

# The Effects of Interictal Spikes on Single Neuron Firing Patterns in the Hippocampus during the Development of Temporal Lobe Epilepsy

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**Abstract**—The interictal spikes (IS) are characteristic signatures of epileptic tissue, and relevant in presurgical evaluation of epilepsy. However, the mechanism and effects of IS remain unclear. In this study, we examined the relationship between IS and single neuron firing patterns, using an epileptic rat with temporal lobe epilepsy (TLE). We investigated the firing of interneurons and pyramidal cells in the CA3 region of the hippocampus. The results show that IS are associated with decreased single neuron firing rates compared with IS-free epochs. Furthermore, the paroxysmal interictal-spiking patterns are associated with sustained decrease in single neuron firing rates. We also found that IS stopped as approaching to seizures. These results demonstrate that IS might be responsible for the development of TLE and ictal events by changing the firing patterns of hippocampal neurons.

## I. INTRODUCTION

Interictal spikes (IS) are important indicators of epileptic foci. As described in [1], [2], they are expressed by high-amplitude and fast EEG transients, and habitually followed by a slow wave that lasts for several hundreds of milliseconds. In addition to being a marker of the location of epileptic tissue [3], [4], IS are believed to have other effects, including their relationship with ictal events and epileptogenesis. For these reasons, much attention has been devoted to analyzing the effects of IS on the generation of seizures. It has been proposed that interictal spiking prevents seizure precipitation in some animal models [5], [6], [7], [8], although the exact mechanism and relationship are not clearly understood yet.

Even more elusive is the relationship between IS and single neuron activities. Are interictal spikes simply an aggregate of single neuron firing? Zhou et al. showed [9], in two seizure models (pilocarpine-induced status epilepticus and recurrent flurothyl models), that IS were associated with a decreased likelihood of action potentials compared with IS-free portions of record in a cell-specific manner. This indicates some more complicated relationship between IS and single neuron firing. Wozny et al. [10] demonstrated that subicular cells displayed spontaneous, rhythmic activity that correlated with the occurrence and frequency of interictal discharges recorded in the EEGs of epileptic patients. These findings are important to understanding the effects that IS

exerts on neurons, and if and how IS may impair cognitive function in epileptic patients.

In this work, we analyzed single neuron firing patterns associated with IS in an epileptic rat with temporal lobe epilepsy. The status epilepticus was induced by an implanted twist electrode, and single neuron discharge and local field potentials were recorded synchronously from multielectrode recordings in the CA3 region of the hippocampus. By estimating the IS triggered average of the single neuron firing rates in a time interval of 100 seconds prior to and 100 seconds following the IS, we verified decreased firing rates associated with IS. A t-test was utilized to test the hypothesis that the single neuron firing rates within 10 seconds prior to and 10 seconds following the IS negative peaks are lower than those within IS-free epochs. The t-test produced sufficient significant level to demonstrate this hypothesis for both types of neuron, although their firing patterns were not exactly the same. We also found that IS stopped as approaching to seizures. These results suggest that IS might effect the development of TLE and ictal events by changing the single neuron firing patterns.

## II. DATA COLLECTION

### A. Electrode implantation and induction of status epilepticus

Thirty-two microwire electrodes for single cell recordings were implanted into the left hippocampus of an adult (57 days old) Sprague Dawley rat. The electrodes consisted of 57  $\mu\text{m}$  tungsten wires insulated with polyimide arranged into an  $8 \times 2$  array. The anterior array was implanted in the pyramidal layer of CA3 of the left hippocampus, and the posterior array was implanted straddling the dentate gyrus and CA1 of the left hippocampus. The data analyzed in this paper were recorded from CA3.

To create the animal model of temporal lobe epilepsy [11], a 330  $\mu\text{m}$ , Teflon coated, stainless steel, bipolar twist electrode was implanted into the right hippocampus. 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 [11].

### B. Multielectrode potential recording

The multielectrode potentials were collected synchronously using a TDT RX7 stimulator base station (Tucker-Davis technologies, Alachua, FL) sampling at 24414.1 Hz. To emphasize the frequencies contained in action potentials, the raw waveforms were bandpass filtered between 500 Hz and 6 kHz. The local field potentials were extracted by a bandpass filter from 0.5 Hz to 200 Hz.

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TABLE I

CHARACTERISTICS OF INTERNEURONS AND PYRAMIDAL CELLS.

Cell type	Amplitude	waveform width	mean firing rate
Interneurons	$\approx 20 \mu\text{V}$	$< 0.6 \text{ ms}$	1.357 spikes/s
Pyramidal cells	$> 60 \mu\text{V}$	$> 0.8 \text{ ms}$	0.566 spikes/s

### C. Neuronal spike sorting

Spike detection and spike sorting were carried out by an experienced experimenter using Spike 2 (Cambridge Electronic Design, Cambridge, England), and spike times were extracted for 1.5 hours leading up to each seizure. This time period was chosen to guarantee that the signals for analysis were recorded from interictal states. In addition, it could help us to investigate how interictal spiking changes before seizures. Spike detection and sorting were done for four channels (channels 1, 6, 8 and 14) in CA3. The neurons were sorted into two types: interneurons and pyramidal cells. The discrimination has been based upon spike amplitude, waveform width, and firing rate using the identification guideline in [12]. The characteristics used to identify these two neurons are listed in Table I.

### D. Interictal spike detection

Using similar methods but different criteria, we detected interictal spikes using Spike 2. The interictal spikes were detected from one of the four channels mentioned above. Following the definition given by [13], we set up our criteria: the amplitudes are in the range of  $250 \mu\text{V}$  to  $550 \mu\text{V}$ ; the spike is followed by a slow wave; the duration of the spike together with the following slow wave is approximately 140 ms. The occurrence times of IS were marked at the IS negative peaks. Fig. 1 shows one interictal spike detected in this experiment.

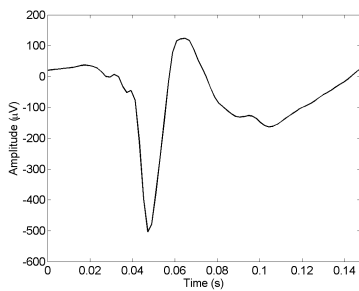


Fig. 1. A typical interictal spike detected in this experiment. The amplitudes of the interictal spikes in this experiment are in the range of  $250 \mu\text{V}$  to  $550 \mu\text{V}$ , and the duration of a spike together with the following slow wave is approximately 140 ms.

## III. DATA ANALYSIS AND RESULTS

For 19 days, 9 seizures were recorded. For the reasons stated in section II-C, We analyzed 1.5 hour interictal period leading up to each seizure. For each cell, spike times were binned to obtain the firing rates. The bin size was chosen to be 10 seconds considering the mean firing rates of the two

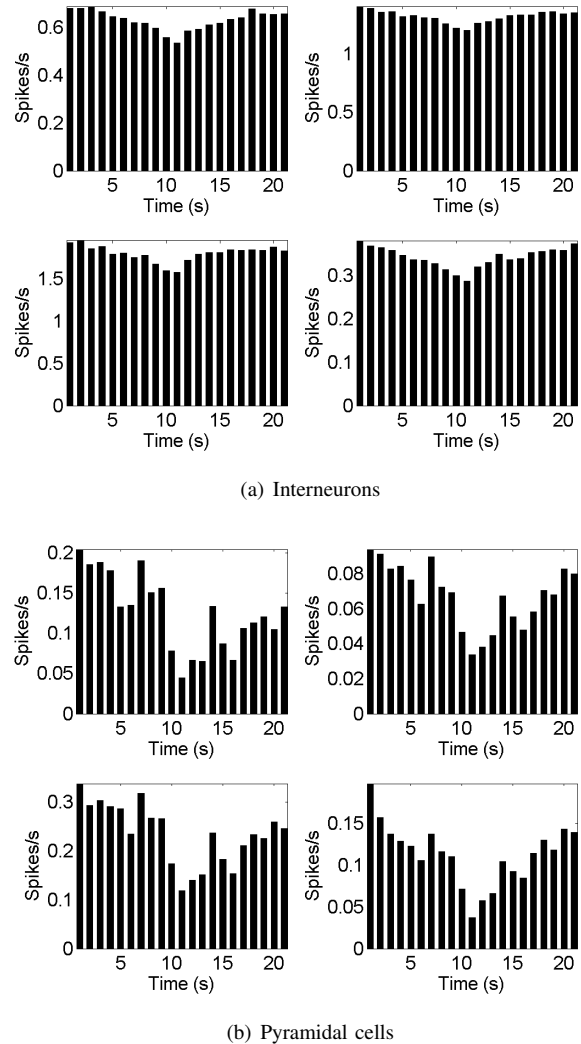


Fig. 2. The interictal spike triggered average of neuron firing rates prior to and following IS. This was obtained by averaging neuron firing rates within 100 seconds prior to and 100 seconds following IS over all 9 interictal time periods for each neuron. (a) are the IS triggered average of firing rates of 4 interneurons from microelectrode channels 1, 6, 8 and 14, respectively. (b) are the IS triggered average of firing rates of 4 pyramidal cells from the same channels. The bin size is 10 seconds. Time 0 is at the IS negative peak.

types of neuron (1.357 spikes/s for interneurons and 0.566 spikes/s for pyramidal cells). Then, the IS triggered average of firing rates during 100 seconds prior to and 100 seconds following IS were averaged over all 9 interictal periods. Fig. 2 displays this result for each cell. It shows that reduced firing rates are associated with the IS occurrence times. Of note, time 0 in this figure corresponds to the IS negative peak so that the values at time 0 in Fig. 2 are the mean firing rates 10 seconds after the IS negative peaks. For interneurons, the firing rates seem to reduce linearly prior to IS, and follow a trend of an arctan function during their increasing after the IS. The firing rates of pyramidal cells fluctuate a lot around the occurrence of IS. For pyramidal cells, the peak 40 seconds before an IS might serve as an indicator of the IS

TABLE II

T-TESTS COMPARING THE MEAN FIRING RATES OF SINGLE NEURONS 10 SECONDS BEFORE IS AND AT RANDOM TIMES, AND COMPARING THE MEAN FIRING RATES OF SINGLE NEURONS 10 SECONDS AFTER IS AND AT RANDOM TIMES. AND PAIRED T-TEST COMPARING THE MEAN FIRING RATES OF SINGLE NEURONS 10 SECONDS BEFORE IS AND 10 SECONDS AFTER IS.

Cell indices	10 sec before IS	10 sec after IS	At random times	P-value (before)	P-value (after)	P-value (paired)
Interneuron 1	$0.62 \pm 1.26$	$0.60 \pm 1.22$	$1.35 \pm 2.32$	$< 0.0001$	$< 0.0001$	0.053
Interneuron 2	$1.27 \pm 1.50$	$1.26 \pm 1.50$	$1.87 \pm 2.00$	$< 0.0001$	$< 0.0001$	0.203
Interneuron 3	$1.69 \pm 1.10$	$1.64 \pm 1.08$	$2.50 \pm 1.73$	$< 0.0001$	$< 0.0001$	0.055
Interneuron 4	$0.31 \pm 0.39$	$0.30 \pm 0.37$	$0.60 \pm 0.75$	$< 0.0001$	$< 0.0001$	0.047
Pyramidal cell 1	$0.08 \pm 0.56$	$0.05 \pm 0.51$	$0.48 \pm 1.18$	$< 0.0001$	$< 0.0001$	0.100
Pyramidal cell 2	$0.05 \pm 0.24$	$0.04 \pm 0.20$	$0.39 \pm 1.00$	$< 0.0001$	$< 0.0001$	0.179
Pyramidal cell 3	$0.18 \pm 0.76$	$0.14 \pm 0.72$	$1.12 \pm 2.60$	$< 0.0001$	$< 0.0001$	0.095
Pyramidal cell 4	$0.07 \pm 0.39$	$0.04 \pm 0.31$	$0.62 \pm 1.41$	$< 0.0001$	0.005	0.038

although we are not ready to explain the mechanism under it yet.

Based on this observation, we employed t-tests to test our hypothesis that the neuron firing rates associated with IS are lower than those at random times. We constructed surrogate firing rates by selecting from random locations an equal number of 10 second time intervals as IS for each interictal period. These random times were guaranteed not to be within 10 seconds prior to or 10 seconds following any IS. Then, we applied a one-sided t-test to compare the mean firing rates 10 seconds before the IS negative peaks and the mean firing rates within 10 seconds at random times. The same test was applied to compare the mean firing rates 10 seconds after the IS negative peaks and the mean firing rates within 10 seconds at random times. The mean, standard deviation and p-values are given in Table II. In this table, 'P-value (before)' gives the significance of the difference in the two mean firing rates of the first t-test described above, and 'P-value (after)' gives the results of the second one. If p-value less than 0.05 is considered significant, we can accept our hypothesis, and conclude that an IS is associated with a profound and prolonged single neuron refractory period for all the neurons we analyzed. We also noticed that, in Table II, the mean firing rates before the IS were slightly higher than those after the IS. Therefore, we compared the mean firing rates 10 seconds after the IS with those 10 seconds before the IS, using a paired t-test. The p-values are  $\leq 0.05$  only for interneuron 4 and pyramidal cell 4. This paired t-test does not show statistical significance to claim that the mean single neuron firing rates 10 seconds before the IS are greater than those 10 seconds after the IS for all neurons.

We then observed the firing pattern of IS. The raster plot in Fig. 3 displays paroxysmal interictal spiking for each interictal period. For this, we questioned whether these flurries of IS can cause sustained decrease of single neuron firing rates. Fig. 4 shows the perievent time histograms of interneurons and pyramidal cells aligned with the raster plot of IS for the ninth interictal period. It is easily verifiable that a flurry of IS is associated with a sustained suppression of neuron firing. In fact, for all the interictal periods, we

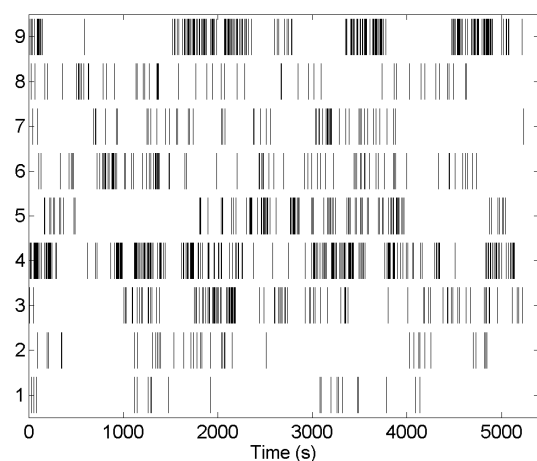


Fig. 3. Raster plot of interictal spike trains for 1.5 hour of each interictal period. The short vertical lines in each row mark the times when an IS appears. Each row displays one interictal spike train, with the bottom row being the first interictal period and the top row the ninth interictal period.

observed that the single neuron firing also had paroxysmal patterns, switching between long term high firing and long term low firing phases. It is interesting that these phases match very well with the firing pattern of IS in an inverted way as shown in Fig. 4.

Finally, we found that, for all the interictal periods, IS stopped as approaching to a seizure. No interictal spikes were observed within 1264.6, 557.35, 176.14, 265.6, 357.18, 664.85, 167.78, 768.96, 182.22 seconds before a seizure for the 9 pre-seizure periods, respectively. We noticed that there were also other epochs when IS did not appear for a certain period of time, but we could show that these gaps were due to randomness, by giving the mean firing rates of IS over all the pre-seizure periods. Fig. 5 displays the perievent time histogram of IS, which was obtained by binning IS firing times of all the pre-seizure periods into 20 second bins. There are no interictal spikes for a duration of approximately 168 seconds before the seizure while other time portions do not have such significant gaps during which no IS appear.

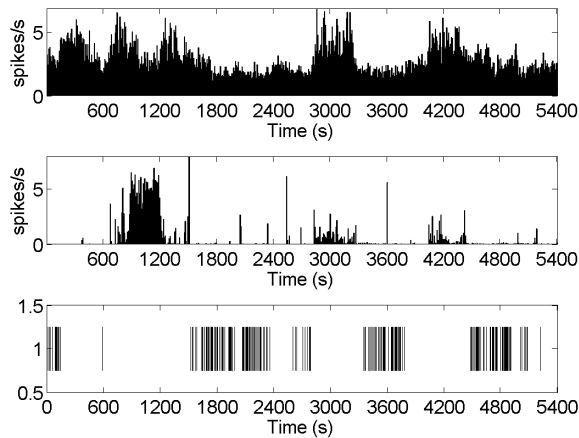


Fig. 4. Perievent time histograms of interneurons (top) and pyramidal cells (middle), aligned with the raster plot of IS (bottom). The bin size for the histograms is 10 seconds.

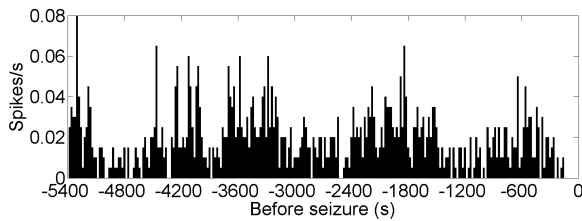


Fig. 5. Perievent time histogram of interictal spike trains. The interictal spiking stops approximately 168 seconds before the seizure. The bin size is 20 seconds.

#### IV. DISCUSSION

To examine the relationship between interictal spikes and single neuron firing, the firing rates of interneurons and pyramidal cells associated with IS were analyzed. The t-tests demonstrate that the single neuron firing rates are reduced within 10 seconds before and 10 seconds after the IS, compared with those at random times. This result is consistent with [9], in which decreased interneuron firing associated with IS was demonstrated for signals recorded in CA1 of pilocarpine-induced status epilepticus and recurrent flurothyl models. In [9], place cells in CA1 did not show distinguishable decrease at the occurrence times of IS, while we observed that firing rates of pyramidal cells in CA3 were reduced within at least 10 seconds after the negative peaks of IS. In addition, we compared the mean firing rates of single neurons 10 seconds before the IS with those 10 seconds after the IS. The paired t-test does not show statistically significant evidence that the mean firing rates of single neuron 10 seconds before the IS are higher than those 10 seconds after the IS for all the neurons. We also found that IS had paroxysmal firing patterns for each interictal period precursing a seizure. By noting that single neurons had distinct long term high-firing and low-firing periods, we showed that flurries of IS correlated well with the suppression phases of single neuron firing in time. Previous studies [14] demonstrated that status

epilepticus is followed by cell and cognitive impairments. Although not confirmed, our results place a possibility that these impairments are caused by the contribution of IS in changing the single neuron firing rates. It has also been proposed that interictal spiking prevents seizure precipitation in some animal models [5], [6], [7], [8]. According to our results, this might be due to the fact that IS suppress the hyperexcitability of certain types of neuron.

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