UNIVERSITY OF CALIFORNIA, SAN DIEGO

CA1 Interneurons Robustly and Consistently Increase their Action Potential Firing Rates in the Minutes Preceding Seizures in the Chronic Model of Temporal Lobe Epilepsy

A Thesis submitted in partial satisfaction of the requirements

for the degree Master of Science

in

Biology

By

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2013

The Thesis of Liang Liang is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego
2013

Dedication

I dedicate this thesis to my family, who has supported me endlessly

To my father, who pushes me to strive for more every day,

To my mother, who has helped me through thick and thin,

To my grandparents, who have cared for me since I can remember,

To my brother and sister, who brighten my day

I would also like to dedicate this to Carmen Yu, who pushed me to take on this endeavor

I would not be here today without you.

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Acknowledgements

I would like to thank Dr. Jill Leutgeb for giving me this chance to learn and study in her laboratory. Under her guidance, I was able to flourish as a scientist and gain a new perspective of the neuroscience field and the scientific method.

I would also like to thank Dr. Laura Ewell for all of her help through my duration as a Masters' student. Both she and Dr. Veronica Piatti have been patient and understanding while teaching me how to become a better scientist. They have always provided me with answers when I was lost or stuck. Because of them, I have learned an extraordinary amount from my time at in the Leutgeb Laboratory.

Lastly, I would like to thank Dr. Stefan Leutgeb and everyone else in the laboratory. Dr. Stefan Leutgeb took time from his busy schedule to personally help me with coding for analyses that I wanted to perform. Many of the other people in the laboratory, including Mandy Wong, Emily Mankin, and Vicky Lam, helped me with various aspects of my project for the past two years. I could not have finished my thesis without them.

Abstract of the Thesis

CA1 Interneurons Robustly and Consistently Increase their Action Potential Firing Rates in the Minutes Preceding Seizures in the Chronic Model of Temporal Lobe Epilepsy

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Liang Liang

Master of Science in Biology

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Professor Jill Leutgeb, Chair

Seizures reflect abnormal synchronized activity of a neuronal network, however, the activity dynamics preceding seizure onset are still poorly understood and algorithms for seizure prediction typically rely on local field potential recordings. Recent research has asked whether single-unit recordings might improve the predictability of seizures. Neuronal activity was found to change inconsistently before behavioral seizure onset

such that an increase in variability was observed in the minutes before the onset. GABAergic interneurons integrate excitatory inputs from local and afferent networks. Such convergence of input may allow interneurons to be more sensitive to widespread neural synchrony that leads to seizures compared to principal cells. We asked whether interneurons might be more reliable predictors of seizures than principal cells. To record activity patterns of interneurons and principal cells before behavioral seizures, we used the repeated low-dose kainate model of chronic temporal lobe epilepsy in male Wistar rats. We implanted animals that developed epilepsy ($\geq$ 2 spontaneous seizures) with tetrode arrays and video monitored the rats (n=3) while local field potentials and single unit activity were recorded from CA1 and CA3. We identified a total of 20 behavioral seizures (rat 1: 4 seizures across 4 days, rat 2: 5 seizures across 3 days, rat 3: 11 seizures across 5 days). We assessed the activity patterns of hippocampal pyramidal cells (n=193) and interneurons (n=69) during the 5 minutes preceding the behavioral seizure onset. First, we characterized baseline firing rates during 100 second epochs. We found that only ~4% of principal cells exhibited firing rates that deviated by more than 3 standard deviations from the baseline in the minutes before the seizure onset, whereas ~25% of interneurons deviated from baseline up to 2 minutes before seizure onset. The average increase in firing rate of all interneurons was +60% during the 2 minutes before the seizure onset compared to baseline. Interneurons are thus a much better predictor of seizures during the minutes before the seizure onset. Because of the large effect size, even small numbers of recorded interneurons can reliably predict a seizure. Together, these data suggest that knowing the change in firing from a previous seizure improves

prediction, but that the gains from using interneurons for detection algorithms would be much more robust.

1.0 Introduction

1.1 Epilepsy

Epilepsy is a common neurological disease that affects 50 million people worldwide (World Health Org., 2012). Epilepsy can result from a variety of factors; high fevers during infancy, insult to the brain, or even an initial unrelated seizure can cause people to develop epilepsy (World Health Org., 2012). There are various types of epilepsy, including Temporal Lobe Epilepsy, which is one of the most interesting types due to the region of the brain that is affected. The Temporal Lobe of the cortex is primarily responsible for memory and emotions, and any insult to this region can cause memory impairments.

The main symptom that is associated with epilepsy is seizures. Seizures are spontaneous neurological discharges that can be associated with a behavioral component. Generalized seizures can cause a patient to lose consciousness and violently convulse. About a third of epileptic patients have seizures and half of all new causes of epilepsy are associated with frequent seizures, called idiopathic epilepsy (Epilepsy Foundation of America). Because seizures are spontaneous, the main issue for these patients is the effect of possible seizures on their lifestyle. Driving becomes dangerous, and laws are in place to prevent epileptic patients who have reoccurring seizures from driving. Even daily activities are accompanied by the possibility of having spontaneous seizures. There has been extensive research into the mechanisms behind seizures and the possibility of predicting oncoming seizures. In order to predict seizures, researchers had to first define the mechanisms behind seizures and the changes to brain activity that may occur before a

seizure. Because of the prevalence of Temporal Lobe Epilepsy, researchers have focused their attention to the temporal lobe in seizure studies.

1.2 Medial Temporal Lobe

The medial temporal lobe is a region of the cortex that includes the hippocampus, medial entorhinal cortex, perirhinal cortex, and parahippocampal cortex. These structures are all important for episodic memory, and damage to this region can result in significant memory impairments (Rempel-Clower et al., 1996). The hippocampal substructure of the medial temporal lobe has been found to be especially important in the formation of new memories, and any damage to the hippocampus can itself cause impairment in memory acquisition (Milner & Scoville, 1957; Milner, Corkin, & Teuber, 1968).

One of the most profound cases in the study of epilepsy was the case of Henry Gustav Molaison (H.M.) H.M. had severe seizures that originated from his medial temporal lobe, which he then elected to surgically remove to prevent future seizures (Squire et al., 2002). Although this surgery may seem radical, surgical treatment for epilepsy is a common occurrence in the U.S. and worldwide (Smith & Cole, 2010). With Temporal Lobe Epilepsy, surgeons remove part of the medial temporal lobe, resulting in memory deficits. This surgery is a last resort for those patients who do not respond to traditional anti-seizure medication. Without the ability to predict the onset of seizures, epileptic patients who have seizures often choose to have surgery to eliminate spontaneous seizures.

It is hypothesized that the majority of the sustained changes that occur in temporal lobe epilepsy are in the hippocampus. During epileptogenesis, the hippocampal network is thought to be reorganizing after the extensive cell death within the structure. The changes in the hippocampal circuit, combined with abnormal neuron behavior, are thought to be the driving force behind the development of spontaneous seizures. Thus, the hippocampus has been a frequently visited structure in the epilepsy field.

1.3 Hippocampal Function

The hippocampus is a structure within the limbic system that deals primarily with the encoding of spatial and episodic memories (Tulving & Markowitsch, 1998). By associating the various parameters of the context, time, space, and emotions during an autobiographical event, the hippocampus is thought to encode episodic memories. We know that without the hippocampus, we are unable to create these autobiographical memories, resulting in anterograde amnesia (Milner & Scoville, 1957). This was seen in H.M., who elected to have part of his temporal lobe removed to prevent the frequent seizures associated with his epilepsy.

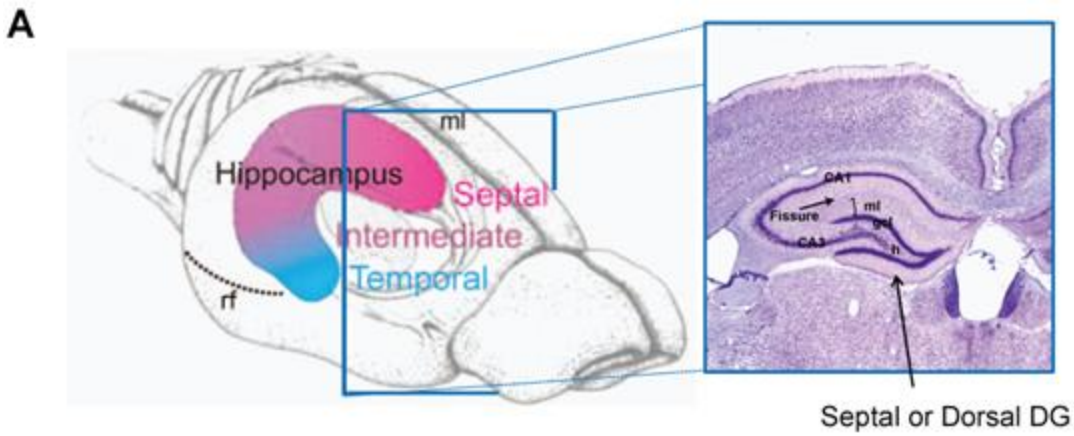

Figure 1 Hippocampus Anatomy. Figure 1, adapted from Bast et al. (2009), represents the rat hippocampus (colored shape) within the cortex. An example of the coronal slice is taken of the dorsal hippocampus and stained with cresyl staining to show the regions of the dorsal hippocampus. The darker regions represent the granule cell layers, where the soma of granule cells or principal cells is located. The regions labeled in the figure are: ML: molecular layer, GCL: granule cell layer, H: hilus, CA1: Cornus Amonnis 1, CA3: Cornus Amonnis 3.

1.4 Hippocampal Anatomy

The hippocampus and associated areas can be divided into four main sub-regions; the dentate gyrus, the hippocampus proper, the subicular complex, and the entorhinal complex (Amaral & Witter, 1989). The hippocampus proper can be further divided into sub-regions CA1, CA2, and CA3, and dentate gyrus (DG) (Amaral & Witter, 1989). Within the hippocampus, the DG, CA1, CA2, and CA3 regions create circuits that encode memory.

The hippocampus receives external input from layer II of the medial entorhinal cortex (MEC II) and the lateral entorhinal cortex (LEC II). These layers project inputs into the dentate gyrus and the CA3 layers via the perforant path input. The CA1 layer of the hippocampus also receives external input from layer III of the medial and lateral

entorhinal cortexes (MEC III and LEC III). These inputs send information from sensory lobes during every day events to the hippocampus for memory encoding.

Once inputs reach the hippocampus, the layers of the hippocampus establishes intrinsic circuits to maintain and encode the sensory inputs into memories. The dentate gyrus is the region that receives inputs from the entorhinal cortex and other cortical areas. The hippocampus has a larger circuit that is thought to be the mechanism of memory encoding, called the trisynaptic circuit. In this circuit, the dentate gyrus projects onto CA3 which projects axons called Schaffer collaterals onto CA1. In addition to input from CA3, CA1 also receives input from CA2. The CA1 region receives input from CA3 via Schaffer collaterals as well as input from the CA2 region. CA1 projects out into the subiculum and entorhinal cortex, which completes the trisynaptic pathway.

During the acute phase of temporal lobe epilepsy, we see widespread cell death in the dentate gyrus, CA3, and CA1 layers of the hippocampus. In the dentate, the local network is reorganized into a recurrent seizure-prone network (Scharfman, 2007). The surviving dentate cells begin to reorganize by sprouting their mossy fiber axons during the latent period. This reorganization is thought to be the origin behind seizures; it creates a circuit that is easily excitable due to a recurrent network

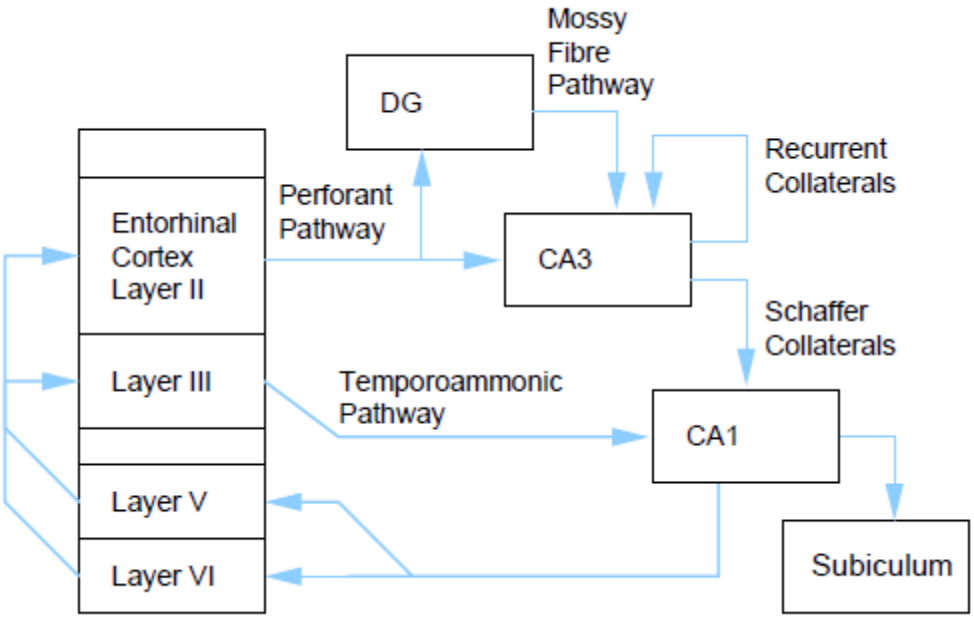

Figure 2 Temporal Lobe Circuit This is a simplified outline taken from Longden (2005) showing the connections within the hippocampus and its immediately adjacent structures. The trisynaptic loop consists of the entorhinal cortex, dentate gyrus, CA3, and CA1, respectively. Layer III's connection to the region CA1 is often called the termporoammonic pathway to distinguish it from the perforant pathway of the trisynaptic loop. CA1 then projects onto the subiculum to the rest of the cortex as well as Layers IV and V as a regulatory loop.

1.5 CA1 Anatomy

The area CA1 is especially interesting in the study of seizures because of its extrinsic connections. The region CA1 is a layer of pyramidal cells within the hippocampal trisynaptic pathway that receives input from the region CA3 and layer III of the entorhinal cortex. This region of the hippocampus is responsible for the majority of extrinsic projections from the hippocampus. CA1 projects not only to the subiculum, which acts as a relay from the hippocampus to the rest of the cortex, but it also projects onto the entorhinal cortex (Amaral & Witter, 1995). This projection can then affect

Layers II and III of the entorhinal cortex, creating a loop (Figure 2). Additionally, CA1 has very few intrinsic loops or in other words, CA1 has few local excitatory inputs (Freund & Buzsaki, 1998).

CA1 can be separated into laminar sub-regions; stratum oriens, stratum pyramidale, stratum radiatum, and stratum moleculare (Figure 3). The stratum pyramidale layer contains the cell bodies of the excitatory pyramidal cells of the CA1 region. This layer also contains many cell bodies of certain types of interneurons (axo-axonic cells, bistratified cells, basket cells, and radial trilaminar cells) of the hippocampus. The stratum oriens layer contains the cell bodies of additional inhibitory interneurons (basket cells and trilaminar cells). The stratum radiatum layer contains the CA3 afferents to the CA1 region (Schaffer collaterals). Lastly, the stratum moleculare layer is where projections from the perforant pathway synapse onto the pyramidal cells of CA1 (Freund & Buzsaki, 1998).

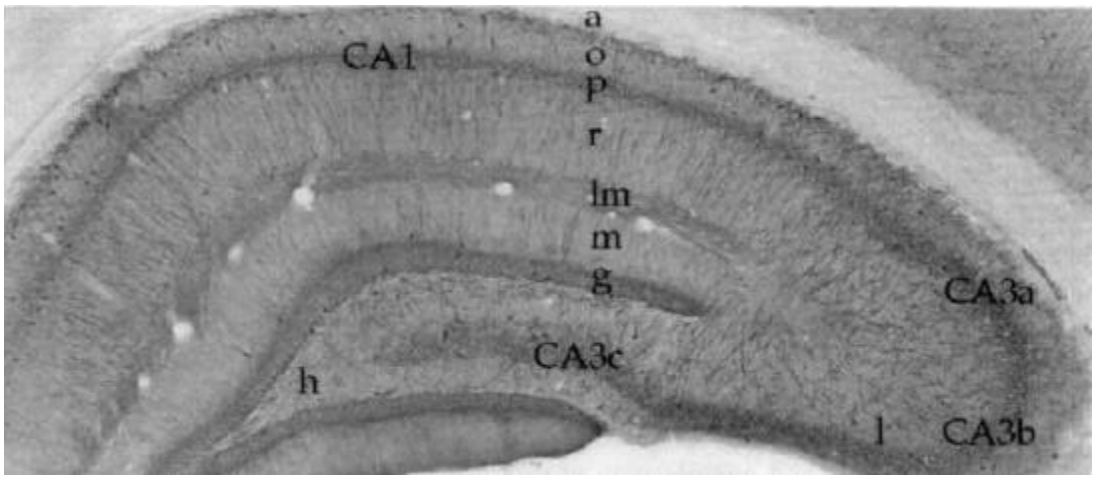

Figure 3 Dorsal Hippocampus Anatomy This figure adapted from Freund & Buzsaki (1998) shows a coronal section of the dorsal hippocampus. CA1 is shown to be separated into different layers with distinct properties. The dentate gyrus and CA3 are also shown in figure, distinguished as; m (dentate molecular layer), g (granule layer), h (hilar region), and CA3.

1.6 CA1 Cells

Within the hippocampus, there are also subsets of cell types. The main group of cells that is often studied in seizure prediction is principal excitatory cells called pyramidal cells or granule cells depending on the sub-region. This cell type can undergo synaptic changes for memory function. The other main subset of cells are interneurons which can have a variety of functions; some interneurons act as relaying stations and others act as regulatory units that project onto pyramidal cells. Most of these interneurons are GABAergic, which mean that they release the inhibitory neurotransmitter gamma-Amonibutyric acid (Freund & Buzsaki, 1998). Interneurons integrate excitatory input from local and afferent networks to regulate the intrinsic hippocampal and CA1 network. Because interneurons can integrate local and extrinsic inputs as well as regulate pyramidal cells, we focused on interneurons in our study.

1.7 Types of Interneurons

Interneurons within the hippocampus can either project onto the dendrites of other neurons, or they can project perisomatically (around the cell body of a neuron). Axo-axonic cells, chandelier cells, and basket cells are all considered to be perisomatic interneurons, which mean that they project on or near the cell bodies of the pyramidal cells. Perisomatic cells are inhibitory in nature and are thought to be tied to oscillations within brain waves (electroencephalograms or EEG's).

There are several types of interneurons that project to and from the region CA1 within the hippocampus. The first type of interneuron, basket cells, was originally described by Ramon y Cajal and named due to large basket-like distribution of axons and

dendrites (Freund & Buzsaki, 1998). Basket cells have their dendrites and cell bodies mainly located within the stratum radiatum, with the excitatory afferents coming from CA3 and the entorhinal cortex. More interestingly, these basket cells receive local excitatory input from recurrent pathways. The axons of these basket cells reach down into the stratum moleculare layer to synapse onto the local pyramidal cells. These basket cells are thought to play a regulatory role through feedback or feed-forward inhibition (Freund & Buzsaki, 1998).

Bistratified and trilaminar cells are additional interneurons that also project into CA1 and onto the dendrites of CA1 pyramidal neurons. Bistratified interneurons send out dendrites to both the stratum radiatum and the stratum oriens, while trilaminar cells project into CA1 and some regions outside of the hippocampus (Toth and Freund, 1992). Both of these interneurons control CA3 input by projecting axons parallel to the Schaffer collaterals synapsing onto the CA1 pyramidal cell dendrites.

One type of interneuron described by Attila Sik (1995) called back-projection interneurons, is largely restricted to the stratum oriens. These interneurons stay within the oriens and project horizontally to other pyramidal cells within the CA1 as well as the region CA3. The inhibitory nature of the back-projection interneurons act as a regulatory factor within the hippocampal circuit. The firing pattern of these interneurons may play a role in regulating the synapses of the hippocampal circuit, creating the opportunity for memory encoding through synaptic plasticity (Freund & Buzsaki, 1998).

Lastly, there is a class of interneurons within the hippocampus that synapse onto other interneurons, called interneuron-selective cells (IS cells). Although IS cells are located throughout the hippocampus, they are most densely populated within the CA1

region. There are multiple classes of IS cells, but the region CA1 is mainly composed of IS-1 cells. These IS-1 neurons have their cell bodies located within the stratum radiatum and project onto dendrites of various other interneurons. The IS-1 cells are thought to receive excitatory input from CA3 and entorhinal cortex, creating a feed-forward regulatory system. When this system of feedback and feed-forward regulation is interrupted, it can result in seizures shown in epileptic patients.

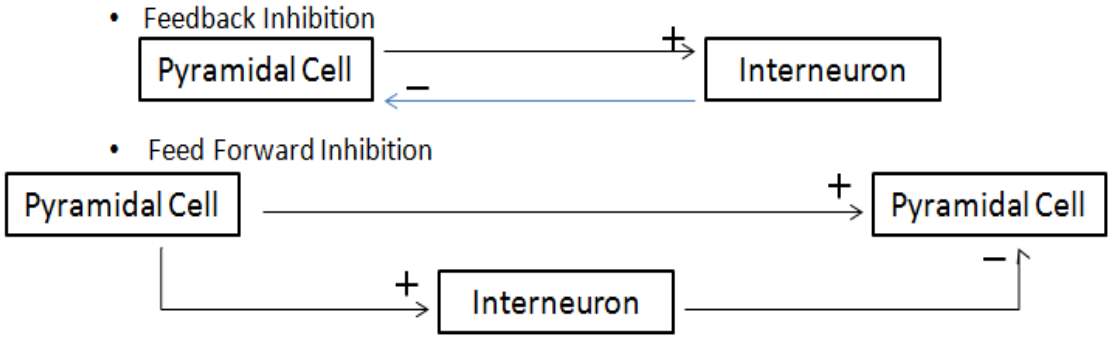

Figure 4 Interneuron Pathways This figure shows possible connections made by interneurons of the hippocampus and CA1. Interneurons are generally inhibitory, but this inhibition can be by feedback or feed forward. Additionally, interneurons can also inhibit other interneurons that project onto pyramidal cells.

1.8 Medial Temporal Lobe Epilepsy

Medial Temporal Lobe Epilepsy (MTLE) is a type of epilepsy that affects the circuits of the medial temporal lobe. Approximately 60% of epileptic cases are classified as Temporal Lobe Epilepsy, with Medial Temporal Lobe Epilepsy accounting for 80% of those TLE cases; the other 20% of TLE cases are neocortical TLE (World Health Org., 2012). Temporal Lobe Epilepsy is generally acquired through injury to the brain (i.e. head trauma, high fever, etc.) that can cause an initial seizure which later develops into

MTLE. The initial cause of MTLE is the widespread cell death within the different layers of the hippocampus, including the Dentate Gyrus, CA1, and CA3. Additionally, neurogenesis is decreased significantly, and the hippocampal circuit reorganizes from a feedback inhibitory system into a recurrent pathway (H.E. Scharfman, 2007).

Although little is known about the cause or specific origin of seizures, we know that they occur as a result of simultaneous activity of a population of neurons (Wu & Leung, 2003). In CA1, various studies have shown that the hippocampal CA1 neurons have increased excitation (Ashwood et al. 1986). Wu and Leung (2003) showed that excitatory Schaffer collateral inputs from CA3 and perforant path inputs from the entorhinal cortex had an increased response in the dendrites of CA1 neurons. It was also shown that inhibition within the hippocampal CA1 is decreased; this loss of inhibition is not a decrease in the activity of GABAergic interneurons or postsynaptic response to inhibition, but rather a decrease in the number of GABAergic interneurons (Ashwood & Wheal, 1985). Pairing of the decreased inhibition and increased excitation could be involved in the onset of epileptic seizures.

Previous research on seizure prediction has been focused mainly on the local field potential (LFP) taken from depth electrodes. These LFP recordings are a summation of all neuronal activity from the local area of an electrode. A previous study looked at predicting seizures in rats using complex computational analyses, with some success (Rajdev, 2010). The main issue with using the LFP as a basis for seizure prediction is that the LFP can be very difficult to analyze, requiring complex computations. Changes to a small proportion of the local neurons would not be identifiable solely through the LFP; these changes are masked by the general variability of the local neuron population. Only

large uniform firing by the local neurons will result in a noticeable change in the LFP. To further understand the mechanisms behind seizure generation, researchers need to look at the cortical activity at a higher resolution to correlate the local LFP with the activity patterns of single neurons. This lead to the study of single units, or individual neurons, using electrodes placed deep into the cortex.

Single unit recordings allow researchers to look at a small population of neurons, each of which can be separated and analyzed for their firing rates. This is generally done by implanting electrodes into the cortex and recording from the small population of neurons surrounding that electrode. In the seizure prediction field, single unit recordings of hippocampal neurons have been mostly focused on pyramidal cells, which are the primary neurons that fire bursts of action potentials followed by a period of inactivity. Using single units, researchers can look at the action potential firing rates for individual neurons surrounding a spontaneous seizure. In their paper, Buckmaster & Bower described changes in the activity of single neurons during periods of spontaneous seizures in a rodent model of temporal lobe epilepsy. They found that there were changes in the firing rate in several groups of DG neurons immediately preceding a spontaneous epileptic seizure (Buckmaster & Bower, 2008). These changes, however, were diluted by the consolidation of all single unit data into one population. This consolidation decreased the significance of the changes by introducing different cell types to a single data set; neurons showing varying changes in their firing rates were combined in one group. As a result, the changes to the population firing rate observed in the Buckmaster paper were minimal.

Another study by Cash et al. was one of a few studies that focused on single units in human patients. Their findings were similar to Buckmaster & Bower; the recorded hippocampal cells showed increased variation immediately preceding the onset of focal seizures in human patients (Cash et al., 2012). Similarly, the number of neurons that reflected an abnormal change preceding the seizure onset was small. Additionally, single neurons are extremely difficult to track during long periods of time in human patients. Because human studies are more invasive and not guaranteed to find consistent pre-ictal changes, we performed our experiments in rats. In rats, we can perform systematic implantations and locate an area or subset of neurons that is the best candidate for seizure prediction. This would give researchers a more specific target in their electrodes implants in human studies. In our study, we wanted to find a better candidate for seizure prediction than just the general population of neurons in the hippocampus.

Our study focused on separating the neuron population into subpopulations of pyramidal cells and interneurons. The previous papers by Buckmaster & Bower and Cash et al. both consolidated the hippocampal population into a single population, and their results showed that there were only a few groups of neurons that reflected a firing rate change before a seizure. We wanted to observe a similar region and observe these firing rate changes by specific cell types. Because interneurons are the integrative and regulatory units within the hippocampal circuit, we were especially interested in examining their firing rates preceding epileptic seizures. If these interneurons are crucial to controlling the circuit, then any abnormal firing rates by the interneuron population could cause a seizure. We think of seizures as abnormal widespread electrical activity that is associated with behavioral components.

A seizure can begin as a local seizure with the neurons of the region firing abnormally. Interneurons could be inhibiting the local seizure from expanding. If these interneurons are affected by the seizure in epilepsy, the local seizure can spread into a generalized seizure. This characteristic of interneurons makes them a prime candidate for a seizure prediction population. Our hypothesis is that interneurons change their firing rates at a higher rate and a higher consistency than principal cells in the chronic model of Temporal Lobe Epilepsy. We will test our hypothesis with two main objectives that aim to establish interneurons as a possible predictor for seizure activity. We will first determine if there are changes in the firing rate of CA1 and CA3 interneurons preceding the onset of motor seizures in Temporal Lobe Epilepsy. Once we establish that there is a rate change in the interneurons, we will ask if these changes in the firing rate of CA1 and CA3 interneurons preceding seizures more robust and consistent than pyramidal cells of the same layers.

In this study, we will record hippocampal single units in awake animals resting in a monitoring box. To mimic the traumatic brain injuries in human patients with temporal lobe epilepsy, we will use the excitotoxic drug kainate acid (KA) in Wistar rats. This drug replicates the insult to the temporal lobe and development of epilepsy similar to that seen in traumatic brain injuries (i.e. acute injury, latent period, chronic epilepsy) by over stimulating kainate receptors (ionotropic glutamate receptors) found exclusively in the DG (Moloney, 1998). The loss of DG interneurons and the sprouting of excitatory axons create a recurrent circuit that is very prone to overexcitement and seizures.

The KA treated animals will then be monitored for spontaneous seizures after a latent period of several months. If a rat is identified as chronic epileptic ($\geq$2 spontaneous

seizures), we implant the rat with a 14 tetrode array recording device. The single unit action potential firing rates preceding, during, and following the seizure will be plotted and compared to a baseline firing rate taken from several minutes up to an hour before the seizure onset. Understanding the possible changes that precede motor seizure onsets can provide researchers with a powerful tool for future clinical studies in humans. The ability to identify, predict, and prevent seizures is a crucial element in providing epileptic patients with a normal lifestyle.

2.0 Materials and Methods

2.1 Animals

A total of 3 Wistar rats originating from Charles River Labs were used in this study. The rats were induced at around 40 days and weighed 190-220 grams at the time of induction and kept in individual plexiglass cages from induction to perfusion. The rats with at least 2 behavioral seizures were implanted with hyperdrives and monitored with a Neurolynx Cheetah recording system with simultaneous video recording. The rats were kept in individual plexiglass cages during monitoring sessions which lasted between 3 to 6 hours with minimal disturbance. The animals were kept in a vivarium with a 12 hour light cycle during which animals were monitored during their dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego.

2.2 Low-Dose Kainate Administration

For induction of epilepsy, we followed a procedure devised by Hellier and Dudek (2005), in which they used male Sprague-Dawley rats. For our experiment, we used male Wistar rats. The rats were given hourly injections of low dose Kainic acid until they developed continuous seizures for three hours. We injected kainic acid intraperitoneally at a dose of 5mg/kg prepared with 0.9% saline. The rats were then reassessed after 60 minutes to determine the next dosage. Our assessments were based on a behavioral Racine scale (Hellier and Dudek, 2005), which categorized motor seizures into five separate stages; Stage I, facial automatisms/spasms and freezing; Stage II, wet dog shakes

and head nodding; Stage III, lordotic posture and forearm clonus; Stage IV, forearm clonus with rearing; Stage V, forearm clonus with rearing and falling over (Hellier and Dudek, 2005).

We administered 5 ml of saline subcutaneously to rats that survive 3 hours of continuous seizures. The saline alleviates dehydration caused by a food and water deprivation the rats undergo for the induction. The rats were then allowed to recover in the vivarium on surgical bedding to prevent them from choking on normal wood chip bedding.

2.3 Monitoring

After the rats are induced, we regularly monitored them for 1-2 days due to the possibility of overnight fatalities. After this initial monitoring, we leave the rats in a vivarium for approximately 5 months, which is sufficient time for animals to develop chronic epilepsy. After 4 months, we began to monitor the rats with a surveillance system I set up in the vivarium. Up to 6 rats were monitored at a single time via two video cameras set up for 4 hours of recording per week. We played back the videos at 4x speed to detect seizures of Stage III or higher. Rats were determined to be epileptic if we observed two or more Stage III seizures during monitoring.

Figure 5 Monitoring Setup The figure above is a picture of our setup for simultaneous video recording and single unit recordings. The rat is placed into the plexiglass box, which has an opening overhead to allow for the system to connect to the headstage of the animals. The animals are monitored in these boxes for up to 6 hours daily in a dark room.

2.4 Surgical Implantation

After animals were identified as chronic epileptic, they were selected for surgical implantation. During surgery, the rats (600-800g) were placed under isoflorance anesthesia (2-2.5% in O_2). The hyperdrives were permanently implanted above the right dorsal hippocampus closely to the following coordinates: 3.9mm posterior to bregma and 2.5mm lateral to the midline. All of the surgical procedures were adopted from J.K. Leutgeb et al. (2007) and met the guidelines set by the National Institutes of Health and

the University of California and were approved by the Institutional Animal Care and Use Committee.

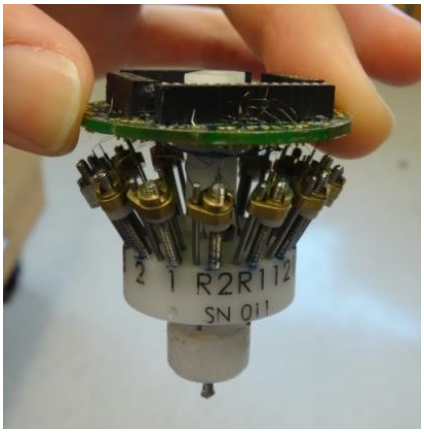

Figure 6 Multi-tetrode Hyperdrive. Our hyperdrives consist of 14 independent tetrodes. We implanted the hyperdrives above the right dorsal hippocampus on the surface of the brain following a craniotomy and dura removal. Two tetrodes were designated as references (R1 and R2); R1 was left in the cortex and used as a differential reference to eliminate cortical noise and R2 was lowered to the hippocampal fissure as a visual reference for LFP. The hyperdrive is connected to a multi-channel headstage and single units and LFP traces are recorded by a data acquisition program, Neuralynx.

2.5 Recording Device

Rats that were selected as chronic epileptic were implanted with a 14 tetrode hyperdrive built by Mandy Wong, a lab technician, and myself. The implanted hyperdrive could then be connected to a multichannel headstage that records the local field potential and single unit activity through a program Neuralynx. The local field potentials were amplified by a factor of 3000. Single unit activity (extracellular action potentials) that had a potential higher than 40μV were acquired through Neuralynx at 32 kHz and recorded in 1ms bins.

The hyperdrive has 14 individually controlled tetrodes; the tetrodes were made from 19μm polymide-coated platinum-iridium (90/10%) wire and tipped with plated platinum for minimal electrode resistance. All tetrodes are initially implanted directly on

the surface of the brain after a craniotomy and dura removal. These tetrodes are then turned 3 revolutions, which corresponds to 320µM per turn for a total of 960µM. Over the subsequent days, the tetrodes are individually lowered (turned) through the cortex into the hippocampus. We used the local field potentials of the tetrodes for guidance cues during the turning process. Two tetrodes were used as references; one tetrode was left in the cortex as a differential reference to eliminate cortical noise and the other tetrode was lowered into the hippocampal fissure as a hippocampal reference. Half of the 12 remaining tetrodes were lowered into the hippocampal CA1 on the first day and immediately brought back up to create tracts for subsequent recordings. This was repeated for the remaining half of the tetrodes the following day. The next step was to bring the tetrodes down to the CA1 layer by using distinct spiking and sharp wave patterns in the local field potential. The 120 Hz oscillation caused by the summation of CA1 population activity is a good indication of where the layer is, with these oscillations inverting when the tetrodes pass the layer. Due to the aberrant LFP of epileptic Wistar animals, we often relied on using theta rhythms to identify the hippocampal region. We begin monitoring once we had a consistent set of CA1 cells. Recording of the rats consisted of simultaneous video recording paired with the LFP and single cell recording system.

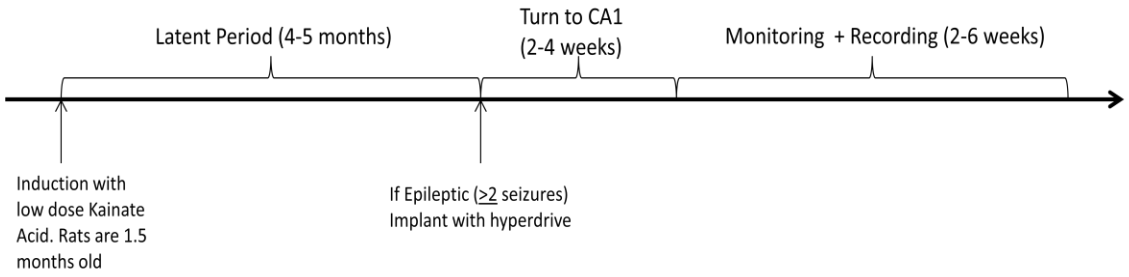

Figure 7 Experiment Timeline Our study uses Wistar rats in an experiment timeline shown above. We begin with 1.5 months old rats, which are induced with Epilepsy using kainate acid, and keep them in the vivarium for 4-5 months. After 4-5 months, during which the latent period will have passed, we implant rats with 2+ seizures and turn to CA1 for single unit recordings and video monitoring. As CA1 single units become unstable, we turn individual tetrodes to CA3 while recording.

2.6 Identifying Seizures

We used the local field potential as a basis for the identification of seizure activity. I scanned through each day of LFP data in LFP viewing software, Neuroview, for abnormal electrophysiological signatures. I marked the timestamps of every abnormal LFP event, which were then translated into video timestamps for seizure confirmation. I then visually confirmed the seizure activity in the video to eliminate abnormal EEG signatures due to noise. Each seizure event was then marked for the video time of the seizure onset, which was defined as the moment of forearm clonus. We used the video monitoring system to confirm the forearm clonus as the onset of seizures for consistency. Previous literature failed to establish a method to confirm the onset of seizures for consistency. This process is especially important to establish as the LFP can be inaccurate in establishing the onset of seizures. These moments were converted from the video time to a timestamp correlating to the Neurolynx software. We then went back to Neuroview to mark the timestamp defined as the behavioral seizure onset. In addition, we used

seizure identification software developed by Ivan Soltesz's lab (University of California, Irvine) as an alternative tool to find seizure onsets. However, we decided that using the behavioral seizure onset as our "seizure onset" was the most consistent method for characterizing a seizure episode.

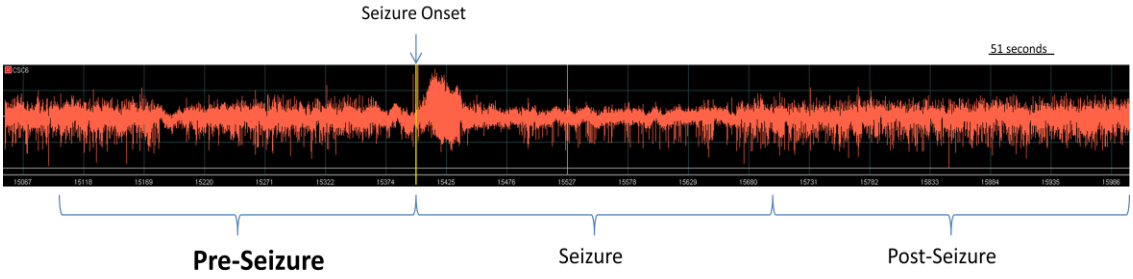

Figure 8 Segmenting Peri-Ictal Data The above figure is an example of how we segmented our peri-ictal data in the local field potential, or LFP. The yellow bar represents the motor seizure onset, and 5 minute segments are established surrounding the motor seizure onset. Pre-seizure: 5 minutes preceding motor seizure onset, Seizure: 5 minutes beginning at motor seizure onset, post-seizure: 5 minute recovery period.

2.7 Segmenting Data

In order for us to study the characteristics of single cells surrounding a seizure event, I segmented the data into three 5-minute blocks (preSeizure- 5 minutes up to behavioral seizure onset, Seizure- segment including behavioral seizure, and postSeizure- 5 minute recovery period following seizure). Single cells were not always stable and the recordings lasted up to 6 hours so trying to define single neurons from an entire day's worth of recordings was not efficient or feasible, therefore, in most cases we limited cell tracking to the three segments previously defined. After these 5 minute blocks were identified and marked, I could then continue to spike sort for single cells.

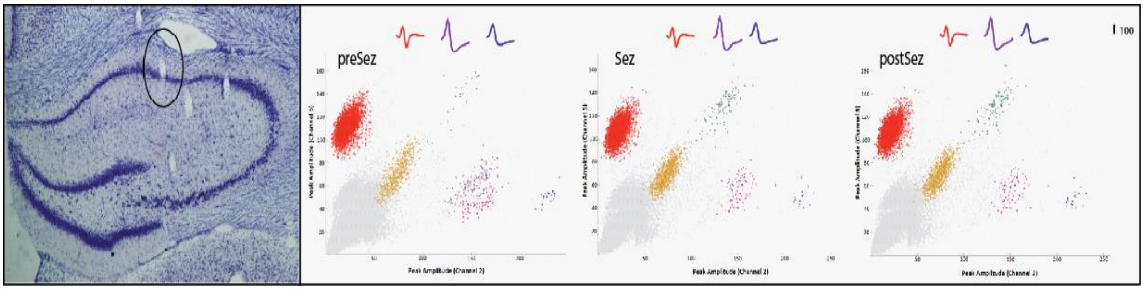

Figure 9 Tetrode Locations and Single Unit Cluster Tracking. Hippocampal CA1 single units were identified using MClust, a 2D graphical analysis program. This software analyzes different extracellular features of the action potentials of nearby neurons. These features are plotted in different electrode channels. Because we use tetrodes with 4 individual electrodes bound together, we can compare amplitude, energy, etc. by comparing them in different electrodes. . These features are determined by the distance the neurons are from each electrode. We can then isolate neurons by their location depending on how their features are plotted among the four electrodes. A single neuron will create a point on the plots every time it fires an action potential, and these points can be consolidated to create clusters. We isolate these clusters and define each cluster as an individual cell. In this example, we can see that the cells of this tetrode from CA1 are stable in the pre-ictal, ictal, and post-ictal segments of the data.

2.8 Defining Single Cells

I identified single cells by spike sorting using cluster cutting software, MClust. MClust allows us to use the different features of a neuron's action potential (amplitude, energy, etc.) to isolate different neurons. We use tetrodes (four bound electrodes in a square formation) so the system can plot these different features in graphs that compare them in the different electrode channels. If a neuron fires an action potential, a point will be plotted on the graphs dependent on the proximity of that neuron to each electrode. The same neurons will then always create points in the same area if the tetrode stays in place. These points create a cluster, which we can then isolate and define as individual neurons. I used autocorrelations (timing between spikes), waveforms (length and duration of spikes), and spike rates as methods to categorize neuronal cell types (e.g. pyramidal cells

and interneurons). I later used histology to confirm the location of tetrodes to confirm the layer of cells that we recorded from.

2.9 Data Analysis

We identified interneurons and pyramidal cells from different aspects of the waveform and spike timing of the neurons. Interneuron action potentials have faster repolarization rates than pyramidal cells (Figure 9). Interneurons are also fast spiking, while pyramidal cells have longer refractory periods of inactivity with instantaneous high rates of action potentials.

Spike times of individual neurons were binned (bin = 1 second) to determine spike rates (Hz) before, during, and after seizure events. Two baseline rate distributions were used for our analysis. To measure variation in our firing activity in the whole population of neurons, we used a 100 second period from 300 seconds to 200 seconds prior to the onset of the motor seizure onset to establish a baseline average firing rate for each individual neuron. We then calculated standard deviations for each neuron and defined a threshold for variation as 3 standard deviations above the mean firing rate. If the rate of a neuron during a given second (momentary rate) exceeds 3 standard deviations above the mean rate taken over the first 100 seconds of the recording epoch, then we would count that as a deviation from normal firing activity. A figure was made showing the percentage of neurons at every second during the peri-ictal period that had deviations from their normal firing rates for interneurons and pyramidal cells.

We then calculated a slightly shorter base-line firing rate to identify the sub-population of interneurons that were changing their firing activity on a minute by minute

basis. Base-line rate distributions were calculated for a one-minute segment from 300 seconds to 240 seconds prior to the start time of a seizure. Subsequent rates with z-scores greater than two or less than negative two were considered as significant deviations from baseline. We calculated z-scores by using the following equation:

Z-score (current bin) = [rate (current bin) – meanRate (baseline)] / std (baseline)

The z-scores compares the current firing rate of a cell to the mean firing rate of a cell by measuring how many standard deviations the current firing rate deviates from the mean firing rate. This calculation is done for every 1 second bin for every interneuron. We then defined a cell as changing over a given minute if the z-score of that cell is larger than 2 for at least 50% of the time (30 seconds out of 60 seconds).

2.10 Histology

We perfused the rat with 200 ml of PBS followed by 200 ml of 4% paraformaldehyde (PFA) in 0.1M PBS. The brains were extracted and kept in 4% PFA for 24 hours, then the brains were transferred to 30% sucrose in 0.1M PBS.

The brains were sagittally split and the left side of the brain stored in 0.2% sodium azide solution for future histology. The right half of the brain was frozen with dry ice and sectioned into 40 μm slices using a microtone. These slices were mounted onto microscope slides and stained using Nissl staining (cresyl violet for tract viewing).

The cresyl staining allowed us to reconstruct the structure of the hippocampus and identify the final locations the tetrodes. These final locations were identifiable by the tracts the tetrodes produced by damaging the brain tissue it was lowered through.

3.0 Results

The basis behind seizure prediction research is that there are abnormal changes leading up to the seizure. These changes may not be noticeable to an observer, or even the patient themselves, until the seizure begins. In our experiment, we recorded EEG, single unit activity, and video of rats to observe the changes in hippocampal interneuron firing rates before spontaneous epileptic seizures. Three Wistar rats were used for our study. These rats were induced at 1.5 months and implanted with a 14 tetrode array at 5-8 months after having two or more spontaneous seizures. After the implantation of the array (hyperdrive), we lowered the tetrodes individually to the putative cell layers in CA1 and began recordings when single units were identified (Figure 9). The animals were monitored 4-6 hours daily for several weeks. We gathered data from a total of 20 spontaneous motor seizures rated at Stage III (Forearm clonus and lordonic posture) or higher. There were a total of 25 distinct interneurons and 149 distinct pyramidal cells recorded during our video monitoring. For most of our analysis, we considered each seizure as an independent event and the same interneuron could be analyzed as independent in different seizures. Using this protocol, we expanded our pool of data to 69 interneurons (CA1, n=52; CA3, n=17) and 206 pyramidal cells (CA1, n=176; CA3, n=30). For every seizure, we assigned 5 minute segments peri-ictally and analyzed cells both in the entire peri-ictal period as well as solely in the pre-ictal segment.

3.1 Changes in the Firing Rate of CA1 and CA3 Pyramidal Cells and Interneurons

Studies into the events preceding spontaneous motor seizures have been popular, but they have mainly focused to looking into the electroencephalographs of humans with

Temporal Lobe Epilepsy. Some researchers have found success in using complex computations to identify seizures in rats (Radjev et al., 2010). However, many of these LFP studies are not able to discern the changes to the activity patterns of individual neurons preceding a seizure. The LFP is summation of the general variability in the population activity and the changes that individual neurons may present are masked in this variability. As a result, using the LFP as a basis for seizure prediction would require the system activity to be uniform or synchronous across a majority of the population. Additionally, in Radjev's paper, they were only able to identify an oncoming seizure 27 seconds before the onset of seizures. This would not be ideal for human patients because of the short amount of time before the seizure onset. We would need to look at the pre-ictal period at a higher resolution than the LFP if we want to extend the timeline of seizure prediction.

More recently, researchers have begun to look at the single unit firing activity preceding seizures (Buckmaster & Bower, 2008; Truccolo et al., 2011). Their data, however, did not consider subpopulations of hippocampal neurons separately even though different classes of neurons have different function and firing properties. Buckmaster and Bower (2008) showed that there were groups of neurons that increased their firing rates and other groups that decreased their firing rates before the onset of seizures. This variability in the firing rate of neurons in Buckmaster & Bower's paper and the small population of neurons that reflect any changes in their firing rates makes it difficult to predict seizures from several minutes before the onset. From the knowledge of interneurons being integrative and regulatory factors in the hippocampal circuit, we wanted to look at the action potential firing activity of interneurons.

We used the cheetah system to record single units, which were then analyzed and isolated using Mclust, a 2D graphical software. Interneurons were separated from pyramidal cells by looking at waveforms and spike timing. Interneuron action potentials have faster rates of repolarization (Figure 9). Pyramidal cells fire action potentials in instantaneously high rates followed by long periods of inactivity (Alger & Nicoll, 1980). We looked at the raw single unit firing data by plotting a raster plot of every action potential in the peri-ictal timeline (Figure 10) for one specific seizure. Interneurons have consistently high rates, which masks any changes in the firing rate in raster plot analysis. We observed changes to the pyramidal cell firing rates, which show that several cells show an increase in firing rates while others show a decrease in firing rates in the minutes preceding a motor seizure onset (Figure 10).

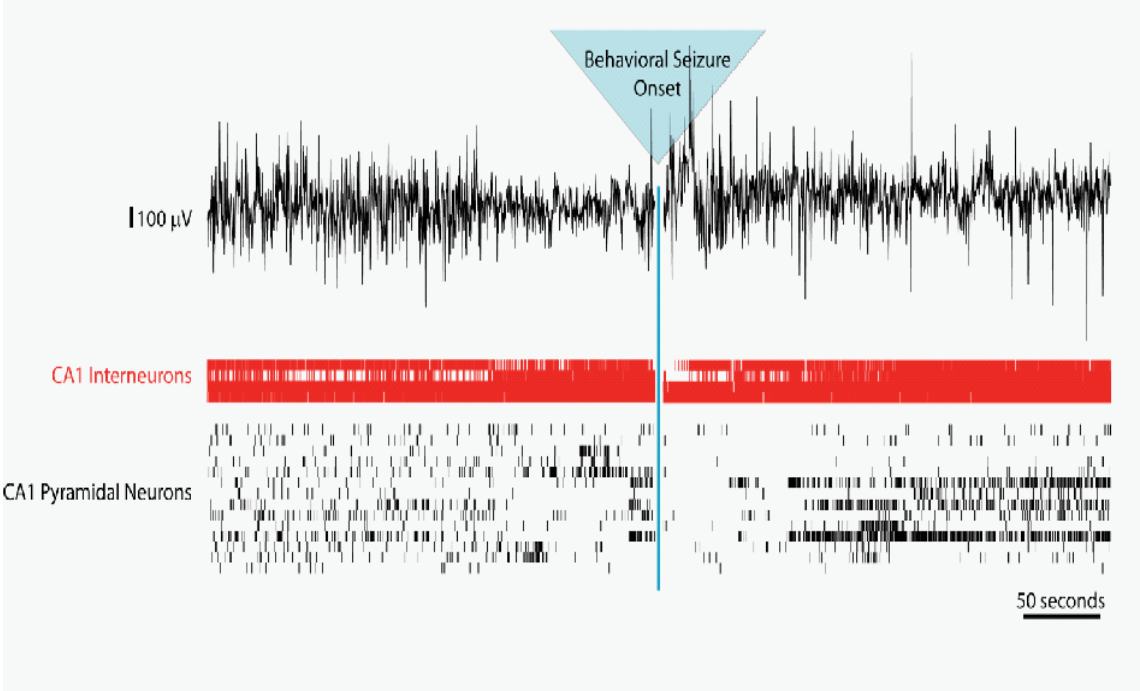

Figure 10 Raster Plot of a Single Seizure Onset. The above figure shows the raster plot of all single units of a sample tetrode spanning the 5 minutes preceding and 5 minutes following the motor seizure onset. This specific tetrode contains 4 interneurons (Red) and 15 interneurons (Black). Each action potential is shown by a single tick. High interneuron rates mask any change in the firing activity of interneurons, but we can see several pyramidal cells changing their firing rates preceding the motor seizure onset, marked in blue.

To observe changes in the firing activity of interneurons, we plotted the average firing rate of all interneurons at each second in the entire 15 minute peri-ictal period (Figure 11). This average was taken by summing the total firing rate of all interneurons at each second and dividing by the number of neurons. We had 40 interneurons and 120 pyramidal cells that were trackable across the entire peri-ictal period. In Figure 11, we can clearly see a change in the average interneuron firing rate that begins two minutes before the motor seizure onset. The average firing rate is the average rate across all interneurons that were trackable across the entire peri-ictal period (15 minutes) for all

seizures. In these 40 interneurons, there was up to a fifty percent increase in the average firing rate of interneurons immediately preceding the seizure. This augmented firing activity of the interneurons suddenly drops at the onset of the motor seizure and recovers again about 3 minutes following the motor seizure onset. Although the change in the pyramidal cell firing activity preceding the motor seizure onset is minimal, we observed a rapid decrease in the average firing rate before the firing rate returns to normal.

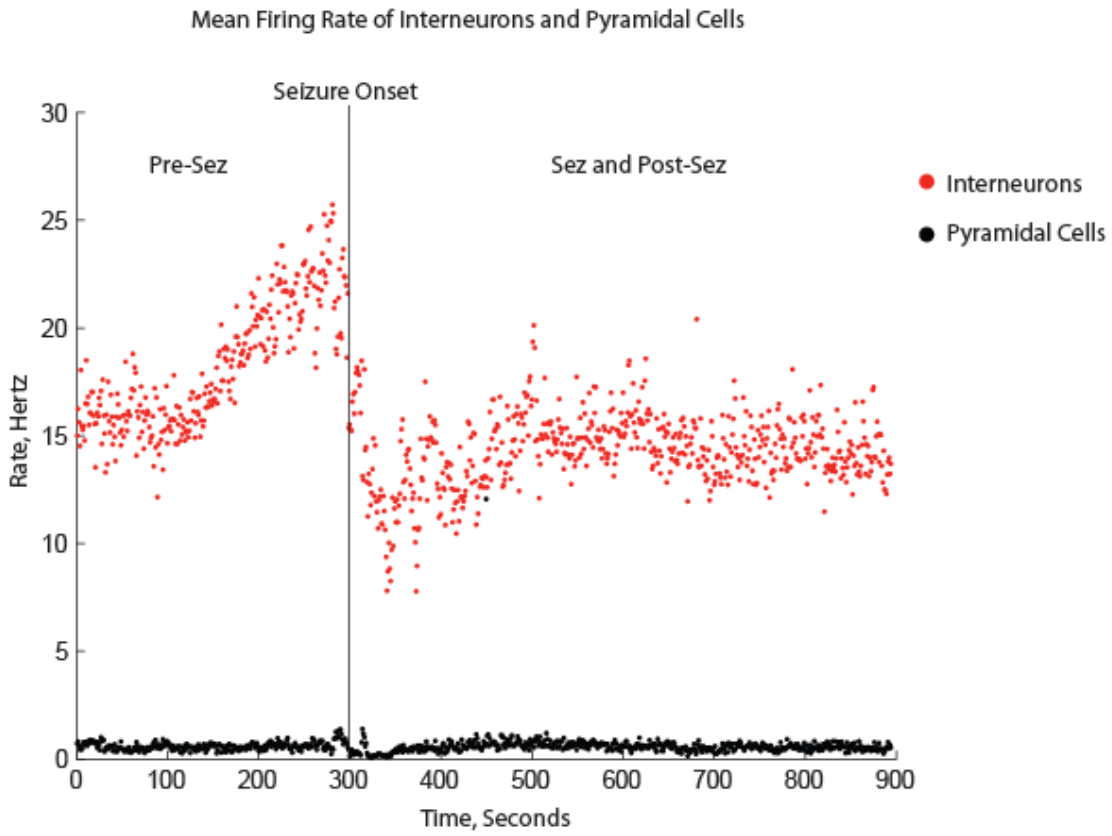

Figure 11 Mean Firing Rate of Interneuron and Pyramidal Cells. The figure above shows the mean firing rate of all interneurons and pyramidal cells that were trackable across the entire peri-ictal timeline. Interneurons, shown in blue, are shown to have a 50% increase in the mean firing rate that in the moments preceding the motor seizure onset. Pyramidal cells show no significant increase in their mean firing rates. Both sets of neurons show a significant decrease in their mean firing rates immediately following the motor seizure onset.

If we look exclusively at the 5 minute pre-ictal segment, we can expand our cell set to 69 interneurons and 193 pyramidal cells. This extra set of neurons is only present during the pre-ictal segment because they are lost during the motor seizure. Violent motor seizures can cause the tetrodes to move out of position, resulting in the loss of neurons during the motor seizure. With these extra neurons, we see a similar effect of an increase in the average firing rate of interneurons with no discernible difference in the average firing rate of pyramidal cells. This data indicates that the extra neurons that are lost during the motor seizure have no significant effect on the results.

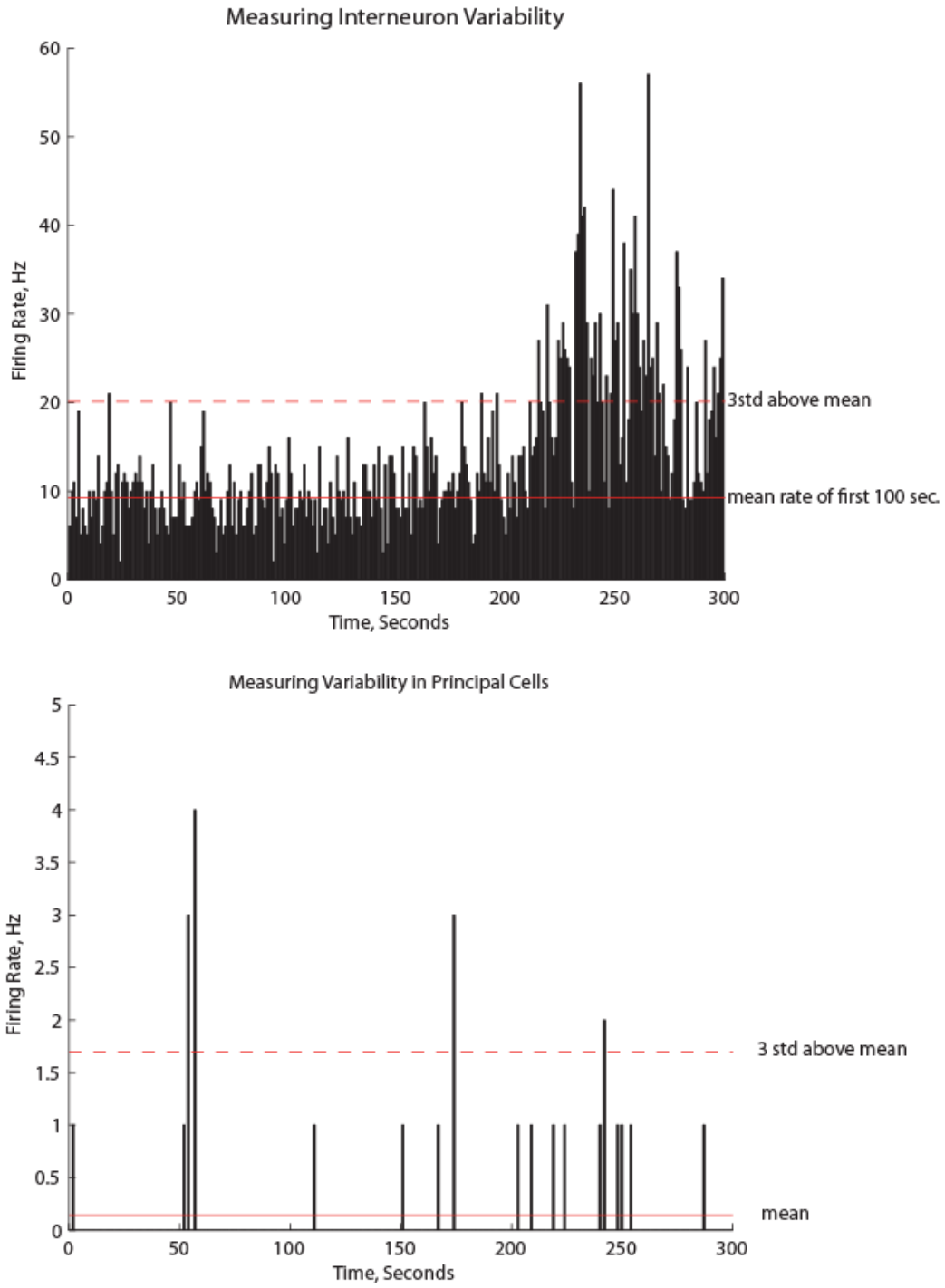

Figure 12 Establishing a Baseline Mean Firing Rate and Standard Deviation. The figure above represents the baseline firing rate and 3 standard deviations of a sample interneuron and pyramidal cell. The baseline mean firing rates and standard deviations were measured for each individual neuron. Each time the instantaneous firing rate of the neuron reaches above 3 standard deviations above the mean firing rate, we consider it a deviation from the normal firing rate.

3.2 Establishing a Threshold for Firing Rate Variation

From Figure 11, we know that there is a more robust increase in the average firing rate of interneurons than in pyramidal cells preceding the motor seizure onset. The next step was to identify the percentage of the interneuron population contributing to this increase in the firing activity. In order to identify the percentage of interneurons that show rate increases, we need to look at variation in the population firing activity. This is done by finding a baseline firing rate for neurons and recording each time the firing rate deviates from the baseline firing rate.

We first defined a baseline for the average firing rate for every individual interneuron and pyramidal cell. This is done by averaging the firing rate across the first 100 seconds of the pre-sez segment; this corresponds with the period from 5 minutes preceding the motor seizure onset to approximately 3 minutes preceding the motor seizure onset. After we established a baseline firing rate for individual neurons, we found the standard deviation for each neuron and set a threshold as 3 standard deviations above the mean firing rate. If a neuron's firing rate for a given second is higher than 3 standard deviations of the mean firing rate of that neuron, then that neuron's firing rate is deviating from the norm. We took the number of times interneurons reached or surpassed the threshold value and divided by the total number of interneurons at each second to get the percentage of interneurons that were deviating from the baseline at each second. This same procedure was done for pyramidal cells. We observe, however, that pyramidal cells fire with instantaneously high rates (Figure 12). The interneuron, however, has intrinsically high rates that ramp up at around 100 seconds before the seizure (Figure 12).

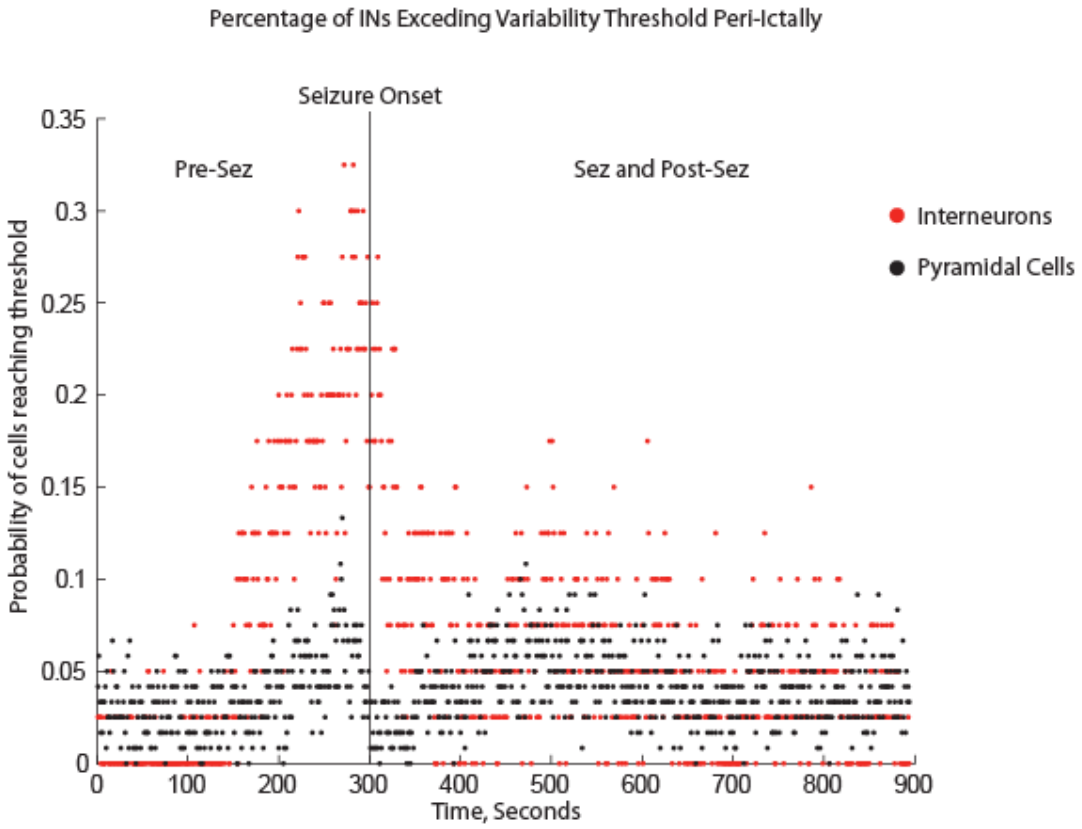

Figure 13 Variability of Pyramidal Cell and Interneuron Firing Rates in the Peri-Ictal Period. The above figure is a plot of the probability of the neuronal populations reaching above the 3 standard deviation threshold we set for individual neurons. The plot spans the 15 minute peri-ictal period separated into 5 minute segments. There is a marked increase in the variation from baseline of interneuron firing preceding the motor seizure onset that is not seen in pyramidal cells.

3.3 Pyramidal Cells have High Baseline Variation in their Firing Rates

Pyramidal cells tend to fire a burst of action potentials with a refractory period while interneurons are fast spiking (Wang, 2002). This characteristic of pyramidal cells creates low baseline mean firing rates due to the long periods of inactivity and high standard deviations because of high rates achieved during action potential bursts. The result of this characteristic causes pyramidal cells to have high baseline variation, shown in Figure 13. We can see that in the entire 15 minute peri-ictal timeline that pyramidal

cells (n=173 pyramidal cells) show consistency in the percentage of neurons that are deviating from the baseline. This result, however, is insignificant because the percentage of pyramidal cells that are deviating from baseline does not change even during the 100 second period in which the baseline firing rate was established (Figure 13).

Furthermore, we looked exclusively at the 5 minute pre-sez segment, which had a larger sample size (n=203 pyramidal cells). The result was similar to the peri-ictal result, with no significant difference in the percentage of pyramidal cells that reflect changes in their firing rates.

3.4 Interneurons show an increase the variability of firing rates preceding motor seizure onset

Interneurons have intrinsically high action potential firing rates. These high firing rates can mask any increase in rates in a raster plot, but we can see from Figure 11 that interneurons (n= 53) have an increase in average firing rate. The example shown in Figure 12 shows the increase in firing rate of a single interneuron. This increase begins around halfway through the presez segment (~150 seconds) and, for this example, exceeds the 3 standard deviation threshold more often as the timeline approaches the motor seizure onset. Figure 13 shows the percentage of the entire population of interneurons that reflects this increasing variation in their firing rates preceding the seizure onset. This increase in the percentage of the interneuron population showing deviations in firing rates begins around ~150 seconds preceding the motor seizure onset, with up to 40% of the interneuron population showing deviations from their mean firing rates. One noticeable characteristic of this variation is that the variation observed in the

interneurons is exclusively increasing; there are no instances when the instantaneous firing rate of an interneuron decreases below 3 standard deviations under the mean firing rate.

If we look exclusively at the 5 minute pre-sez segment, we can increase the sample size to 69 interneurons. These extra interneurons increases the percentage of interneurons that show deviations from normal firing rates even higher to 45-50%.

3.5 Interneuron Firing Rate Increases are Sustained Preceding the Seizure.

The next step in our analysis was to determine if there is a sustained change in the interneuron firing rates in the minutes preceding the seizure onset. We not only want to find populations of neurons that change their firing rates before the onset of a seizure, but we also want to confirm that these changes in the firing rates are abnormal and not due to a random burst of activity. This can be done by looking at the z-scores of the interneuron firing rates at any given second over the 5 minute pre-ictal segment. The z-score is calculated by first establishing a baseline mean firing rate over the first 60 seconds of the pre-ictal segment. We then used the equation:

$$Z - score = \frac{Rate(now) - \ Mean\ Rate\ (Baseline)}{Std\ (Baseline)}$$

We defined an interneuron as varying when its z-score is higher than 3, which means the rate at a given second is 3 standard deviations away from the mean rate at baseline. We plotted this data using a cumulative distribution of z-scores separated by each minute.

As the timeline approaches the seizure onset, we see more interneurons with a sustained high z-score. That means that these interneurons have high firing rates for a sustained amount of time in a given minute. For example, in the minute preceding the seizure onset, we can see that approximately 10 of the 44 interneurons spend at least 30 seconds with z-scores higher than 3. This means that these 10 interneurons have sustained high firing rates for more than 30 seconds preceding the seizure onset. Compared with the baseline, during which there are no interneurons that sustain their high z-scores (firing rates) for more than several seconds, we can see that this sustained high firing rate in a group of the interneurons is abnormal.

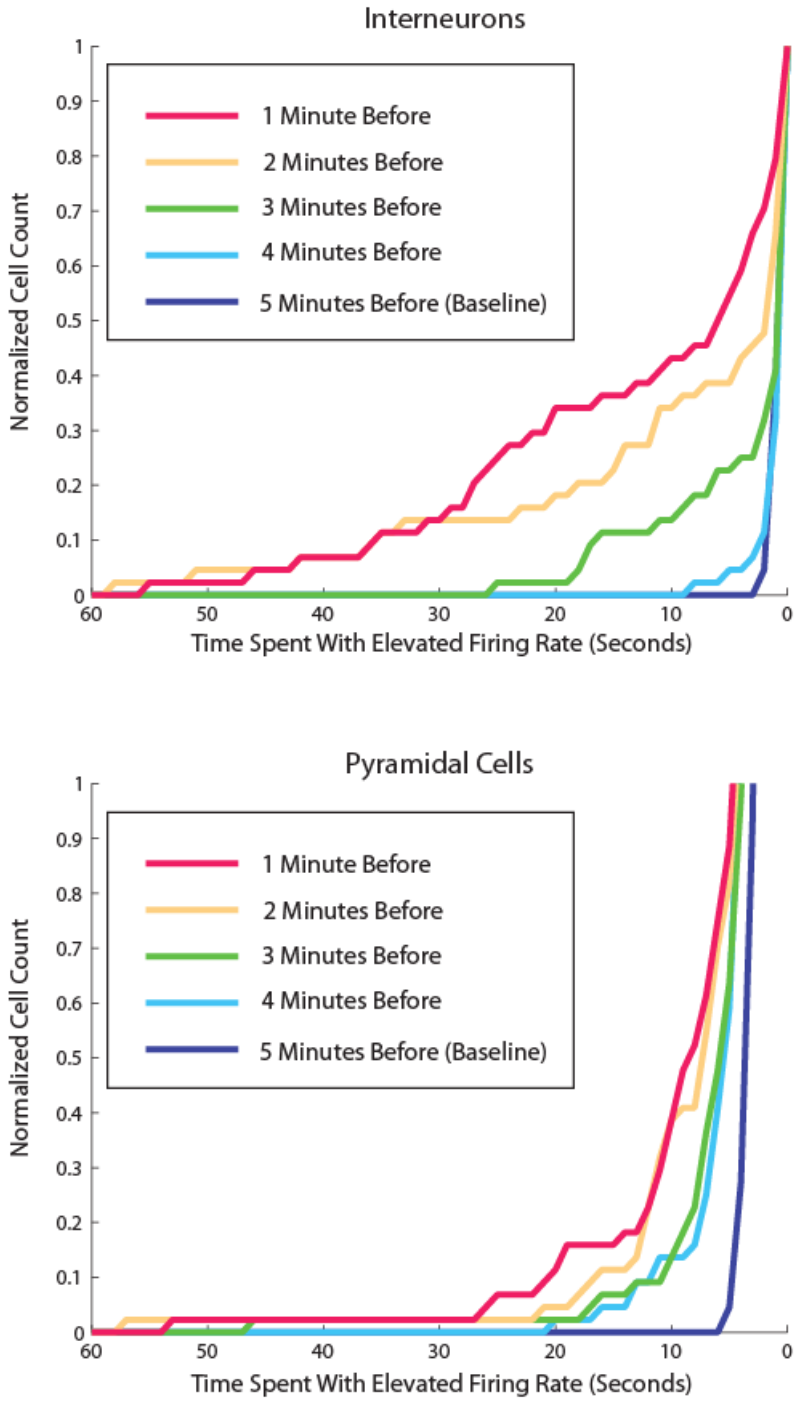

Figure 14 Sustained Rate Changes in Interneurons. The figure above shows the cumulative distribution of the z-scores of interneurons and pyramidal cells in the minutes preceding the seizure onset. You can see that in both groups, there are more cells that have sustained increases in their firing rates. Using three standard deviations above the baseline mean as a threshold, we see that interneurons are sustaining their high rates as the timeline approaches the seizure onset. The proportion of interneurons that has a sustained rate is also much more pronounced than that of the pyramidal cell population.

We defined an interneuron as changing its firing rate when the z-score was greater than 2 or less than 2 for 50% of one minute. We separated the presez segment into 1 minute bins to measure for the change in interneuron firing rates and found that the increase in firing rates begins halfway through the 5 minute segment. As the timeline progresses, an increasing number of interneurons show positive rate changes (Figure 14). In our preliminary results, we see a very high population of CA3/DG interneurons that show a positive rate change in the minutes before a seizure, while the number of interneurons that show a positive rate change was fewer for CA1.

4.0 Discussion

4.1 Average Firing Rate

Previous studies have shown that hippocampal pyramidal cells change their firing rates in the minutes preceding seizure onset (Buckmaster & Bower, 2008). These studies have shown that groups of neurons had changes in their firing rates before a seizure onset, but these changes were inconsistent and variable; some neurons showed increasing firing rates while other neurons showed decreasing firing rates. This pre-ictal variability makes the neuron population used by Buckmaster & Bower unreliable for seizure prediction. The origin behind the triggering of seizures is not widely known, but it is speculated that seizures could be a result of pathological changes to neurons that cause synchrony in their firing activity. In in-vivo studies using a different model, researchers have found that neuronal synchrony occurs in the moments preceding ictal spikes, a physiological indicator of seizure activity (Grasse, Karunakaran & Moxon, 2013). Because of the low percentage of hippocampal pyramidal cells that show a change in rate preceding seizure onset, we focused on a subpopulation of the hippocampal neurons, interneurons. Interneurons provide regulation to the hippocampal network through feedback and feed-forward inhibition, modulating the connections between pyramidal cell networks. This important trait leads us to assume that the onset of epileptic seizures could be correlated with abnormal firing of the hippocampal interneurons. We looked at both CA1 and CA3, but the focus was more on CA1 due to its connections to the local hippocampal circuit and the extrinsic temporal lobe network.

When we looked at the action potential firing rates of interneurons, we can see a clear increase preceding the seizure onset that begins 150 seconds preceding the seizure.

In the three rats, we see a 50% increase in the average firing rate of interneurons. We did not observe significant changes in the average firing rate of pyramidal cells preceding seizure onset. This result supports our hypothesis that abnormal interneuron rates can be used to predict the onset of seizures. In a recent paper by Grasse et al. (2013), they found that the firing rate of CA3 interneurons increases by 50% several seconds before the onset of seizures in Temporal Lobe Epilepsy. Our results are in line with the recent paper by Grasse et al., but our results suggest a longer timeframe of increasing firing rates preceding a seizure than the several seconds seen in Grasse's paper. An additional crucial difference between our study and Grasse's paper was the use of a video recording system to confirm the motor seizure onset. Without a video monitoring system to confirm that a behavioral seizure has taken place, it is difficult to determine how changes preceding the onset of hippocampal ictal spikes could be interpreted to predict the onset of motor seizure. From our results and those published from the Buckmaster lab, it is clear that motor seizures precede the onset of hippocampal ictal spikes. Interestingly, the increase in the interneuron firing rate is not related to any change in the pyramidal cell firing rate. We would think that an increase in the firing rate of inhibitory interneurons is a result of increased pyramidal cell input. The resulting increased inhibition from the interneurons should also decrease the rates of pyramidal cells, but we don't observe this either. We can assume that this is a result of the pathology of epilepsy; interneurons could be increasing their sensitivity to excitatory input or a physical change in the dendritic spines that receive input from other cells (Gulyas et al., 2006). The lack of a pyramidal cell response to the increased interneuron inhibition suggests that the axonal synaptic outputs are also affected; either these synapses had changes to their GABA release mechanism or if there

are modifications to the synapse itself is up for debate (Gulyas et al., 2006). In future studies, we hope to develop a detection method based on sustained increased interneuron firing rate that can accurately detect the onset of motor seizures.

An additional interesting result in our data shows a dramatic decrease in all neurons at the beginning of the seizure onset that was followed by several minutes before recovering. It could be that the interneuron increase could have been trying to stem a local seizure before the interneurons reach a threshold and fail. The failure to hold back the local seizure correlates with the sudden drop in the firing rates and the onset of a motor seizure. The subsequent increase in the hippocampal neuron firing rates could then be the widespread neural activity returning to the hippocampus. This is speculative based only on the analysis of the average firing rates.

4.2 Population Variability

After establishing that there is an increased average firing rate in interneurons, we wanted to look at the variability within the interneuron population. In the paper by Buckmaster and Bower, they only found a fraction of the pyramidal cell population that showed changes to the firing rates (Buckmaster & Bower, 2008). The goal for our project was to find a more suitable subpopulation of neurons that can be a better indicator of a seizure onset. We know that not all interneurons are recruited and/or affected in the ictal or pre-ictal segments (Figure 10). This is possibly due to the continued development of the disease pathology; we may have seen more extensive interneuron recruitment if the rats were studied at an older age.

Our results show that as the timeline approaches the motor seizure onset, more interneurons are recruited into firing abnormally. These interneurons generally receive and integrate input from other areas of the temporal lobe, such as the medial septum and entorhinal cortex (Buzsaki et al., 1983). These theta inputs from the other areas of the temporal lobe could be driving abnormal firing in the interneurons. Previous researchers have found that there is increased theta synchrony before the seizure onset (Grasse et al, 2013). This would explain the increasing interneuron firing preceding the seizure onset. Our next step would be to analyze the theta modulation in the pre-sez segment, which will let us know if our increased interneuron firing is driven by theta modulation. Another hypothesis for the increased interneuron firing is that these interneurons are trying to inhibit the increasing signals from within the hippocampus. At the point of the seizure onset, these interneurons fail to inhibit the internal signals from the hippocampus, resulting in the widespread cortical activity that is observed as the motor seizure. Future studies can test this hypothesis by cutting off the inputs to the hippocampus from other areas of the temporal lobe. Then the interneurons can be recorded from and analyzed for any increases in their firing activity. An increase in interneuron firing rates independent from extrinsic input from the medial septum and entorhinal cortex would suggest that these interneurons have intrinsic pathology that causes them to have abnormal rates.

4.3 Changes in Sub-populations

In our previous analysis, we found that a percentage of the population shows increased variation preceding the seizure onset. In our data, we looked at the entire interneuron set and found that about 40% of the hippocampal interneurons showed

increased firing in the minutes preceding seizure. We combined the interneurons from CA1, CA3, and DG in this result, but we wanted to look at the individual percentages of each subpopulation. A recent paper looked at CA3 interneurons, finding that about 43% of the CA3 interneurons had increased activity immediately preceding the seizure onset (Grasse, Karunakaran & Moxon, 2013).

We established a separate baseline and threshold to measure the separate groups of interneurons. Our results show that interneurons in both CA1 and CA3/DG showed higher percentages of the population that had increased firing rates than observed in the pyramidal cells. Our results, however, show that a higher percentage of the CA3/DG population reflected an increase in their firing rates compared to baseline. We believe that this could be attributed to the addition of DG interneurons into our CA3 group. The kainate acid directly affects the dentate gyrus by killing kainate sensitive neurons within the DG, and therefore could have caused interneurons within the DG to have increased pathology. The next step for this project would be to expand the data set to confirm the results that we have observed in our three animals.

4.4 Changes in Sub-populations are Sustained

Plotting the cumulative distribution of the z-scores allowed us to observe for sustained changes in the firing rates of interneurons. We found that as the timeline approaches the seizure onset, interneurons have increasingly sustained high firing rates, reflected by a sustained high z-score. This result confirms that these increases in the interneuron firing rates are pathological and not due to chance. During the first minute, interneurons do not sustain their high firing rates for more than a few minutes. We

assume that if there are random bursts of interneuron activity, the cumulative distribution of z-scores would be similar to the first minute of the pre-ictal segment.

The sustained high z-scores confirm that this change in interneuron firing rates is abnormal and possibly pathological. This result can be an additional feature for future seizure prediction studies because normal random increases in the firing rate of interneurons are not sustained.

5.0 Conclusion

Because of the importance of the role of interneurons' as regulators and relaying neurons in the hippocampus, we assume that abnormal interneuron activity would be correlated to the onset of a seizure. Additionally, we believed that CA1, with its extrinsic inputs from the entorhinal cortex and local inputs from CA3, would be a prime candidate for abnormal interneuron activity. Although we did find that the interneurons from CA1 had a higher percentage of the population showing increased action potential firing rates than pyramidal cells, the region CA3/DG had a much higher interneuron population that reflected abnormal interneuron rates preceding the seizure onset. Our study also shows that these changes in the increased rates in the interneurons are also sustained in the minutes preceding the seizure onset. We theorize that these increased rates seen in the interneurons of all regions could be due to an increase in the theta inputs from the medial septum and entorhinal cortex. This hypothesis could be further tested by severing those connections and observing for similar changes in the pre-ictal firing activity of interneurons. The higher rates in CA3 could be supported by this hypothesis, since CA3 receives much of its input from the entorhinal cortex via the perforant pathway (Figure 2).

By deciphering the changes in the single units of the hippocampus, we can better design processes that recognize the onset of seizures. This research could be greatly beneficial to neuroscientists and physicians who research the mechanisms of seizure onset in human patients. It provides a basis for future clinical research into the importance of certain cell types when trying to predict motor seizures. Hopefully in the near future, there will be seizure prediction software that can accurately and consistently

predict seizures. This could provide relief for the millions of epileptic patients who are affected by spontaneous seizures.

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