate that the mechanism in which neural resetting takes place may be found at the termination of a seizure episode, called the post-ictal activity. In previous research, our group has shown that the state transitions associated with SLEs spontaneously triggered by Mg-free experimental condition can be represented by the modified CRG model with different parameter combinations [8]. Three different CRG-generated postictal activities were selected (see Fig. 3) and scaled to a range of ±200μV, similar to the physiological recorded data amplitude. These oscillator-model generated signals were treated as field potential stimulation to the slice preparation through the MEA, when the onsets of SLEs were detected (accompanied by an increase in spiking frequency and a reduction in signal complexity). One of the three candidate postictal signals was randomly selected. Finally, a two-sided rank sum test was performed on the inter-seizure interval to evaluate the Null Hypothesis that the negative control (NC) and postictal stimulation (PS) come from the distributions with equal medians.

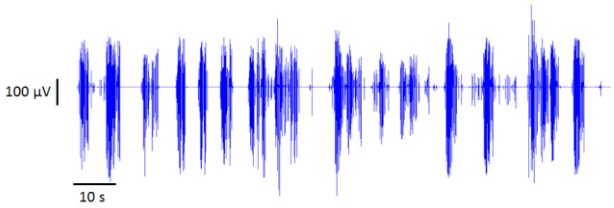

Figure 2. Simulated field potential generated using the modified CRG model, illustrating spontaneous generation and termination of SLEs.

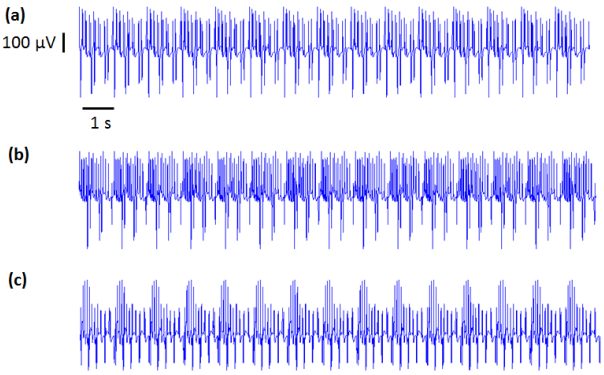

Figure 3. Oscillator-based postictal field data was repeated to create the stimulation signals that are 15s in length.

III. Results

A. Negative Control Experiment

The negative control experiments were performed using the Mg-free solution on rat hippocampal slice preparation, consisted of over six hours from three animals. Recurrent seizure-like activities (as shown in Fig. 4) can be seen throughout. A total of 100 SLEs was observed. On average, the SLEs occurred 8 to 10 times every 10 min and each SLE lasted 0.5-2 min.

B. Suppression of Seizure-Like Events

Once the NC trials have been completed, over two hours of PS was performed under identical experimental condition. Postictal stimulation was applied to the recording electrode when the ictal onset was detected. In most cases, the subsequent SLEs after a PS were delayed by over a minute. Occasionally, a long period of interictal spiking activity would appear after PS. In these cases, the inter-seizure time was increased significantly. As an illustrative example in Fig. 5, after a 15s postictal stimulation, the subsequent SLE was prolonged for over 6 min by the stimulus in Fig. 3b and before resuming again. The second PS waveform in Fig.3 is more effective than the other two even though the exact reason is not fully evaluated in this paper. A ranksum statistic was performed using MATLAB to compare the medians of the inter-seizure time between the NC and the PS trials (Fig. 6). The SLEs were found to occur more frequently in the NC situation than with PS ($p < 0.002$).

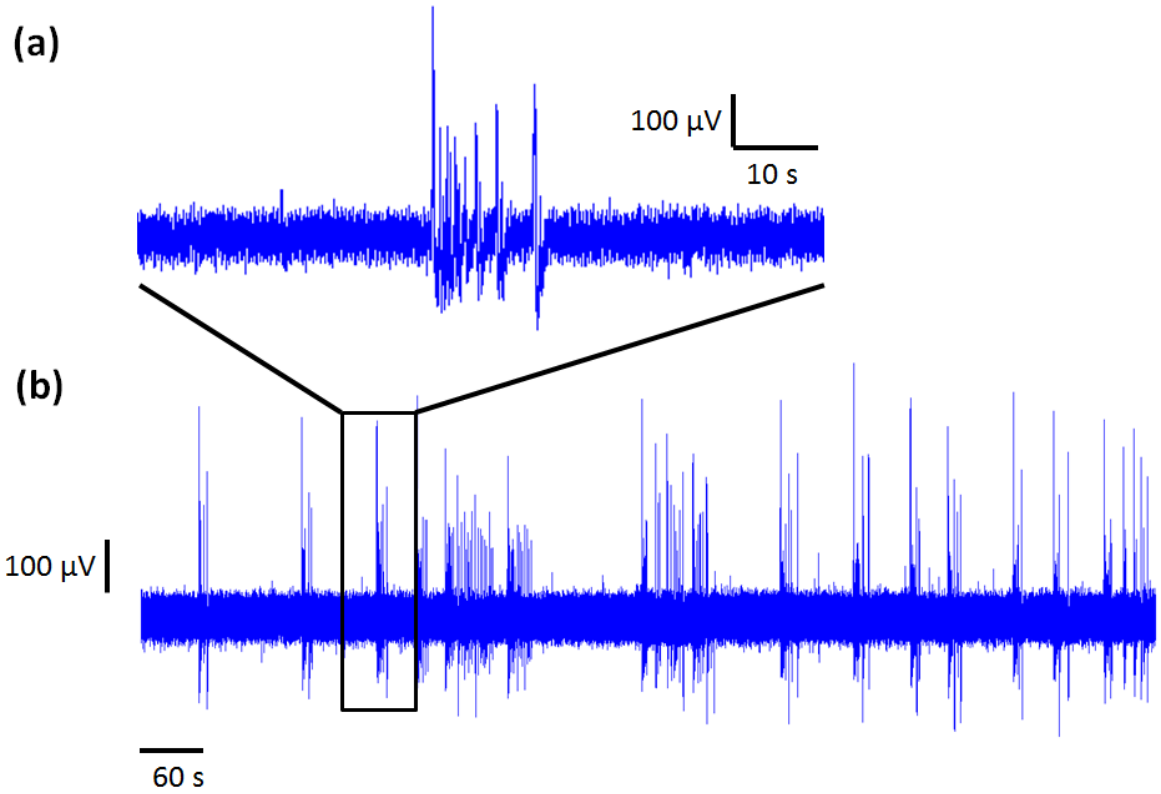

Figure 4. Sample field measurement from the negative control experiment showing repeated SLEs at approximately 8.30 ± 2.23 times every 10 min (n = 100).

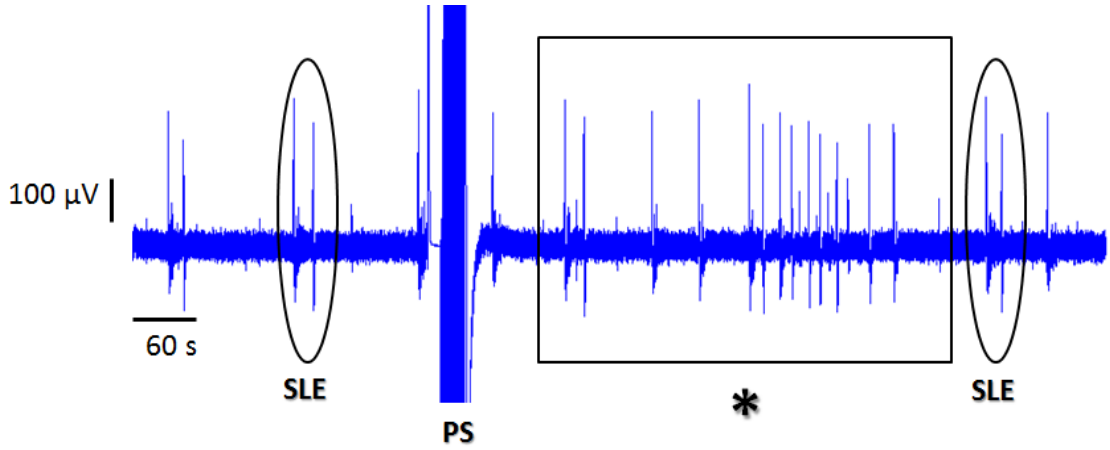

Figure 5. The effect of postictal stimulation (PS) on Mg-free *in vitro* hippocampal slice seizure model is shown. The seizure-like events are denoted as SLE. After stimulation (PS), interictal spiking events (*) were generated, leading to delayed or prolonged inter-seizure time.

IV. Discussion

Oscillator-based stimulation protocol was tested on its effectiveness to suppress seizure episodes in an *in vitro* hippocampal slice preparation. The Mg-free setup was chosen because it was able to generate a high volume of recurrent seizure-like activities, which makes it a valuable model to evaluate our postictal stimulation protocol. After the stimulation, most subsequent SLEs were delayed by approximately one minute. In a few instances (about 20% of the time), long interictal spiking activity would appear after PS, which would significantly prolong the onset of the next SLE. The manifestation of the interictal spikes after postictal stimulation can be considered a temporary resetting of the slice and then the magnesium-free environment would induce seizure-like activities again.

In the modified CRG model, different parameter combinations offer seizure patterns with various adjustable durations, firing rates, and spiking amplitudes. More variety of the postictal stimulus patterns must be included for future

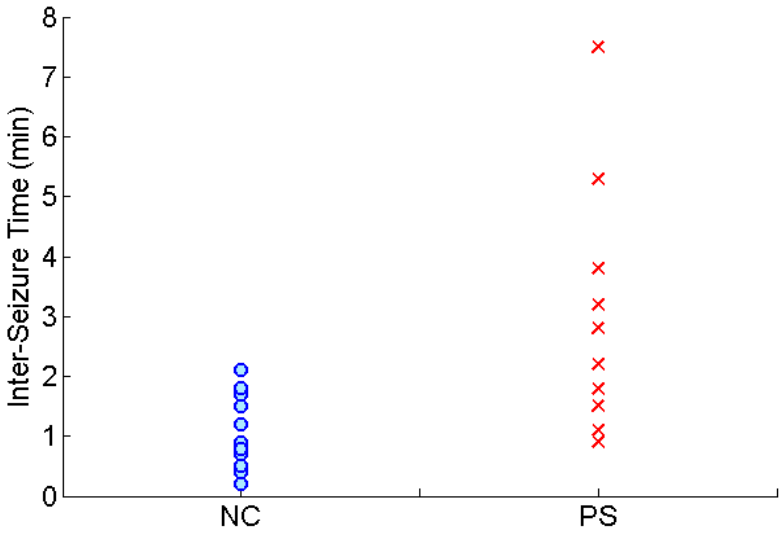

Figure 6. Ranksum statistics was performed on the negative control (NC) and postictal stimulation (PS) trials, showing that PS stimulation can prolong the inter-seizure time ($p < 0.002$).

testing. Potential neural resetting factors may be obtained in the future by carefully studying the impacts of these stimuli.

Those factors may enable the suppression of seizures through forced resetting of the neuronal network activities. However, this effect appeared to be short-lived, before the next imminent SLE would start again. The full analysis of why certain PS stimulus works better than other is currently underway. The next step of this study would also need to involve changing the magnitude and duration of the PS. At this stage, the postictal stimulus appeared to help reset the neuronal network into normal activity. However, more work is needed to evaluate the long term effect of such stimulation on the neurodynamics of the subjects.

This research may have potential implication in the therapy for epileptic patients, related to a better design for an adaptive deep brain stimulation strategy. However, further *in vivo* test is needed. Selecting the resetting dynamics from the epileptic patient's own brain signal is a possible way to enable a more natural way to provide stimulation.

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