

Are the Spatio-Temporal Firings of Pyramidal Cells and Interneurons Markers of Impending Seizures?

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Abstract—Current pharmacological, electrophysiological, and surgical treatments are not always effective for all epileptic syndromes. In analyzing the clinical utility, traditional EEG analysis provides a coarse representation of the neuronal activity and we hypothesize that for chronic, *in vivo* epilepsy research more specific electrophysiological techniques are necessary. In order to increase our understanding of single unit behavior in an epileptic network, this study recorded pre-seizure single unit firing rates from pyramidal cells and interneurons in an epileptic rat model of temporal lobe epilepsy. The information gained from this study seeks to aid in the development of new seizure warning and control methods.

I. INTRODUCTION

EPILEPSY is a neurological disorder that affects approximately 2.7 million people in America and costs \$15.5 billion in medical treatment and lost wages annually [1]. Temporal lobe epilepsy (TLE) is one of the most common forms of epilepsy refractory to medical treatment (pharmacotherapy, surgery) limiting the quality of life for these patients. This high cost of care and the resistance to treatment for TLE create a great need for the development of novel diagnostics and therapies. Current treatments for epilepsy include pharmacological treatments, surgical resection of brain tissue, vagus nerve stimulation [2], and deep-brain stimulation (DBS) [3]. Despite their widespread clinical use, these treatments are not completely effective in all cases [4]. Many current methods for seizure detection, prediction, and analysis only look at the EEG and/or ECoG level [5, 6]. Although proven diagnostically useful, these techniques do not provide an explanatory neurophysiological mechanism because they are based on macroscopic brain activity (i.e., ECoG or EEG). From a cellular perspective, the EEG signal is a coarse representation of the total activity in groups of interconnected neurons and we hypothesize that for chronic, *in vivo* epilepsy research more specific electrophysiological techniques are necessary.

The cellular mechanisms for how an epileptic brain behaves are still not well understood. Specifically it is still not well known how neurodegeneration and gliosis, notably in the CA1/CA3 areas of the hippocampus and in the hilus of the dentate gyrus [7, 8] affects the activity of abnormal

neural networks *in vivo*. We hypothesize that a seizure prediction paradigm based on the analysis of local field potentials and single unit neural activity will complement the accuracy of current seizure prediction methods, and contribute to a better understanding of the cellular mechanisms underlying changes prior to seizure onset.

In this work, we present a novel framework for studying the onset of seizures that is based on a level of abstraction rooted in the time-varying interactions of neural ensembles. We seek to identify and quantify the ensemble of spiking neurons that constitute seizure onset. By analyzing the richer information available in the spatiotemporal spiking activity of neurons, we hope to improve our understanding of the functional modifications underlying epileptic brain circuits, and ultimately derive new seizure warning and control methods. The neural engineering approach implemented here may also provide an explanation of underlying mechanism governing the impending of seizures

II. DATA COLLECTION AND ANALYSIS

A. Electrode Implantation and Neuron Targeting

The parallel and distributed processing capabilities of the brain suggest that neural information is spread across populations of neurons [9]. Therefore, simultaneous recordings from large numbers of neurons should extract more information compared to serial single neuron recordings. This basic hypothesis of systems neuroscience has sparked the development of a variety of invasive recording techniques and electrode arrays that are capable of recording the activity of tens to hundreds of neurons chronically for long periods of time. The functionally representative modulation of activity in neural assemblies is the signal of interest. For TLE, the trisynaptic loop which includes dentate granule cells, CA3/CA1 pyramidal cells, and entorhinal cortex is well known for its role in generating epileptiform activity [10]. To quantify and track the neuronal firing that precedes the onset of seizure, we employed techniques to obtain large-scale sampling of the hippocampal structures that are involved in epileptiform activity. Thirty-two microwire electrodes for single cell recording were implanted into the left hippocampus of an adult (57 day old) Sprague-Dawley rat. The electrodes consisted of 50 μm tungsten wires insulated with polyimide arranged into an 8X2 array. Within each row, the electrodes were spaced 250 μm with 500 μm between the two rows.

The anterior array was implanted in the pyramidal layer of CA3 of the left hippocampus with the center of the array - 2.4 mm AP, and 2.5 mm lateral to Bregma and 2.8 mm

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below the surface of the cortex [11]. The array was rotated so the long axis was 62° off the AP axis with the anterior end more medial to conform to the morphology of the hippocampal pyramidal cell layer. The posterior array was implanted straddling the dentate gyrus and CA1 [11] of the left hippocampus (-4.3 AP, 1.7 left, 2.6 mm deep, rot. 70°).

To create the animal model of temporal lobe epilepsy used here [12], a 330 μm , Teflon coated, stainless steel, bipolar twist electrode was implanted into the right hippocampus. This electrode was used to induce status epilepticus. The electrode was implanted -5.3 AP and -4.9 lateral to Bregma, 2.9 mm below the cortical surface [11].

B. Induction of Status Epilepticus

Twenty-five days after electrode implantation, the animal was subjected to an event of status epilepticus by stimulating through the bipolar twist electrode using well established techniques [12]. A 410 μA , 1 ms pulse width bipolar square wave was delivered in 10 s trains at 50 Hz with 2 s between each train. After the start of stimulation, the subject displayed “wet dog shakes” and seized 4 times during stimulation. Thirty min after first seizure, stimulation was turned off and the subject continued to have self sustained seizures for the next 4 hr. Seizures were graded behaviorally on the Racine scale to validate the protocol for generating the animal model of TLE [13].

C. Data Collection

Multielectrode recordings were collected synchronously using a TDT RX7 stimulator base station (Tucker-Davis Technologies, Alachua, FL) and two synchronous streams of data were preprocessed. The first stream was used to collect local field potentials which could be used to define the onset of each seizure. Using the onboard DSPs, this low-frequency stream first was bandpass filtered from 0.5 Hz to 200 Hz using biquad filters and downsampled to 500 Hz. The second stream, a high-frequency recording used to analyze single neuron activity, was also bandpass filtered from 500 Hz to 6 kHz (biquad) and digitized at 12207 Hz. Time synchronized, infrared video recordings were also collected. The animal was monitored 24 hr a day, for 60 days. This demanding schedule generated over 1 TB of data for each animal.

D. Identification of Pyramidal Cells and Interneurons

Extensive analysis of hippocampal electrophysiology have characterized the differences in interneuron and pyramidal cell waveform shapes and firing rates for identifying the cell types *in vivo* [14]. Discrimination has been based upon spike amplitude, waveform width, and frequency of firing rate [14]. Using these guidelines, cells here were classified as either interneurons or pyramidal cells using the same criteria.

Interneurons were characterized by smaller amplitude (approx 20 μV), and a shorter waveform width (< 0.6 ms) and increased firing rate (mean firing rate 1.357 spikes/s).

Pyramidal cells had a larger amplitude (> 60 μV) and a much larger waveform width (> 0.8 ms) and lower firing rate (mean firing rate 0.566 spikes/s). The firing rates are similar for pyramidal cells as previously reported, but lower for interneurons [14]; however, differences between animal states may account for these firing rate differences. Waveform averages for one interneuron and one pyramidal cell are shown in Fig. 1. Spike times were isolated and extracted off-line from the high-frequency stream using Spike 2 (Cambridge Electronic Design, Cambridge, England), then exported to MATLAB. Spike times were extracted for the 1.5 hrs leading up to each seizure.

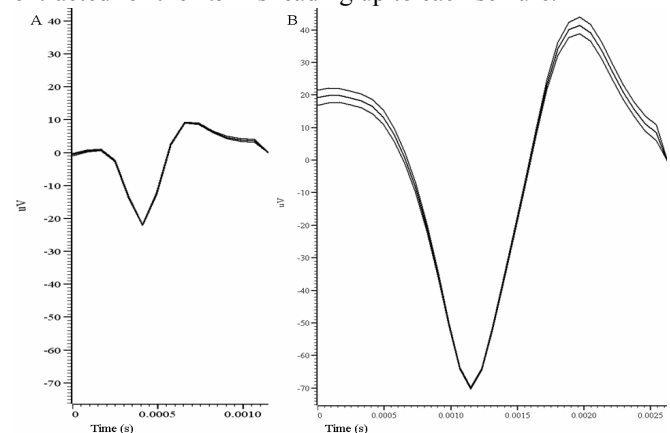


Fig. 1. Waveform shapes for interneuron and pyramidal cell. A. Waveform for interneuron. Note the smaller amplitude as well as decreased width of the waveform. B. Waveform for pyramidal cell. The amplitudes for these cells are much larger, and the waveform is much wider. Time scales for A and B are not equal.

E. Data Analysis

To compare the firing of each cell type across multiple seizure realizations, the reference point of seizure onset was first defined using established techniques [6]. This step allowed for time alignment of all seizures during the 1.5 hr before this common reference point. Seizure detection was performed on the low-frequency data stream by summing the absolute power of the recording into one-second epochs in a method similar to those used for EEG recordings [6]. This function was then plotted, and seizures are indicated by a sustained increase in power for the duration of the seizure. We note here that increases in power are common throughout the circadian cycle of the rat; however, during seizures the amplitude of the power curve remains at an elevated level for durations of up to 1 min. After electrophysiological analysis, all seizures were confirmed behaviorally through video recording. During the 22 days reported here, 10 seizures were identified and behaviorally scored as between 3 and 5 on the Racine scale [13].

III. RESULTS AND ANALYSIS

The firing rate of the cells just prior to seizure onset was quantified to see if a change occurred. Spike times for the 30 min prior to each seizure were binned in 10 min bins for each cell. The firing rate for each cell was then averaged across seizures, and the average for each cell type (interneuron and pyramidal cell) was found. Interneurons

showed a significant increase between the firing rate 30 min prior to seizure onset and 10 min prior to seizure onset (paired t-test, $p < 0.05$). The distribution of firing rates for the pyramidal cells was found to be a non-normal distribution therefore, a Wilcoxon rank sum test was used to test for significant change in firing rates leading up to seizure onset. Pyramidal cells showed a significant increase between 20 min and 10 min prior to seizure (Wilcoxon rank sum test, $p < 0.05$). The mean firing rate and standard deviation are shown in Table I. For all following statistical tests, a paired t-test was used for interneurons, and a Wilcoxon rank sum test was used for pyramidal cells.

TABLE I
AVERAGE FIRING RATE (SPIKES/S) 30 MIN BEFORE SEIZURE

Time Before Seizure	Cell Type			
	Interneuron		Pyramidal Cell	
	Mean	SD	Mean	SD
30 min	1.005	0.715	0.422	0.208
20 min	1.203	0.674	0.432	0.225
10 min	1.297	0.724	1.365	0.669

The animal model used here creates an epileptic state that develops over time. To assess this development, firing rates 30 min before each seizure onset were averaged across the cell types and plotted in Fig. 2. Interneurons show a slow increase in firing rate as seizure number increases up to seizure #5 (Fig. 2A). There is a significant change in firing rate between each seizure from seizure #5 to seizure #8.

Pyramidal cell firing rate shows little overall change across the 10 seizures, although there is some variability from seizure to seizure (Fig 2B). Most notably, there is a significant decrease in firing rate from seizure #3 to #4 and again from seizure #9 to #10.

Processing the data in 30 min and 10 min bins gives a coarse overview of the data right before seizure onset, but analysis at a finer temporal resolution and over a longer time period may provide greater insight into the changes in firing rates leading up to a seizure. With this in mind, mean firing rates for each cell were calculated in 10 s bins for the 90 min leading up to seizure onset. Peri-event time histograms (PETH) for each cell type were created by averaging across cell type and across all seizures. Fig. 3 shows these PETH time locked to seizure onset for the 90 min before seizure onset. By using PETH, any noisy firing is averaged out (reduced by \sqrt{N}), and significant changes in firing rates are shown as peaks.

While Table I shows an increase in firing rate of interneurons leading up to seizure onset, we see in Fig. 3A that there is a significant peak in activity 490 s before seizure onset, before decreasing and starting to increase again just before seizure onset. Table I also shows a large increase in pyramidal cell firing leading up to seizure onset. However, Fig. 3B shows that pyramidal cell firing rate peaks 160 s before seizure onset, then decreases sharply leading up to seizure onset. The peak in interneuron firing rate directly

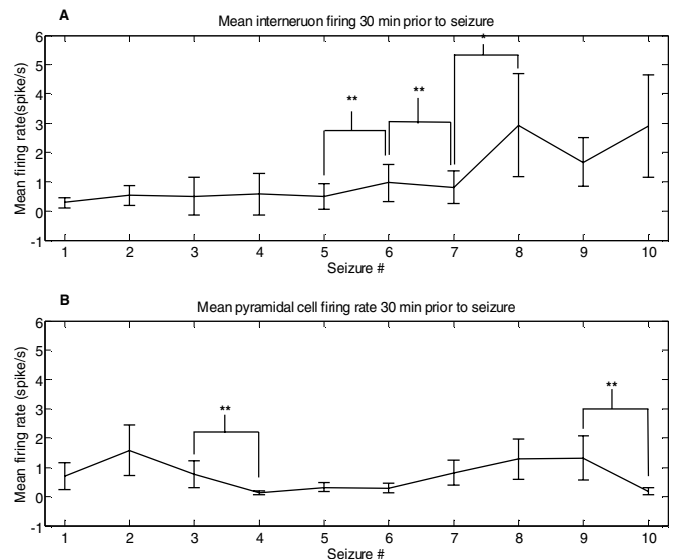


Fig. 2. Mean firing rates of interneurons (A) and pyramidal cells (B) 30 min prior to each seizure. Significant increase in mean firing rate is seen from seizure #5 to seizure #8 for interneurons but not pyramidal cells. ** = $p < 0.05$, * = $p < 0.10$.

precedes the build up of firing rate before the peak in pyramidal cell firing rate. Also in Fig. 3A, there is an apparent slow oscillation in firing rate between 3170 s and 1710 s prior to seizure onset. The peaks of the oscillation occur approximately every 200 s.

IV. DISCUSSION

To better understand how an epileptic system functions, and to help develop new seizure detection and control methods, the firing rates of interneurons and pyramidal cells in the CA3 region of an epileptic rat were analyzed. Initial quantifying of firing rates in the 30 min prior to seizure onset showed that there is an increase in firing rate of both interneurons and pyramidal cells (Table I). However, there was great variability in the firing rates between each of the cells. This variability could possibly be explained by a change in firing rates throughout the development of epilepsy. Fig. 2 shows that there are significant increases in the firing rates of interneurons over the course of 10 seizures, but there is not for pyramidal cells. This suggests that the development of epilepsy in this network is due to interneuron activity, not pyramidal cell activity.

To increase the temporal resolution of the data, PETHs were created for the mean firing rates for 90 min prior to seizure onset (Fig. 3). The PETH for the pyramidal cells showed a large increase in the firing rate just minutes prior to seizure onset (Fig. 3). This increase in activity is expected, as a seizure is caused by firing of many cells at the same time, but there is a drop off pyramidal cell firing rate immediately before the seizure onset.

There is also a peak in interneuron firing rates prior to the increase in pyramidal cell firing rates (Fig. 3). This increase in interneuron firing could create the synchronicity needed for seizure development. The ability of interneurons to reset

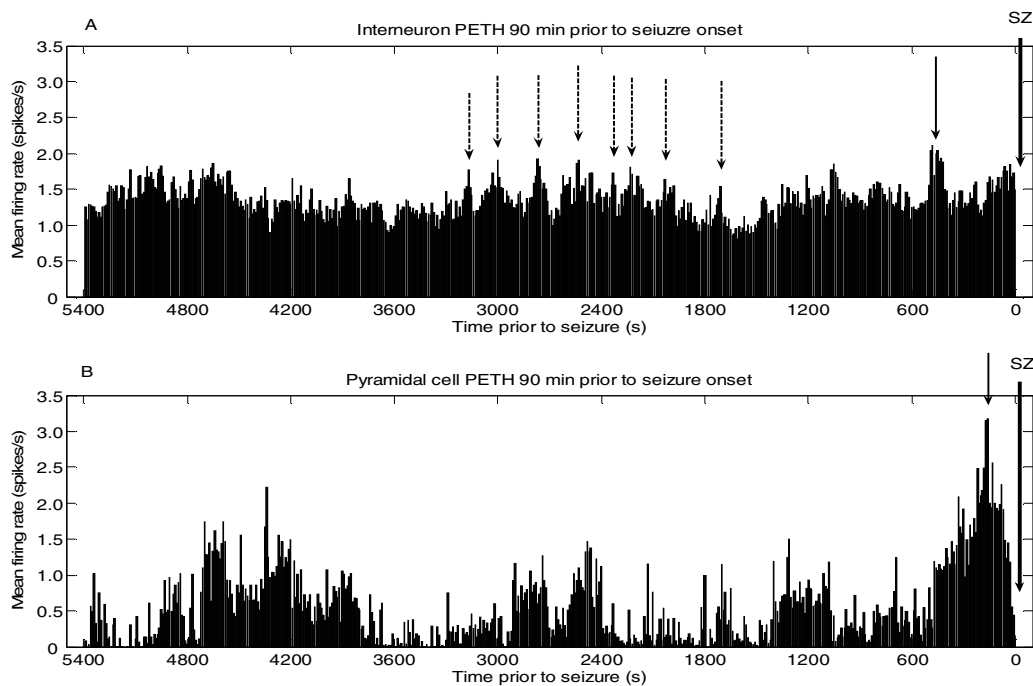


Fig. 3. Mean firing rate of interneurons (A) and pyramidal cells (B) 90 min prior to seizure onset (Bold arrows). Firing binned in 10 s windows. A. Interneuron firing rates show a peak of activity that starts 490 s before seizure onset (Thin arrow). Interneuron firing rates also show oscillation of firing prior to peak in activity (peaks of oscillation shown by dashed arrows). B. Pyramidal cell firing rates show a peak in activity 170 s before seizure onset (Thin arrow). Build up to this peak in activity starts as the peak in activity in A starts to decrease

and synchronize the firing of pyramidal cells has been shown in the CA1 region of the hippocampus [15] and similar behavior may be exhibited here in the CA3 region. The timing of these peaks have an interesting correlation to a method of seizure prediction using EEG recordings. When using the STLmax method of seizure prediction, the STLmax score shows a decrease starting around 10 min prior to seizure [5]. The STLmax also shows a plateau of minimal score around 5 min prior to seizure. These periods are very similar to the time frames for the peaks observed in the firing rates of interneurons and pyramidal cells (Fig. 3).

Prior to the increase in interneuron firing, oscillation in interneuron activity can be seen. This oscillation could be the interneurons beginning to synchronize their firing leading up to the increase in firing seen 490 s before seizure onset.

While these findings contribute to our understanding of how neurons behave leading up to seizures, more study is necessary to develop new treatments for epilepsy. This study only looked at one part of the hippocampal circuit, but seizures occur through out the hippocampal network. Understanding how neurons in other parts of the hippocampus behave before seizures is crucial to understanding how the epileptic system functions.

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