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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Dentate Gyrus Network Reorganization During Medial Temporal Lobe Epilepsy Leads to
Dysfunctional Dentate Neural Network in Rats

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Vicky Lam

Committee in charge:

Jill Leutgeb, Chair
James Brewer
Jon Christopher Armour

2012

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

Chair

University of California, San Diego

2012

DEDICATION

I dedicate this thesis to my loving family who has provided me with all their love and support.

To my father, whose ambition I admire.

To my mother, who has taught me to be humble.

To my grandmother and late grandfather, for their encouragement.

To my brother and sister, for watching over me.

I would also like to dedicate this to my boyfriend, Bang, for his patience and understanding.

Through even the most stressful of times, you were always there for me.

EPIGRAPH

“Go Big or Go Home!”

Unknown

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ABSTRACT OF THE THESIS

Dentate Gyrus Network Reorganization During Medial Temporal Lobe Epilepsy Leads to Dysfunctional Dentate Neural Network in Rats

by

Vicky Lam

Master of Science in Biology

University of California, San Diego, 2012

Professor Jill Leutgeb, Chair

Theoretical models have suggested that the dentate gyrus (DG) plays a significant role in pattern separation, an important neural-network computation that is critical for memory formation. Patients with medial temporal lobe epilepsy (MTLE), defined by having chronic unprovoked seizures, typically complain of memory impairments. There are many distinct anatomical changes that occur in many MTLE patients, including mossy fiber (mf) sprouting from hippocampal region the DGs granule cells. It is unknown as to whether the observed anatomical reorganization that occurs in the development of MTLE contributes to changes in dentate network function and consequently memory impairments. I therefore proposed to test network functions of the DG in a rat model of MTLE. In this study we looked at the permanent anatomical changes (mf sprouting) of the DG in relation to the functional changes (pattern separation) of the DG network and its downstream target, cornu ammonis region 3 (CA3), in

awake-behaving animals. Experiments testing the ability of the dentate network to pattern separate included rats foraging in a series of square and circular environments, while electroencephalograph and extracellular action potentials were recorded. The results showed that the increased amount of pathological reorganization found in the DG (mf sprouting) correlated with the DG cell populations' impaired ability to pattern separate. However, there was no effect on the CA3 region in epileptic rats when compared to control rats.

1.0 Introduction

The limbic system is made up of a set of evolutionarily conserved brain structures located above the brain stem mainly consisting of the amygdala, hypothalamus, and hippocampus. Together these structures are involved in processing and regulating emotions, motivations, sexual arousal, and memory. This system is well connected to the autonomic nervous system (ANS) and is essential for the body's response to stressful situations. Damage to the different structures in the limbic system can result in the loss of the ability to control certain feelings, regulating the function of the ANS, or formulating new memories. The hippocampus has been found to be an essential structure for memory formation (Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957). If the hippocampus is damaged or has suffered from a seizure there is a possibility of the patient developing medial temporal lobe epilepsy (MTLE) (Tulving & Markowitsch, 1998). This neurological disorder is characterized by recurrent, unprovoked seizures that originate from the medial temporal lobe (MTL) (World Health, World Health Organization. Department of Mental, & Substance, 2005).

1.1 Hippocampal Function

The hippocampus is a structure that lies within the limbic system, playing a significant role in forming spatial and episodic memories of our everyday lives (Tulving & Markowitsch, 1998). Episodic memories are those that are made up of autobiographical occurrences. This takes into account spatial, temporal, emotional, and contextual aspects involved in an event. It has long been known that without the hippocampus we would not be able to form any new memories, which is known as anterograde amnesia (Scoville & Milner, 1957). This was seen in the famous patient H.M. who suffered from epilepsy and underwent a surgical procedure in which large

portions of his hippocampus were removed as a treatment to prevent any further seizures from occurring (Milner et al., 1968). Due to this treatment H.M.'s MTLE was controlled. However, he suffered from severe anterograde amnesia and some retrograde amnesia (loss of memory for events preceding trauma/lesion) (Milner et al., 1968), illustrating the importance of the hippocampus for episodic memory formation.

1.2 Hippocampal Anatomy

Anatomically the hippocampus has unique architecture that is subdivided into several regions known as the Dentate Gyrus (DG), Cornu Ammonis Region 3 (CA3), and Cornu Ammonis Region 1 (CA1) (Figure 1A). The cortical input to the hippocampus forms the extrinsic connectivity (Figure 1B), where the DG and CA3 (downstream target of DG neurons) both receive perforant path input from the medial entorhinal cortex II (MEC II) and the lateral entorhinal cortex II (LEC II). CA1 also receives input from LEC III and MEC III.

There are three different types of intrinsic connectivity within the hippocampus itself: the tri-synaptic pathway, the micro-circuit of the DG, and the micro-circuit of CA3. The tri-synaptic pathway consists of the connectivity between the DG, CA3, and CA1. As shown in Figure 1B the DG receives information from MEC II and LEC II and sends its granule axonal projections to CA3 (these axonal projections are known as mossy fibers – mf). The projections from CA3 are then sent to CA1 and are known as schaffer collaterals (Figure 1B). The three neuronal synapses between the different regions are what make-up the tri-synaptic pathway.

The DG contains a densely packed layer of cells (D. G. Amaral, Scharfman, & Lavenex, 2007), which consist of a micro-circuit. A characteristic of this network of cells involving excitatory mossy cells (hilar cells – Figure 1A and B) that form an indirect recurrent feedback

circuit to DG cells shown in Figure 1B (D. G. Amaral, 1978). Mossy cells are excitatory intermediate cells that take in information from the dentate and CA3, which then send outputs back into the DG (D. G. Amaral, 1978). The mf from granule cells (gc) synapse onto mossy cells, which send back-projections into the DG. These excitatory back-projections towards the dendritic branches of DG cells can connect distant regions of the DG together as well (e.g. across hemispheres) (Ratzliff, Santhakumar, Howard, & Soltesz, 2002).

Similarly, the CA3 sub-region has a micro-circuit as well, but its network is formed by direct recurrent circuits. The micro-circuit consisting of a collateral fiber system as well as projections back out to cortical areas (D. G. Amaral, and Witter, M.P. , 1995). The CA3 can therefore create its own excitatory feedback loop with itself. Mossy cells also receive back-projections from CA3 neurons and can affect the DG (Figure 1B) (H. E. Scharfman, 1994). These recurrent circuits (both direct and indirect excitatory feedback loops) have been theorized to be the reason why this region is prone to epilepsy.

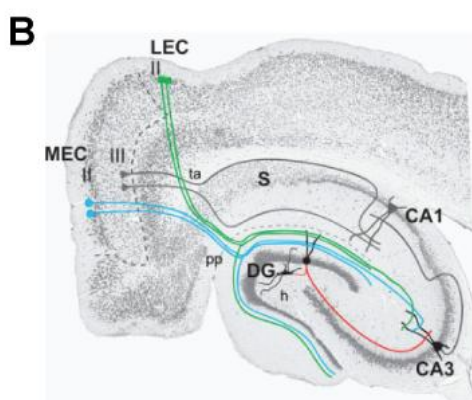
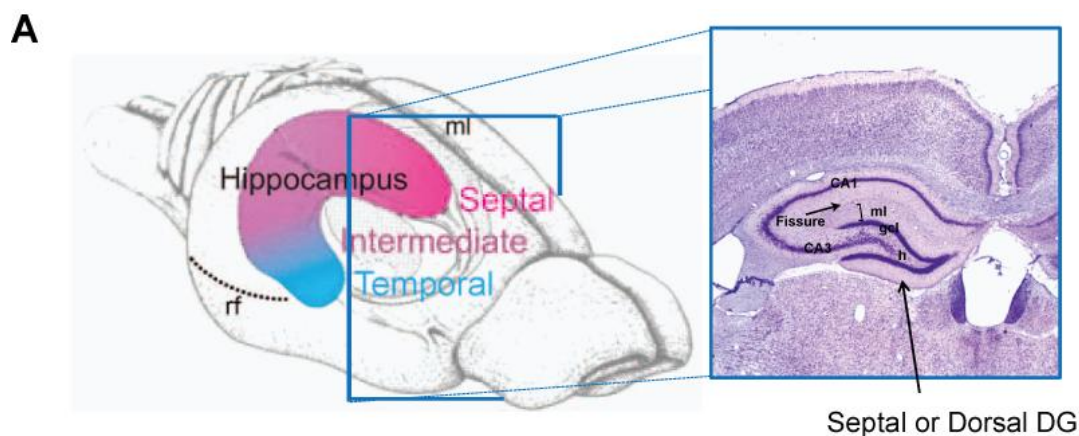


Figure 1 Hippocampus anatomy. **A** This modified figure adapted from Bast, Wilson, Witter, and Morris (2009) shows the orientation of the hippocampus (pink and blue in the background). The Nissl stained coronal slice of dorsal hippocampus is seen in the foreground, where the dark layer of purple are the stained nuclei of granule cell layer and principal cell layer. ML: molecular layer, GCL: granule cell layer, H: hilus, CA3: Cornu Ammonis 3, CA1 Cornu Ammonis 1. **B** A sagittal slice adapted from Jill K. Leutgeb and Leutgeb (2010) illustrates the connectivity of a rat's hippocampus. The dentate gyrus and CA3 pyramidal cell layer receives the same inputs from MEC II (blue) and LEC II (green), whereas CA1 receives input from MEC II. The dentate granule cell axonal projections (mossy fibers- red) synapse onto CA3 neurons, and back-projections from CA3 return to the dentate granule cells via mossy cells. S: subiculum, PP: perforant path, TA: temporoammonic-tract, MEC: medial entorhinal cortex, LEC: lateral entorhinal cortex.

1.3 Dentate Gyrus (DG) and Cornu Ammonis Region 3 (CA3) Function

My study focused on the first of the three sub regions that lie within the hippocampus, the DG. There are several characteristics making the DG a unique brain region and difficult to investigate. The region is composed of about 1.2×10^6 gc's, where less than 1% of the cell population is active at any one time (West, Slomianka, & Gundersen, 1991) making recordings of this region challenging. It is also one of the few areas where neurogenesis still occurs

(subgranular zone) and contains a competitive network, in which the excitation of certain cells will inhibit the activation of other cells. Some theories suggest that the mossy cells (excitatory cells of the micro-circuit in the DG - indirect recurrent network) are involved in this competitive network contributing to the sparse firing in the neuronal network (this is distinctive because this layer is densely packed with cells) of the DG by recruiting inhibitory (Myers & Scharfman, 2009). For this reason the DG is thought to work as a gate or filter for passing along neuronal information to its downstream target CA3, which could avert CA3 from overly exciting its recurrent circuitry and as a result preventing seizures from taking place (Hsu, 2007). This led to the belief that the hippocampus is susceptible to MTLE when there is trauma to the DG (Scharfman, 2007). What is the DG's contribution to memory processing in relation to the entire hippocampus?

Studies have shown that one of the DG's functions is to encode differences of very similar environments by making them dissimilar, a process termed as "pattern separation" (J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; McNaughton & Nadel, 1990). As an example, pattern separation mechanisms allow me to encode two similar but different memories, such as working in lab on Monday versus the memory that was formed when I was working in lab on Tuesday in the same environment. The ability of the dentate to pattern separate is observed in the firing pattern of cells (J. K. Leutgeb et al., 2007).

Additionally, CA3 is thought to be essential for encoding short-term memories, for pattern completion (retrieving stored patterns), in addition to playing a role in pattern separation (Kesner, 2007). These functions are hypothesized to use the interactions between DG and CA3 and the auto-associative network in CA3 (e.g. recurrent circuitry collateral fiber system) (Kesner, 2007; Marr, 1971).

These theories have been supported by the discovery of place cells in the hippocampus. Place cells will fire when the animal is in a specific region of the environment forming place

fields (map) seen in Figure 2 (O'Keefe & Dostrovsky, 1971). These fields are characterized by their spatial tuning in the open environment as well as the firing rate (non-spatial inputs) of action potentials. The important characteristics of these cells are capable of changing when the environment is altered, a process known as “remapping” (Muller, Kubie, Bostock, Taube, & Quirk, 1991). There are two types of remapping that have been identified within the hippocampal network: global remapping (spatial) and rate remapping (non-spatial) (S. Leutgeb, Leutgeb, Moser, & Moser, 2005). Global remapping occurs when both the spatial location of the place fields and non-spatial inputs of firing rate have changed in the new environment (S. Leutgeb et al., 2005). For example the place field of the cell forms a new field different from where it had been firing in the familiar environment along with a new firing rate, when the animal is exposed to a new room. However, rate remapping only consists of the change in firing frequency between cues in the same surroundings (J. K. Leutgeb et al., 2007). The encoding process is thought to be used to form episodic memories. A representative cell of rate remapping is shown in Figure 2, where the place field of the cell does not change between cues (square or circular environments) in the same room but the rate does. What types of information processing drives the global or rate remapping? Is one thought to be more particularly tied to dentate function?

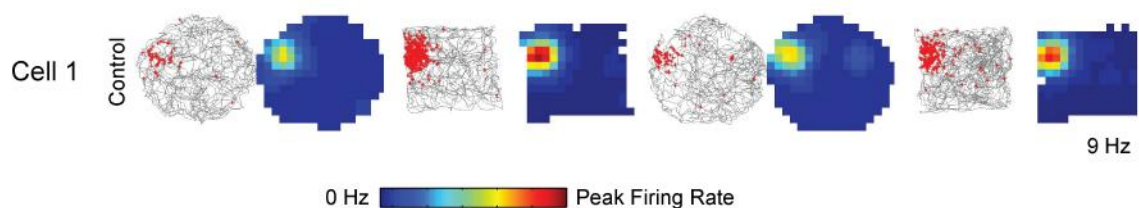


Figure 2 Firing fields and rate maps. This is a representative cell from CA3 illustrating rate remapping, data also shown in Figure 8A, when electroencephalograph (EEG) and single unit recordings of a rat freely forages for food in a series of four random square and circular environments (two of each). In the firing fields (left of each session) extracellular action potential spikes (red) is superimposed over the rats trajectory (grey). Color coded rate maps are shown directly to the right of each firing field session and provides the firing rate of each cell beneath it. Looking at these rate maps we can determine that there is rate remapping occurring because the cells peak rates change between shapes, but remains consistent within the same shapes. Blue = 0 Hz, Red = peak firing rate.

Previous research has proposed that spatial information (global remapping) comes from the MEC (Fyhn, Molden, Witter, Moser, & Moser, 2004; Hafting, Fyhn, Molden, Moser, & Moser, 2005). Input from the MEC changes when the animal is in a new place and therefore forms a new spatial map when in a completely different surrounding. LEC function is still unidentified but is hypothesized to give rise to the firing rate (rate remapping) (J. K. Leutgeb et al., 2007; Rennó-Costa, Lisman, & Verschure, 2010). The other possibility is that rate remapping could originate in the DG and be the mechanism behind pattern separation (J. K. Leutgeb et al., 2007). This question is addressed in my study by analyzing cell population activity of the DG.

The ability of the DG to pattern separate is determined by its ability to encode different cues (defined by color or shapes of the environment in which the rats are placed – we use shape) in the same location through rate remapping (encoding similar events differently making them dissimilar). By altering cues in the surrounding area and observing the difference in cell population activity when the rat is exposed to one cue (square) versus the other (circle), while in the same surroundings, we are able to measure pattern separation in the DG. These measurements are quantified using firing rates, where the highest firing rate occurs when the preferred cue is present. In Figure 2, the preferred cue is the square when the cell fires at a peak rate of 9 Hz as opposed to 3 Hz in the circle.

1.4 Medial Temporal Lobe Epilepsy (MTLE)

Epilepsy is considered one of the most common serious brain disorders that affect people of all race and ages (World Health et al., 2005). About 10% of the global population has had an epileptic seizure in their lifetime, where ~30% of them progress into epilepsy (Anatol Bragin, Engel, & Staba, 2010). One percent of the worlds' population is living with active epilepsy (World Health et al., 2005). Only 60% of those that undergo antiepileptic drug therapy will become seizure free (Anatol Bragin et al., 2010). Although there are several different types of

epilepsy related to certain conditions, the primary focus of my research will be acquired (e.g. cerebral damage) temporal lobe epilepsy (TLE). This disorder was first recognized in 1881 by John Hughlings Jackson, who described seizures from the hippocampus as a “dreamy state” (World Health et al., 2005). There are two forms of TLE, those of which include MTLE (seizures originating in the hippocampus) and neocortical TLE (concerning the outer portion of the temporal lobe) (World Health et al., 2005).

Temporal lobe epilepsy accounts for 60% of all epileptic patients, where 80% of these are classified as MTLE (Sirven, 2011). Patients with MTLE have are the most frequently resistant to treatment (Sirven, 2011). A common complaint among these epileptic patients is memory loss related to seizure events (Smith, 1998; Trenerry et al., 1993). The basis of understanding the pathophysiology of epilepsy can help in the development of preventions, diagnoses, and treatments for patients with this disorder.

MTLE is a chronic neurological disorder defined as having reoccurring episodes of spontaneous complex partial seizures that may develop into secondarily generalized seizures (Engel, Babb, & Crandall, 1989; French et al., 1993). During these seizures a group of neurons experience synchronous excitability. This acquired epileptic disorder is typically linked to some type of cerebral injury (e.g. head trauma, febrile seizures, etc.) or event that leads to the initial seizure which causes an imbalance between excitatory and inhibitory networks in the brain (H. E. Scharfman, 2007). When thinking back to the DG operating as a gate or filter and the indirect/direct recurrent micro-circuitries of the hippocampus described earlier, one can imagine how the network imbalances can lead to seizure activity. This early occurrence of hippocampal damage that can ultimately lead to the development of MTLE is a phenomenon known as the initial insult.

During the development of MTLE there are characteristic anatomical changes observed. Some of which include the disappearance of mossy cells, CA1 and CA3 cell death, the drop off of

neurogenesis, ectopic DG gc's, mf sprouting, and hippocampal shrinkage along with a strong recurrent circuitry are some of the main reorganizations that are thought to contribute to the development of epilepsy (H. E. Scharfman, 2007).

The most pronounced changes seen in MTLE studies are seen in the dentate gyrus, which is the first processing stage of the hippocampus. Interestingly, one of the first things that takes place immediately after an initial insult is mossy cell death (Magloczky & Freund, 1995). When mossy cells become overly excited they tend to die, eliminating a pathway for information to be sent to the dentate from CA3 and also reducing feedback inhibition (Magloczky & Freund, 1995). The more gradual effect after an initial insult is the first increase in neurogenesis and then eventual decrease along with the inappropriate migration of these cells to ectopic areas of the DG (Hattiangady, Rao, & Shetty, 2004; Helen E. Scharfman & McCloskey, 2009). Another gradual effect is the mf reorganization, normally the mf's are axonal projections from D gc's that project to the hilus and CA3 (Represa, Tremblay, & Ben-Ari, 1987; H. E. Scharfman, 2007; Sutula, Cascino, Cavazos, Parada, & Ramirez, 1989; Tauck & Nadler, 1985). Yet in MTLE patients these fibers grow out from the dentate cells and synapse back onto the dentate cell layer, creating a direct recurrent circuitry which did not exist previous to the initial insult (H. E. Scharfman, 2007). The new circuitry allows the DG to become susceptible to seizures (H. E. Scharfman, 2007).

Typically, a delayed period between the initial insult and the first spontaneous seizure occur known as the latent period (Sirven, 2011). It has been hypothesized that the initial insult is what triggers structural reorganization of circuitry the DG, which leads to a change in function and ultimately results in MTLE (H. E. Scharfman, 2007). The variable amount of time lapsed (latent period) after an initial insult before the onset of MTLE, making MTLE difficult to study in humans. This makes it difficult to categorize certain anatomical changes that cause the development of MTLE or if the anatomical changes were a compensatory response to MTLE. It also raises the question of whether the anatomical changes or compensatory responses have lead

to functional consequences underlying memory impairment. Interestingly, not everyone who has had head trauma or a single seizure develops epilepsy. There are several other genetic or environmental factors that could contribute to the development of epilepsy, which is another reason why this disease and its progression difficult to study in humans. Animal models that simulate the same structural reorganizations have been developed for research instead.

Research is still needed in this area because there is much unknown about both the function of the DG and how its dysfunction leads to MTLE. Based on the pathophysiology of MTLE there is a cascade of events that can occur before recurrent seizures begin. Making it very difficult to find out whether or not the observed structural reorganization of mf sprouting seen in the DG of MTLE patients is accompanied by functional loss associated with memory impairment. This is the primary focus of my research project, in which I will test the ability of the DG to distinguish between two different cues by measuring the difference in pattern separation in control and epileptic rats. This has been addressed in my study by observing histology in conjunction with neuronal cell firing patterns. **My hypothesis is that permanent structural reorganization of the DG, irrespective of seizure, is correlated with the functional impairments of the DG leading to memory processing impairments.**

Objective 1:

Determine whether permanent structural reorganization of the dentate circuitry associated with MTLE results in a change in dentate and CA3 network function.

Objective 2:

Are functional changes in dentate network activity directly correlated with permanent anatomical changes of the network?

In this project I will use neurophysiology in awake-behaving animals and immunohistochemistry in a rat model of MTLE to investigate my outlined objectives. Due to the

fact that the majority of MTLE patients develop epilepsy as result of traumatic head injury, we will use the drug kainic acid (KA) that will replicate the same common changes in the network (e.g. cerebral injury, latent period, development of chronic seizures) specific to the hippocampus as the initial insult (Hellier, Patrylo, Buckmaster, & Dudek, 1998). KA is a neuroexcitatory amino acid that over stimulates kainate receptors (ionotropic glutamate receptors), found specifically on the surface of neurons in the DG, causing seizures to occur (Moloney, 1998). The effects of this drug share the common changes in the network seen in humans with MTLE, i.e. mf sprouting, mossy cell death, and development of a recurrent circuitry, creating neuronal circuitry that is prone to seizure activity (Hellier et al., 1998).

Kainate treated rats are then chronically implanted with a recording device made up of 14 tetrodes (Figure 3 – Hyperdrive), which will be lowered into the hippocampus and used for monitoring brain activity. The activity patterns in the hippocampus and the gradual development of epileptic brain activity will be observed via electroencephalography (EEG – Figure 5A and B, lower) and single cell recordings (extracellular action potentials – Figure 5A and B, middle) (discussed in materials and methods section 2.4). We will be able to observe the changes in neuronal network function in these animals by recording action potentials from large populations of dentate granule neurons and CA3 pyramidal cells in awake-behaving animals trained to run freely in open fields for recordings to measure encoding and show representations of stored information processes thought to be important for memory. Some of these network functions include the measurement of pattern separation, where they will explore either a square shaped open field or circular shape (representing the two different cues used). Analysis will be done on the firing pattern (non-spatial) and place fields (spatial maps) of these cells, and then compared to control animals (discussed in materials and methods section 2.8). These findings will be considered as a measure of the DG's ability to pattern separate between the two different cues.

Anatomical changes will be observed through the histology of the brain tissue using immunohistochemistry techniques (Nissl and Timm stain) for each animal. Nissl stains will be used to visually verify the tetrode tracts of recordings (Figure 5A and B, far left), where a separate technique (Timm stain – Figure 6) to anatomically characterize and quantify the amount of mf sprouting that occurs in either a healthy or epileptic rat (discussed further in methods and materials section 2.9). Finally, comparisons will also be done between anatomy and physiology by correlating the measured degree of pattern separation and the amount of reorganization observed (Timm score).

Although patients typically complain of retrograde amnesia immediately after a seizure event, we are more interested in whether long-term reorganization of the network results in memory formation and functional impairment of the hippocampus as a whole after the onset of MTLE. Comparisons between a healthy rat and various time points of an epileptic rat will help give us insight into how this disease will affect the memory processing of the hippocampus as time progresses. Also, by comparing the behavior of rats that develop epilepsy to those that are induced with KA and do not develop MTLE, we can compare the links between the anatomical reorganization of the network, changes in network function, and development of MTLE in the DG. Being able to understanding the disease will not only help develop therapy for future patients, but it will also help us develop a better understanding of the dentate gyrus and CA3 functions.

2.0 Materials and Methods

2.1 Animals

A total of 24 male rats were used in this study from Charles Rivers Lab. There were seventeen Long Evans hooded (LE) rats (350 - 425 g at seizure induction) and seven Wistar (W) rats (350 – 400 g at seizure induction) which were individually housed in transparent Plexiglass after seizures were induced. Rats were food deprived according to their behavior during experiments for motivational purposes. Sixteen of the twenty four rats (thirteen LE and three W) were induced with seizures using a low-dose KA model where only eight (six LE and two W) survived, three (LE) of which had a Hyperdrive implant (Figure 3) and used in experiments (Hellier & Dudek, 2005). There was also one LE rat with a Hyperdrive implant that died during the seizure induction. The remaining eight rats (four LE and four W) were used as controls (6 age-matched), two (W) of which were implanted with a Hyperdrive (not age-matched). Animals that were implanted and used in experiments had free access to water. They were maintained at a minimum of 85-90% of their original body weight. These rats were housed in a vivarium which maintained under diurnal lighting conditions (12 h on/12 h off). Experiments and seizure monitoring occurred in their dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego.

2.2 Seizure Induction

Kainic Acid (KA) Administration

We began following a low-dose kainate model developed by Hellier and Dudek (2005), where they used male Sprague-Dawley (SD) rats (180 – 250 g at seizure induction). We have adapted this protocol to our study based on our observations of the pharmacological effects of the drug on rat strain and age.

In our observations, LE rats were extremely variable in terms of the onset of status epilepticus (SE) and subsequent survival when referring to their reaction towards systemically injected KA (total volume of 2 – 10 mg). There was a survival rate of about 46% in this strain and age group, where only 50% developed epilepsy. To help increase the number of epileptic rats we decided to switch to W rats, which are known to be more sensitive to and exhibit a more reliable, consistent behavioral response to KA compared to LE rats (Golden, Smith, Ferraro, & Reyes, 1995; Golden et al., 1991). They have also consistently developed epilepsy in our collaborators, Ivan Sotlesz's lab. An additional ten W rats (275- 350 g at seizure induction) were induced since the conclusion of my experiments (three W rats), where only 2 survived (administered total volume of 3 – 8 mg of KA). In this case there was only a 31% survival rate. However, we noticed a particular trend that would emerge, beginning with minor or no reaction to the KA for a few hours and then they would suddenly have severe seizures, sometimes lasting longer than 40 minutes at a time. We speculated that the delayed reaction was due to an internal threshold, when reached the rats would enter into SE and remain there much longer than physiologically tolerable. This ultimately resulted in their death. Our assumptions are that this is due to their age at seizure induction.

In our modified protocol, KA was injected into rats intraperitoneally (i.p.) with an initial dose of 5 mg/kg (prepared in 0.9% saline at a concentration of 5mg/ml) and assessed every 30 or 60 minutes for further treatment. Assessments were based on the behavioral manifestation of seizures and classified using a modified Racine scale (Hellier & Dudek, 2005). The degree of motor seizure activity can be divided into five classes: I, facial automatisms that can be mistaken for normal animal behavior and are therefore difficult to recognize; II, head nodding and series of wet dog shakes (WDS); III, forelimb clonus with lordotic postures; IV, forelimb clonus with rearing; V, forelimb clonus with rearing and falling over (Hellier & Dudek, 2005). Those rats with very harsh seizures will jump in and throughout the cage (Hellier & Dudek, 2005). At every

30 to 60 minutes after each injection that rat would be assessed for any seizure-like activity. If the rats begin to show a series of WDS's (class II) then the following injection would be skipped and he would be reassessed in the next 30 or 60 minutes to see if he progresses into class III-V seizures. Repeated injections are administered if the rat does not develop any WDS's or progressed into class III-V seizures, but at half the initial dosage (2.5 mg). This process continues until the rats have experienced having several class III-V seizures successively for 3 or more consecutive hours.

The rats that did survive were injected with 5 ml of saline (distributed on different regions of the animals' body) subcutaneously to aide in dehydration. They were then returned to the vivarium with surgical bedding (to prevent inhalation and choking on wood-chip bedding), the water dispenser removed for their safety (e.g. head trauma during a seizure), and given apple slices, for the first 48 hours post seizure induction.

Monitoring for Spontaneous Epileptic Seizures

After the initial seizure induction follows a latent period of a reported minimum of about three weeks and a mean time interval of eleven weeks (Hellier et al., 1998). Behavioral monitoring, EEG and single unit recordings from the DG (Figure 5B) and CA3 (Figure 5A) were performed to monitor spontaneous seizures (5-7 days a week for 2-4 hours at a time) three weeks after the seizure induction. Rats that had two or more behavioral seizures were classified as epileptic.

Data was analyzed using three different groups of rats Control, Epileptic, and Induced. Control rats were defined by not going through the seizure induction process, Epileptic rats were determined after being monitored, and Induced rats were those that went through the seizure induction but were never classified as epileptic.

2.3 Hyperdrive Preparation and Surgical Implant

Hyperdrives consisted of 14 independently movable tetrodes (Figure 3). The tetrodes were 17 μm polyimide-coated platinum-iridium (90/10 %) wire and the tips were plated with platinum in order to reduce electrode impedances (200 – 300 k Ω) at 1 kHz. These Hyperdrives (Figure 3) were then chronically implanted above the right dorsal hippocampus at the following coordinates of each rat (4.0 mm posterior to bregma, 2.7 mm lateral to the midline, 1.0 mm below dura in LE; 3.8 mm posterior to bregma, 2.7 mm lateral to the midline, 1.0 mm below dura in W₁; and 4.0 mm posterior to bregma, 2.6 mm lateral to the midline, 1.0 mm below dura in W₂). Electrodes were projected to be centered over the DG blade when lowered. At the time of surgical implantation rats (375 - 600 g) were under isoflurane anesthesia (2 - 2.5% in O₂). All surgical procedures were performed according to National Institutes of Health and University of California guidelines and as approved by the Institutional Animal Care and Use Committee. Standard protocols were tailored from J. K. Leutgeb et al. (2007).

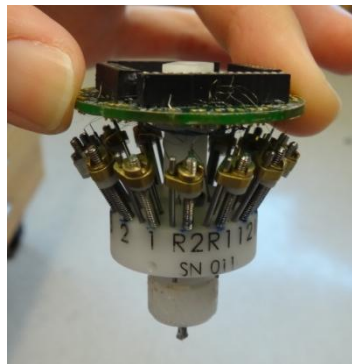


Figure 3 Hyperdrive. A Hyperdrive consists of 14 independently moveable tetrodes. These drives were chronically implanted above the right hippocampus of rats, where the electrodes are projected to be centered over the DG blade when lowered. Two of these tetrodes (R1 and R2) were used as references for all other tetrodes. R1 was used as the differential reference, subtracting cortical noise from all tetrodes. R2 was used as a visual reference, using the EEG as a reference to guide other tetrodes in the hippocampus. The Hyperdrive was connected to a multichannel, impedance matching, unity gain headstage to a data acquisition system.

2.4 Recording Procedures

The Hyperdrive was connected to a multichannel headstage. EEG activity was amplified by a factor of 3000-5000. Spike waveforms (extracellular action potentials) that were above a threshold of 35-40 μV were time-stamped and digitized at 32 kHz for 1ms.

After implantation, tetrodes were gradually lowered into the hippocampus using EEG as a visual reference (Figure 5A and B, lower). During the movement of tetrodes towards the DG and CA3 layers the animal was resting on a pedestal next to the testing environment. Tetrodes were strategically lowered and raised independently based on their anatomical position (turning strategies) for longitudinal experiments, which were different than the strategies for rats that were implanted after they had been classified as epileptic (discussed in section 2.5). Two tetrodes were used as reference signals from the cortex (R1 - differential reference subtracted noise from all other tetrodes) and hippocampal fissure (R2 - visual reference to guide other tetrodes into the hippocampus) (Figure 3). Tetrodes were first quickly lowered to form tracts and all but one (hippocampal fissure reference) were retracted to allow the brain tissue to recover. The next approach would shift the remaining tetrodes towards CA1 pyramidal cell layer (e.g. spike activity and sharp waves seen in EEG), which is a good point of indication towards the DG and CA3 cell layer.

Characteristic changes in EEG and spike activity (above a threshold of 20 μV) were used as a sign that tetrodes were approaching the gc layer. As tetrodes entered the molecular layer of the DG, there were distinct gamma amplitude increases and dentate spiking events (Anatol Bragin et al., 2010; A. Bragin et al., 1995) with the disappearance of sharp waves. When there was an increase in background unit activity tetrodes were turned through the zone in small increments (20 – 40 μm per day) until the putative cell layer was reached and active cells could be recorded from. Those that passed through putative gc layer or were lateral to the DG were lowered further past the hilus (sharp waves were apparent again) and into the CA3 pyramidal cell layer. This

process typically required three weeks until cell layers were found. All spike activity from the DG and CA3 were recorded simultaneously. Standard protocols were tailored from J. K. Leutgeb et al. (2007).

2.5 Longitudinal Study

There were two LE rats that were implanted when they had healthy brains. They had served as controls for themselves as well as MTLE models. This was used to monitor the progressive nature of MTLE development on a healthy brain after an initial insult (e.g. seizure induction) via EEG, single unit recordings, and behavioral observations. A timeline can be found in Figure 4C (purple square).

During the healthy phase all tetrodes were first lowered quickly towards CA1 pyramidal cell layers with half (those consisting of mostly medial tetrodes when referring to the bundle with a few lateral tetrodes) of the tetrodes reaching CA1 (two weeks earlier) and using it as a reference point towards DG and CA3. The second half of the tetrodes were retracted and would linger in the cortex with slight movements to prevent them from being fixed. Two tetrodes were used to record a reference signals and one remained in the cortex for seizure monitoring comparison. The first half would then make their way towards the DG and CA3 layer as the second half would be moved towards CA1. The goal was to locate DG, CA3, and CA1 cells simultaneously for recordings. Once the cells were located experimental recordings of EEG, single units, and behavior would begin.

Once a baseline of control (e.g. normal function of this animal) experimental recordings for three or four days were done and these rats were then induced using the low dose KA model where only one of the two survived (Hellier & Dudek, 2005). After the seizure induction the animal entered into the latent phase of the development of MTLE and experimental recordings were performed as well as monitoring for spontaneous seizures. Since the brain can fluctuate and

tetrodes can move involuntarily the second half of the tetrodes would then make their way down towards the DG and CA3 layers to ensure that these cell layers would be recorded from post seizure induction. The rat entered the chronic phase of epilepsy and was recorded from for the next three months. A timeline can be found in Figure 3B.

2.6 Chronic Phase Study

Two LE rats were implanted after we had established that they were epileptic. The turning strategy for these rats was to move all the tetrodes simultaneously (except the references and cortex tetrodes) towards DG and CA3 (Figure 4C – blue circle).

2.7 Behavioral Procedures

Training in square/ circle experiments started one week after surgery, while recordings began after hippocampal cells were located. To assess the effects of MTLE on pattern separation across two distinct cues (square or circular shapes were used as an indicator for spatial memory), rats were tested in a series of four open squares (1 m by 1 m – Figure 4A, left) and circular shape (1 m diameter – Figure 4A, right). Each environment was presented twice and the order of the shapes was randomly chosen by the experimenter daily (Figure 4B). The environments were enclosed by black walls with a white cue card that was consistently placed in a specific orientation between shapes. Rats were motivated (crushed chocolate pellets) to run around the environment during a ten minute session with a five minute rest in between trials. A baseline recording of cellular and EEG activity was recorded, during a 15 – 20 minute “sleep” session at the beginning and conclusion of the experiment, when the rat was placed in a pedestal outside of the recording environment (experimental timeline seen in Figure 4B).

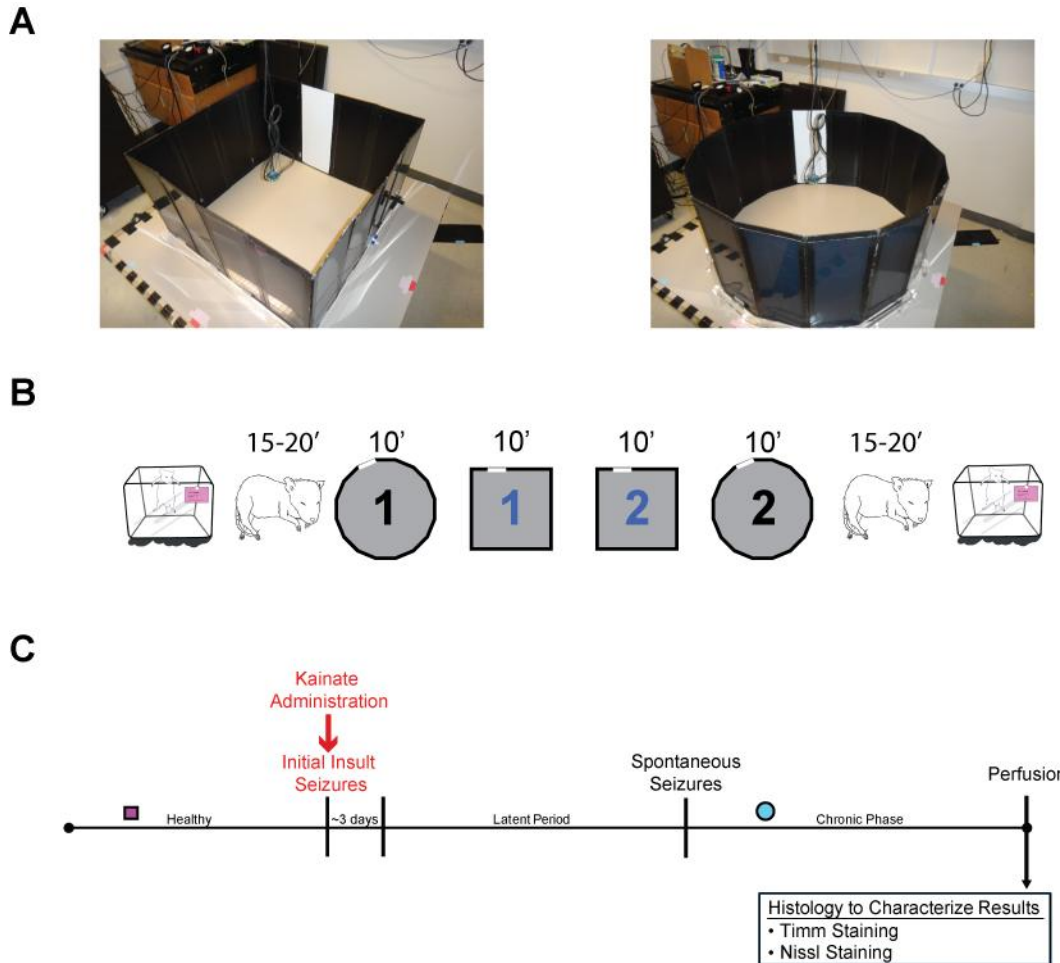


Figure 4 Experimental design. **A** The square (left) and circular (right) environments the rats freely forage in are shown here. **B** Rats are taken from their home cage and are placed into a pedestal. Recordings begin there for a 15-20 minute ‘sleep’ session, then four 10 minute sessions of a randomly sequenced square or circular environments (two of each) with a 5 minute rest in between are run. The rat is once again placed into a pedestal for the last 15-20 minute ‘sleep’ session and are either returned to their home cage or monitored for seizure activity for a few hours at the conclusion of experiments. Time-stamps are used to distinguish the start and end of each session during data analysis. Numbers within each environment represent the order of that particular shape. **C** Schematic of the experimental timeline displays both the longitudinal and chronic phase studies used. Healthy rats were induced using a low-dose kainic acid (KA) model (Hellier & Dudek, 2005). One rat was chronically implanted while they were healthy (purple square) and were induced and recorded from (e.g. single unit recordings, EEG, behavioral) throughout the progression of the disease. Rats that were induced prior to implantation were only implanted after being classified as epileptic (two rats), and used in the chronic phase study (blue circle). Control rats (two) were never induced.

2.8 Data Analyses

Standard data analysis were tailored from J. K. Leutgeb et al. (2007); S. Leutgeb et al. (2005).

Spike Sorting

Spike sorting was performed offline with graphical cluster-cutting software (MClust, D. Redish) (Figure 5). Cluster-cutting was performed manually within two-dimensional projections of the multidimensional parameter space (e.g. waveform amplitudes and energies) (Figure 5A and B, middle). Autocorrelations (presence of bursts), waveforms (spike width – Figure 5A and B, far right), average spike rate, and cross-correlations (separating cells) were some of the tools used to determine putative cell types (e.g. granule cells, pyramidal cells, and interneurons). Histology (Figure 5A and B, far left) and EEG (Figure 5A and B, lower) were used to confirm the tetrode tracts and regions they were in. Spatial firing rate distributions (‘place fields’) for all well-isolated neurons were constructed in a standard manner, by summing the total number of spikes that occurred in a given location bin (5 cm × 5 cm), dividing by the amount of time that the animal spent in that location, and smoothing with a Gaussian centered on each bin. The average rate in each bin x was estimated as

$$\lambda(x) = \sum_{i=1}^n g\left(\frac{s_i - x}{h}\right) \bigg/ \int_0^T g\left(\frac{y(t) - x}{h}\right) dt$$

where g is a smoothing kernel, h is a smoothing factor, n is the number of spikes, s_i the location of the i -th spike, $y(t)$ the location of the rat at time t , and $[0 T)$ the period of the recording. A Gaussian kernel was used for g and $h = 5$ cm. Positions more than 5 cm away from the tracked path were regarded as unvisited.

The numbers of firing fields were estimated based on the summed rate maps for each cell across shapes. The pixel with the peak firing rate was found and contiguous pixels around the

peak with an average rate exceeding 10% of the peak rate were considered to be a firing field. These pixels were then deleted from the rate map and the procedure was iterated until no additional fields with peak rates above 2 Hz were found in the rate map. Recordings in sleep session were also spike sorted to assure recording stability of a cell and identify cells that were silent during behavior.

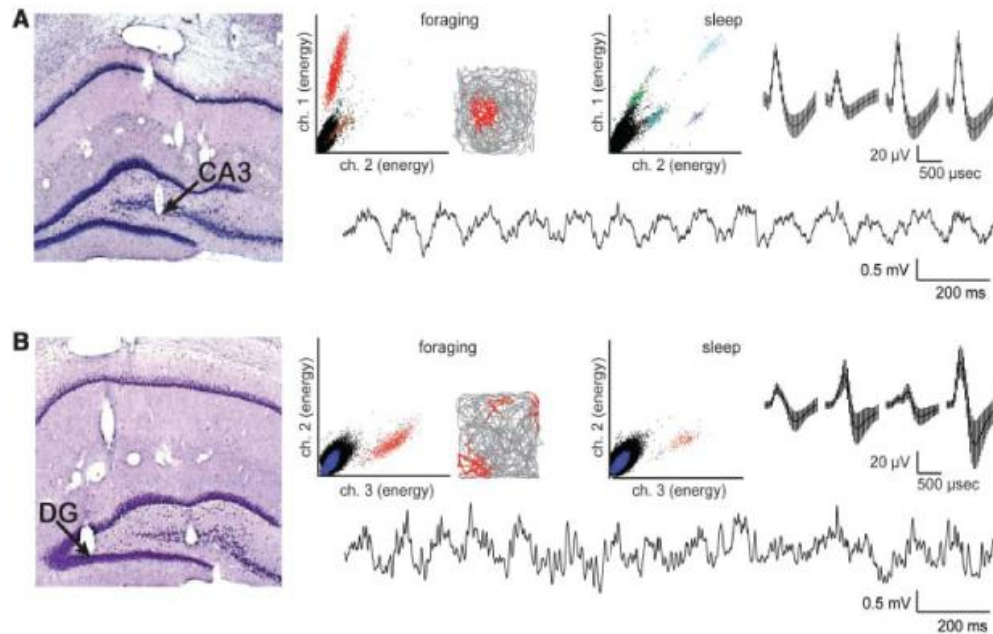


Figure 5 Spike sorting. Figure adapted from J. K. Leutgeb et al. (2007) exemplifies how tetrode positions were determined (histology) along with how place fields are constructed using recorded data. Simultaneous recordings were performed in the dentate gyrus and CA3 pyramidal layer. Tetrode recording locations (arrows) were reconstructed using serial cresyl violet stained coronal sections (left). Examples of both CA3 pyramidal cells (**A**) and dentate granule cells (**B**) are shown. Spike sorting in the two dimensional projections (middle) show clearly separated clusters (extracellular action potentials) when the animals were foraging in a square box during a 10' trial or when they were in a 'sleep' session. Spike clusters (each assigned a color and is representative of one putative cell) were used to show spikes superimposed onto the rat's trajectory (gray) while the rate forages. Waveform of spikes (right) were used as a tool to characterize cells. The EEG traces shown at the bottom of the panels were recorded while rats foraged in the square enclosure.

Population Vector Correlations

Rate vectors were constructed for the entire population of recorded cells for each trial and all animals by arranging the place fields into x-y-z stacks. The x and y represent the two spatial

dimensions (binned into 16 x 16 pixels of 25 cm² each), in which z represents the cell-identity index. Mean rate distributions ran along the z axis for a given x-y location (bin), representing the composite population vector for that location. Only bins that were shared between all configurations (a square of 16 x 16 bins or 80 x 80 cm²) were considered. Cells from DG and CA3 were analyzed separately.

Place Field Correlations

Population vectors were then further analyzed based on individual cell rates and individual place fields seen in a cell. The firing patterns of individual cells were compared with a spatial correlation procedure.

Mean Overlap

The “overlap” between the active place fields in two shapes were calculated by dividing, for each place field, where the mean firing rate in the less-active shape by the mean firing rate in the more-active shape. Fields with peak rates of more than 2 Hz in at least one shape were considered active, and the ratios for active cells were averaged for the place fields. The expected overlap was then used to correct the measured overlap by dividing the expected overlap minus the measured overlap by the expected overlap minus 1. The resulting overlap scores are equal to 1 for the comparison of a cell population with itself and equal to 0 for comparisons between independent place fields.

2.9 Histology

Perfusion

The rats received an overdose of sodium pentobarbital and were perfused intracardially with 0.37% sodium sulphide solution (pH 7.2) followed by 4% paraformaldehyde in phosphate

buffer solution (PBS 0.1M, pH 7.3 - 7.4). The tetrodes were retracted (1 – 3h after perfusion), the brains were then extracted, post-fixed in 4% paraformaldehyde overnight, and then transferred to 30% sucrose in 0.1M PBS. All rats were perfused at 7-10 months of age (4-6 months after seizure induction).

The brains were then cut in half (sagittally) and the left side of the brain was stored in 0.02% azide solution for future histological measurements. The right side of the brain tissue was then frozen with dry ice and coronal sections (40 μ m) were cut using a microtome. All slices through the hippocampus were collected. Alternate sections were mounted on slides with a gelatin solution and stained using the Timm staining and/or Nissl staining (cresyl violet).

The final tetrode positions of recording tetrodes were determined by serial reconstruction across sections. All tetrodes were identified by following the tetrode tracts (tissue damage – Figure 5A and B, far left) across an angular deviation between section planes and tetrode tracts. The tip of the tetrode was determined to be in the last section the tetrode tract was seen. The ending location of the tetrode determined the inclusion of data analysis if they were found in the DG or CA3 layers.

Timm Staining

Mounted slices were washed in distilled water, incubated in developer solution (consisting of 50% gum arabic, 25.5% citric acid and 23.5% sodium citrate aqueous solution, 1.7% hydroquinone, and 0.09% silver nitrate solution) within a light-free container, washed again in warm and distilled water in another light-free container, and fixed in sodium thiosulfate pentahydrate, dehydrated in ethanol, and coverslipped.

Synaptic reorganization of mf within the supragranular region of the dorsal DG was scored on a scale of 0 – 5, adapted from Cavazos, Golarai, and Sutula (1991): 0, no granules between the tips and crest of the DG; 1, sparse granules in the supragranular region in a

continuous distribution between the tips and crest of the DG; 2, more numerous granules with a continuous distribution between the tips and crest of the DG; 3, prominent granules with a continuous pattern between tips and crest alongside the occasional patches of confluent granules between tips and crest of the DG; 4, prominent granules forming a confluent dense laminar band between tips and crest; 5, confluent dense laminar band of granules that extends into the inner molecular layer.

Statistical Analysis

Scores of Control, Epileptic, and Induced rats were independently scored by two observers and averaged. Difference between group mean scores were statistically analyzed for significance by performing a one way ANOVA and Tukey's Multiple Comparison Test post hoc.

3.0 Results

To examine the DG reorganization (e.g. Timm stain) in concurrence with DG and CA3 function in a MTLE model, we recorded EEG and single unit activity while rats foraged for scattered food in either a square or circular enclosure presented in a random order (series of four sessions with two in each form of environment). Four rats were recorded from during experiments. The rats were divided into three different group studies that included Control DG (n = 2), Control CA3 (n = 1), and Epileptic DG and CA3 (n = 2) (one rat was part of the longitudinal study and included in both Control DG and Epileptic DG and CA3 groups). Rats within the longitudinal study were trained daily prior to seizure induction and therefore served as their own control prior to seizure induction, while rats on the chronic phase study were trained in experiments during their diseased state. Recordings began when putative cell layers were reached and continued until rats were perfused. There were a total of 20 gc (Control n = 8, Epileptic n = 12) and 122 CA3 pyramidal cells (Control n = 24, Epileptic n = 98) recorded from. However, only 14 active gc's during behavior from the DG (Control: n = 6, Epileptic: n = 8) and 56 active pyramidal cells in CA3 (Control: n = 15, Epileptic: n = 41) were used in analysis to determine the functional changes of the neuronal network in rats with MTLE in comparison with control data. This was analyzed alongside with anatomical changes to see if there was a correlation between change in the DG ability to pattern separate and the degree of reorganization.

3.1 Anatomical Reorganization

Studies have shown that the mf sprouting is one of the key anatomical changes seen in both human patients and rats with MTLE (Represa et al., 1987; H. E. Scharfman, 2007; Sommer, Roth, & Kiessling, 2001; Sutula et al., 1989; Tauck & Nadler, 1985; Van Paesschen, Revesz, Duncan, King, & Connelly, 1997). This has caused us to take a closer look at the DG reorganization in relation to its function.

The amount of reorganization within the hippocampus when referring to gc axonal projections (mf) was investigated using Timm stained coronal slices. This stain selectively labeled high concentrations of zinc within the neuron axon terminals (the dark brown stain in Figure 6A) of the DG (Slomianka, 1992; Smeijda Haug, 1973). Mossy fiber sprouting is seen in the supragranular region (arrow between crest and tips in Figure 6A) and is rated on a scale of 0-5 (Cavazos et al., 1991). Six control rats (Figure 6B, left panel) had an average Timm score of 0.5 ± 0.55 (Figure 6C) with a typical appearance as seen in Figure 6A (right). The three animals (Figure 6B, middle) that were determined to be epileptic (e.g. behavioral seizures and EEG) had an average score of 2.67 ± 0.58 . An example Timm stain of a chronically implanted epileptic animal illustrates a score of 3 in Figure 6A (middle). Those rats that were induced but never resolved to have developed epilepsy were categorized as the Induced group, who had Timm scores ranging from 2-5 in a total of five rats (Figure 6B, left) and an average Timm score of 3.00 ± 1.22 . A one-way ANOVA and Tukey's Multiple Comparison Test post hoc of mean Timm scores were performed between groups to verify the significance when compared statistically (Figure 6C). Both the Induced and Epileptic groups had significantly higher time scores than Control, with $p \leq 0.05$ and $p \leq 0.01$, respectively. The extent of mf sprouting in animals that underwent seizure induction was not seen in control animals, implying that the initial insult may have led to dentate reorganization but not necessarily accompanied by spontaneous recurrent seizures. Our next question is if the degree mf sprouting results in loss of function or does it somehow compensate for the other anatomical changes that occur (e.g. cells loss, severe decline of neurogenesis)?

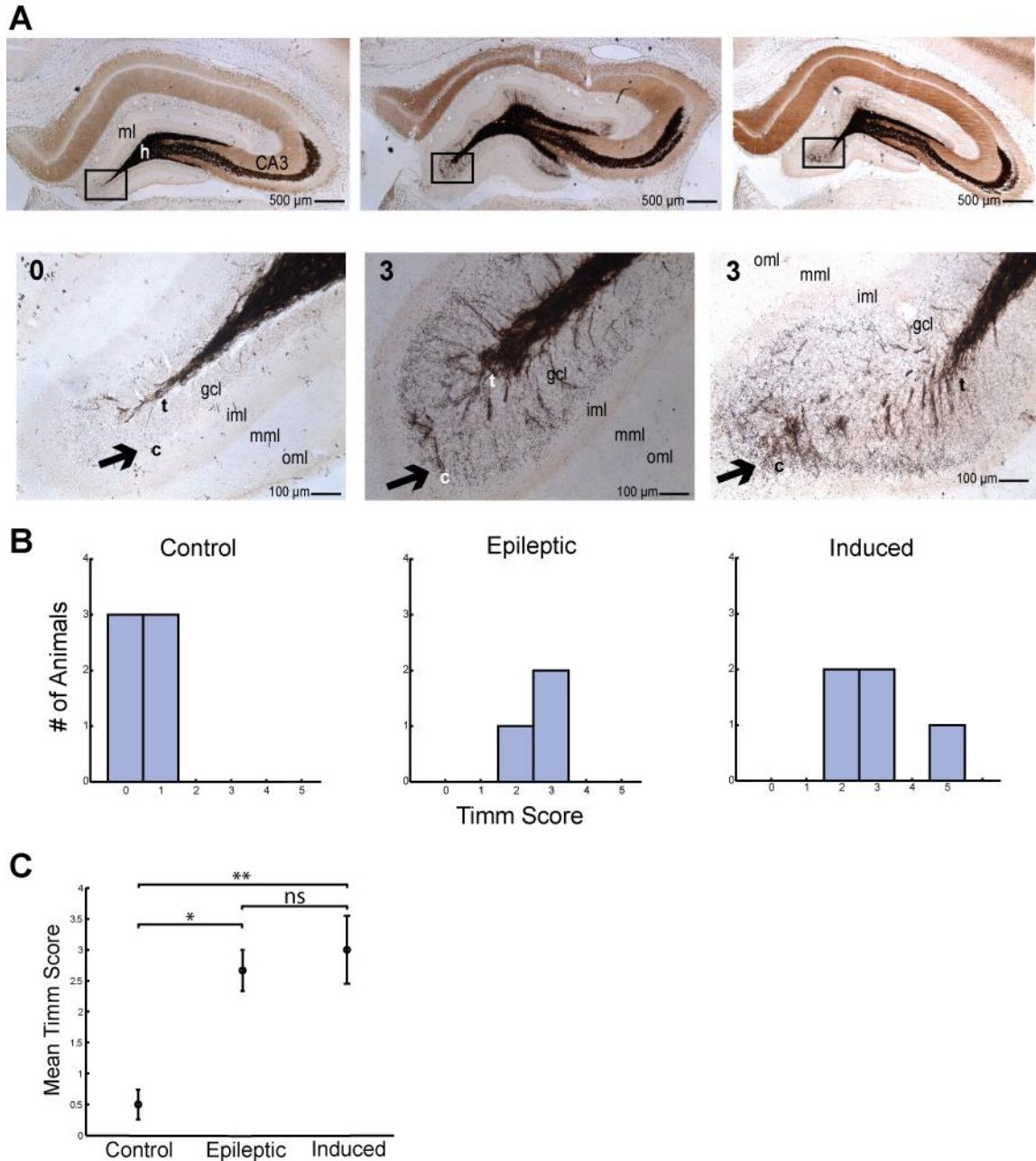


Figure 6 Timm scores. **A** Right Timm stained (stains for mossy fiber sprouting) coronal slices of Control (left), Epileptic (middle), and Induced (right) groups of rats are shown at 2.5 X magnification (top) and 20 X magnification (lower – hippocampal region is represented by the boxed area in the 2.5 X magnification). Timm scores, developed by Cavazoes, Golarai, and Sutula (1991), are displayed in the left corner (20 X magnification). Timm scores are based on the amount of mossy fiber sprouting seen in the supragranular region (arrow). ml: molecular layer, h: hilus, CA3: Cornu Ammonis region 3, gcl: granule cell layer, iml: inner molecular layer, mml: middle molecular layer, oml: outer molecular layer, c: crest, t: tips. **B** Number of animals for each score within Control (n = 6), Epileptic (n = 3), or Induced (n = 5) groups are depicted by the bar graphs. **C** Mean Timm scores are analyzed using a one-way ANOVA. Both the Induced (3.00 ± 1.22) and Epileptic (2.67 ± 0.58) animals had significantly higher Timm scores than Control (0.5 ± 0.55). * = $p \leq 0.05$, ** = $p \leq 0.01$.

3.2 Functional Change in Dentate Gyrus

The unique function of the normal DG seen in rats is the ability to pattern separate between very small changes in an environment (J. K. Leutgeb et al., 2007). A characteristic feature of the gc's are the multiple place fields seen in color coded rate maps (Figure 7) (Jung & McNaughton, 1993). The DG spatial representation of place fields may be compromised due to the structural reorganization of the DG after seizure induction (Figure 6) (e.g. increasing or decreasing the number of fields or field sizes typically seen in Controls). To find the answer to this question we used an experimental design (Figure 4), in which awake-behaving rats foraged for scattered food through a series of square and circle environments (open fields enclosed by black walls) while EEG and single unit data was recorded. Analysis performed on the data was used to determine the map stability (place fields) and ability of the DG to pattern separate (rate maps) in epileptic rats and compared to control animals.

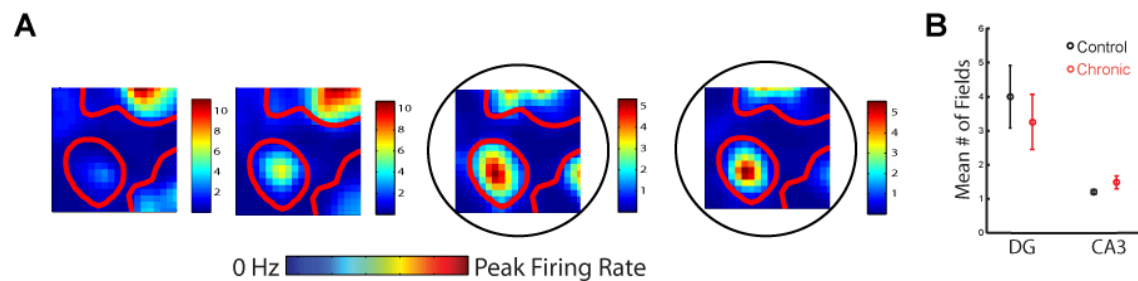


Figure 7 Number of place fields. **A** Color coded rate maps are scaled to the maximum firing rate (red) within the entire test sequence. Peak rates (Hz) vary based on individual fields formed by a cell and can be determined using the color coded rates to the right of each map. A program, MClust, is used to distinguish the individual place fields in each session (represented by red outlines). In this cell there are a total of three place fields within the entire testing sequence. **B** The mean number of fields of active cells in behavior between Control (black circle) and Epileptic groups (red circle) was not significantly different from one another (unpaired t-test of DG and CA3 show $p > 0.05$), but there is a observed difference between the mean number of fields between DG and CA3.

Representative firing fields (Figure 8A, left map in each session) and their corresponding color coded rate maps (Figure 8A, right map in each session) are depicted in Figure 8A. The

firing fields illustrate the rats' trajectory (gray) with spike locations (red), every time a rat runs through the established place field. Rate maps were analyzed to determine the number of place fields found in that particular cell seen in Figure 7A (red circles around place fields). The mean number of these fields (Figure 7B) allowed us to statistically conclude that there is no significance difference in the mean number of place fields in both the DG or CA3 cells, and therefore implying that spatial maps are largely preserved in epileptic rats. However, there is a significant difference in the number of place fields between DG and CA3 cells, which is already known (J. K. Leutgeb et al., 2007). So far the function of epileptic animals show no functional change compared to control rats when only the mean number of place fields is considered.

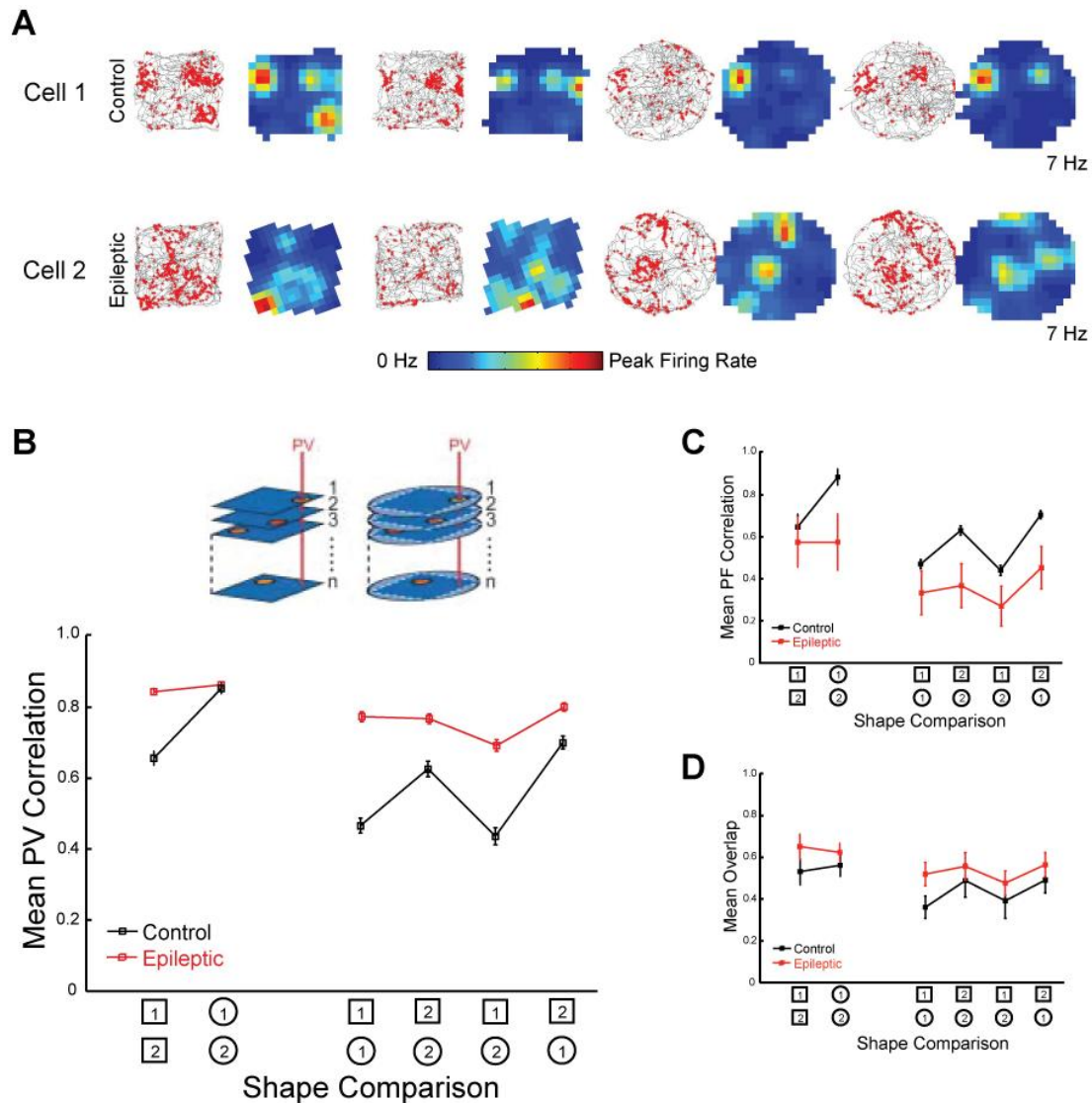


Figure 8 Quantitative assessments of spatial correlations and mean overlap of the dentate gyrus. A Representative firing fields of Control and Epileptic rats, where trajectories with spike locations (right) and their corresponding color coded rate maps (left). The color scale is located at the bottom, areas of blue are when the cells are silent and red when cells are at their peak firing rate. There are multiple place fields in both control and epileptic rats that are observable more stable within control rats in these cells. All rate maps were scaled to their maximum firing rate within all the sessions and average peak firing rates for that trial session are indicated at the bottom of the rate maps. **B** The top of the figure is adapted from J. K. Leutgeb et al. (2007) Fig. 3A showing the procedure for calculating population vector correlations. Mean PV correlations show that the Epileptic (red) group had a higher consistent correlation within the same shapes (e.g. square-square comparison) compared to Controls. In the following segment there is a larger difference in correlation between shapes (e.g. first square and first circle comparison). Implying that Epileptic group had an impairment in pattern separation when compared to Controls. **C** Mean place field correlations were performed in individual cells. Controls have an overall higher correlation within shapes than Epileptics, and a consistent decorrelation between shapes (less apparent in the Epileptic group of rats). Place fields, therefore, seem to be more stable in controls. **D** In this case mean overlap analysis were performed on individual place fields, with only a slight difference between Control and Epileptic rats. For

values refer to Table 1. PV: population vector, PF: place field. Number of cells used in analysis for control $n = 6$, epileptic $n = 8$.

Further analyses were done to test whether pattern separation or map stability was effected in the diseased state. Population vector (PV) correlations (representative of network change), place field (PF) correlations, and mean overlaps were calculated and values (Table 1) were plotted on a graph in Figure 8B, C, and D respectively. The averaged mean PV correlation of within shape comparisons (e.g. first square and second square) of Epileptic animals (0.85 ± 0.01) was slightly higher than Controls (0.75 ± 0.07), but this could have been skewed due to the small sample size (Figure 10A). However, there was more decorrelation observed in Control rats (0.56 ± 0.06) of between different shape comparisons (e.g. first square and first circle) compared to Epileptics (0.75 ± 0.02) (Figure 10A). This preliminary data suggests that the DG of Epileptics has an inferior ability to encode even large differences between two very distinct environments because it appears to have the same network activity regardless of the cue presented.

The PV encompasses both place field and rate information. In order to distinguish which aspect of encoding is impaired, the two parameters were separated by analyzing mean PF correlation (spatial) and mean overlap (non-spatial). The cells map was used for mean PF correlations to see the map stability in the DG (Figure 8C, Table 1). The overall mean PF correlations of dentate gc's seem to be less stable in Epileptic rats than Controls. When looking at the DG ability to rate code in averaged mean overlap calculations (Figure 8D, Table 1) there was only a slight difference between Epileptic and Controls.

Based on these preliminary findings the DG appears to show that network changes are due to impairments seen in spatial encoding and not in rate coding of the MTLE models. This decreased ability to keep stable maps within shapes and between shapes influences the PV correlation, causing the mean PV correlation of Epileptics to remain consistently high between

shapes rather than lower as seen in Controls (Figure 8B, Table 1). The degree of structural reorganization may have something to do with this.

3.3 Functional Change in CA3

The anatomical connection of DG to its downstream region, CA3, via mf projections is the basis for us to investigate whether the measured changes alter the information transferred to this pyramidal cell layer's function. By investigating the only downstream target of the DG we can further analyze the functional impairments seen in the DG. Due to both the permanent structural reorganization of the hippocampus (e.g. mf sprouting, neuronal cell death) and functional impairments distinguished in the DG, one would also expect a functional change when looking at the CA3 region. However, preliminary data has demonstrated that the CA3 function remains intact in the diseased MTLE models.

CA3 data was recorded simultaneously with DG in awake animals freely foraging for chocolate pellets in a series of open environments to see if there were any apparent functional changes. A CA3 cell's place fields are shown as trajectory maps (left map of every session) and color coded rate maps (right map of every session) found in Figure 9A. These maps show only one place field, which is typical for CA3 cells in a healthy brain (Figure 7B) (J. K. Leutgeb et al., 2007; O'Keefe & Dostrovsky, 1971). There is relatively the same mean number of fields found in CA3 cells of Epileptic rats (Figure 7B). The methods to determine the number of fields in a given map is the same used in DG cells (Figure 7A).

Impairment in CA3 function was examined using mean PV correlations (Figure 9B, Table 2), mean PF correlations (Figure 9C, Table 2), and mean overlap analysis (Figure 9D, Table 2). Calculated values can be found in Table 2. There were 15 active pyramidal cells from the CA3 Control group, as opposed to the 41 CA3 pyramidal cells analyzed in Epileptic rats. Mean PV correlations do not seem to show any difference between Control and Epileptic rats in

Figure 9B, Table 2. The averaged mean PV of Control and Epileptic rats within shape (0.86 ± 0.01 , 0.87 ± 0.01) and between shapes (0.63 ± 0.02 , 0.63 ± 0.01) showed no observable differences when compared (Figure 10A). The ability of CA3 to pattern separate gives the impression of retaining its function even in the diseased state. Like the DG data the two parameters of PV correlation were then separated into mean PF correlation and mean overlap to analyze which form of encoding was more affected in an epileptic state. Figure 9C, Table 2 shows the PF correlations reveal that place fields within and between shapes seem to be less stable compared to Controls. When focusing on the CA3 region, rate remapping (Figure 9D, Table 2) mean overlaps showed no visible difference between Control and Epileptic rats. Surprisingly, unlike the DG the CA3 region was capable of preserving its function despite the structural reorganization throughout the hippocampus (known to occur in MTLE models) (H. E. Scharfman, 2007) and functional changes seen in the DG.

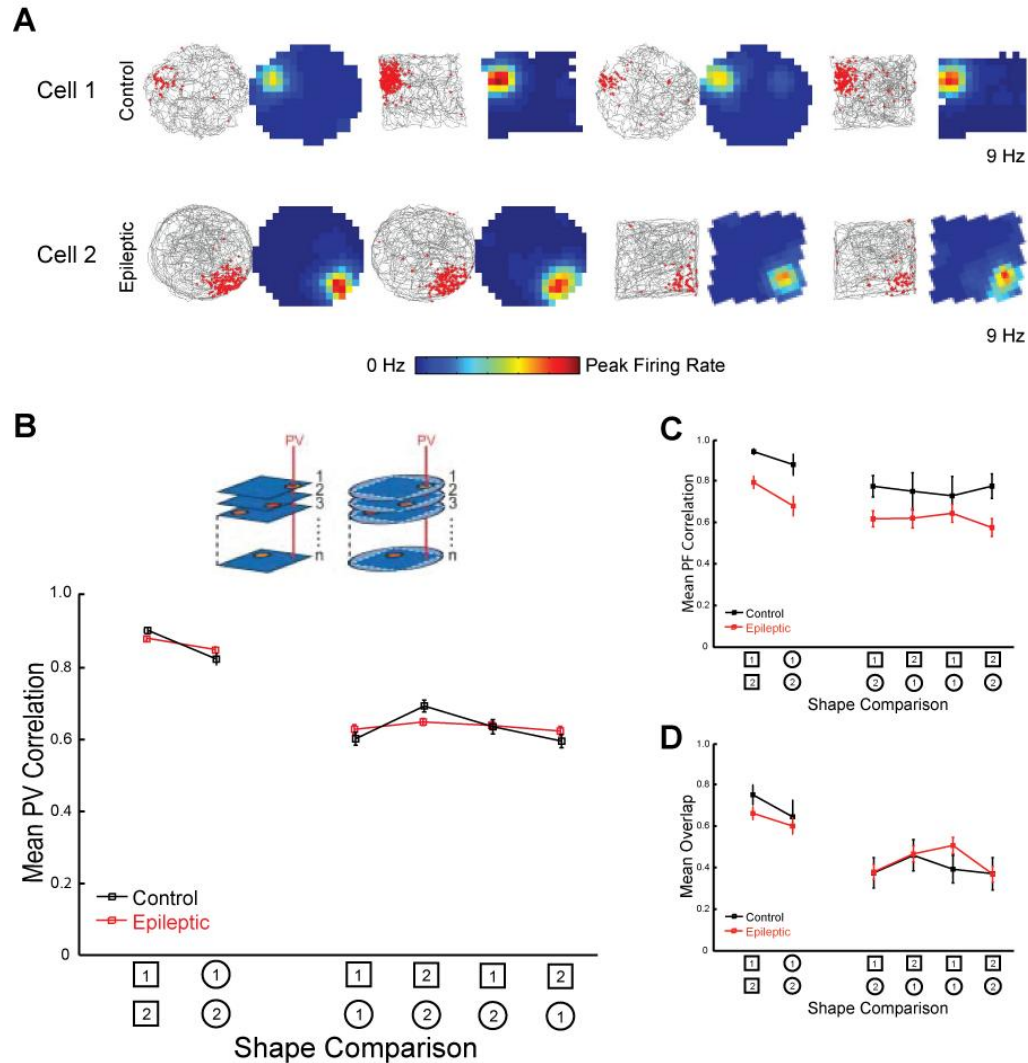


Figure 9 Quantitative assessments of spatial correlations and mean overlap of the CA3. **A** Firing fields (left in each trial) for representative Control and Epileptic CA3 cells are shown with the rats trajectory labeled in gray and spike locations in red. Their corresponding color coded rate maps are to the right of these maps, where no cell spikes is indicated by blue and the peak firing rate as red. Rate maps were scaled to the maximum firing rate within the entire test sequence. The average peak rate for each session is given below each rate map. These cells appear to have one stable place field that rate remaps in both Control and Epileptic animals. **B** The top of the figure is adapted from J. K. Leutgeb et al. (2007) Fig. 3A showing the procedure for calculating population vector correlations. The mean PV correlations reveal that there appears to be no difference between Control (black) and Epileptic (red) rats within the same shapes (e.g. first square and second square comparison) as well as between shapes (e.g. first square and first circle comparison). The pattern separation ability of the CA3 region turns out to be unimpaired. **C** Mean place field correlations were performed in individual cells. Epileptic rats showed lower mean correlations across all comparisons when compared to Controls. Place fields show to be slightly more stable in Control than Epileptic rats. **D** Mean overlap analysis was performed on individual place fields, illustrating a small difference between Control and Epileptic rats' ability to rate remap. The only exception is the comparison between the first square and first circle, where there is slightly more decorrelation in Controls than Epileptics. For values refer to Table 2. PV: population vector, PF: place field. Number of cells used in analysis for control $n = 15$, epileptic $n = 41$.

3.4 Functional Changes Versus Anatomical Changes

In addressing the question of whether functional changes in the DG network are directly correlated with permanent mf sprouting within this network we look at Figure 10B. Data from the two recorded epileptic rats were separately plotted in Figure 10B to show that different degrees of mf sprouting also have different degrees of network function impairments. There is an observable correlation between the increased degree of structural reorganization represented by the animals Timm score and the increased functional network changes expressed by the mean PV correlation. The higher mean PV correlations between shapes illustrate the DGs inability to distinguish the two distinct cues presented to the Epileptic rats. Therefore, the more structural reorganization observed correlates with the DGs impaired network function.

The control data shows much more variability of mean PV correlation between shapes. This can be explained by the DG ability to properly separate the two cues presented to these animals. The control data was not separately plotted because one rat was involved in the longitudinal study and tissue during the control period could not be obtained. The other control rat was originally intended for a longitudinal study, but did not survive the initial seizure induction and we were unable to be perfused according to Timm stain protocol due to the abrupt death. This rat was perfused using Nissl stain protocol instead. Reliably we were able to use the averaged control Timm score ($n = 6$) in place of the actual Timm scores.

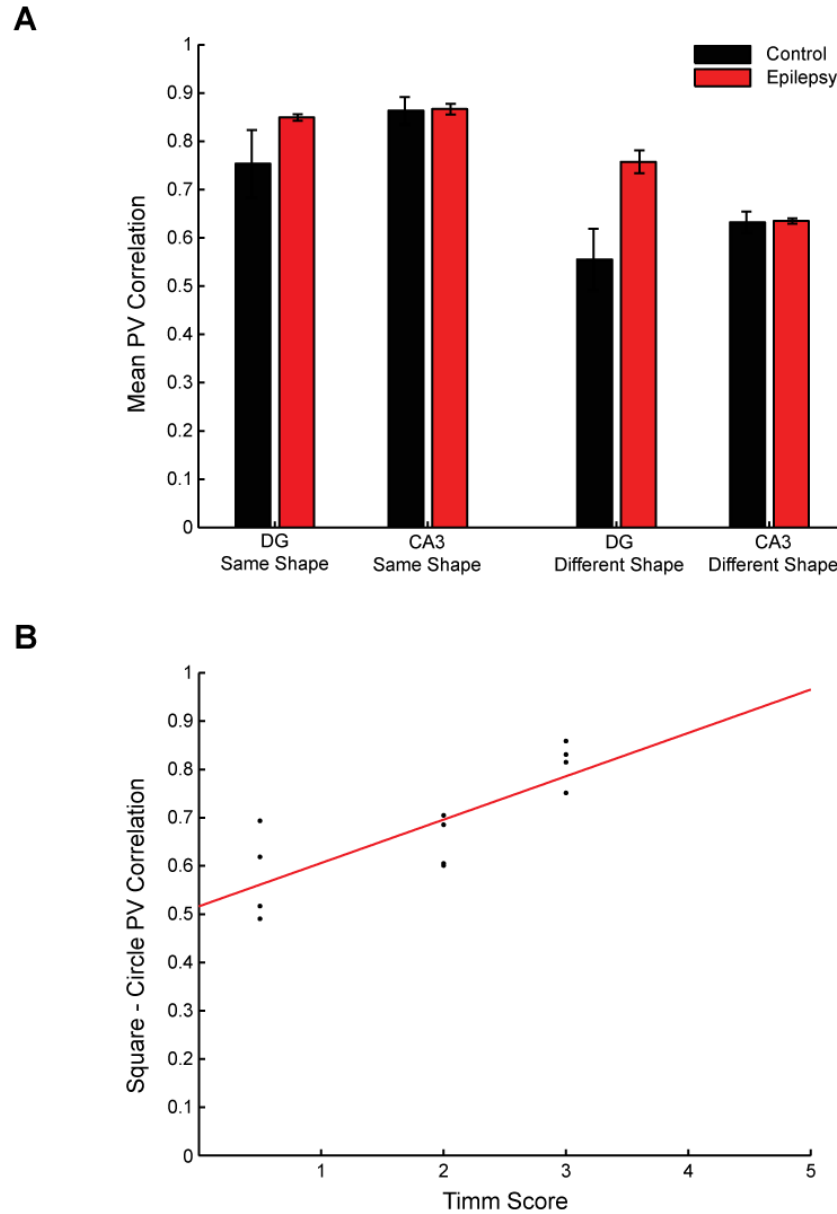


Figure 10 Functional versus anatomical changes. **A** A summary figure of averaged means PV of the same shape and different shapes using data from Figures 8 (DG) and 9 (CA3). This figure illustrates that there is a distinct difference in function within the DG, especially when the animal is in different environments, based on mean PV analysis. However, this change is not apparent in CA3 function. **B** The DG PV of different shapes in comparison to the animals Timm score shows a positive correlation, where the more anatomical change (represented by a higher Timm score) is correlated with higher PV correlations compared to the control. Average Timm scores (0.5 ± 0.55) of all Controls were used as the Timm score for the control rats. The adjusted R-squared value is 0.62 ($y = 0.09x + 0.52$).

4.0 Discussion

4.1 Anatomical Restructuring

The anatomical changes that occur in humans involve hilar neuronal death (e.g. mossy cells and interneurons), mf sprouting into the inner molecular layer, and CA1 and CA3 cell death (Babb, Kupfer, Pretorius, Crandall, & Levesque, 1991; Ben-Ari, 1985; de Lanerolle, Kim, Robbins, & Spencer, 1989; Houser, 1992; Margerison & Corsellis, 1966; Sutula et al., 1989). These effects, although variable depending on the severity and duration of the seizures during the seizure induction, can be mimicked by the KA treatments (Ben-Ari, 1985; Victor Nadler, 1981). Researchers have hypothesized that hilar cell death of mossy cells, which normally function as excitatory cells that are thought to contribute to the competitive network of the DG (D. G. Amaral, 1978), is one of the essential steps causing a cascade of events that ultimately leads to mf sprouting and forming a recurrent excitatory network that did not already exist (Babb et al., 1991; Tauck & Nadler, 1985; Wuarin & Dudek, 1996). Although, this may play a role in MTLE development we focused more on the question of whether this new network affects the overall function of DG and its downstream region, CA3, in terms of pattern separation. We also correlated the extent of mf sprouting with the amount of impairment seen.

When looking at the amount of mf sprouting in Epileptic or Induced groups in comparison with Control rats we see a distinct difference in averaged Timm scores. Even though there were eight rats that survived seizure induction only three of them were classified as epileptic. Our analysis shows that there was no significant difference between Induced (3.00 ± 1.22) and Epileptic (2.67 ± 0.58) mean Timm scores. This raises the question of whether or not the DG and CA3 networks of the Induced animals, if they had been recorded from, would have been more representative of the Control or Epileptic group. However, the Induced rats were not definitively classified as showing no sign of having developed epilepsy because they were only monitored 5-7 days a week for 2-4 hours at a time over the course of a few weeks. We could also

assume that they might have been epileptic, but never witnessed a behavioral seizure in the times they were monitored. Future experiments performed on these Induced rats would shed light onto these questions. We would expect to find the same correlation, where the amount of anatomical restructuring (i.e. Timm score) would positively correlate with functional impairment seen in Figure 10B.

4.2 Pattern Separation

Here we question whether permanent changes, associated with MTLE, to the DG network leads to functional changes within the DG and CA3 of awake-behaving animals irrespective of recent seizure activity. The ability of the DG and CA3 network to pattern separate was determined via place field stability (global remapping) and firing rate (rate remapping) in different environments (J. K. Leutgeb et al., 2007). The DG and CA3 both receive inputs from MEC II and LEC II, as well as from each other (Figure 1B). Based on the connectivity, it can be implied that these two networks work together in memory processing. Since there is obvious anatomical reorganization seen in the DG, we would assume that the new recurrent excitatory circuitry that accompanies neuronal cell death (Babb et al., 1991; Tauck & Nadler, 1985; Wuarin & Dudek, 1996) would affect the normal function of the DG as well as its downstream targets (CA3). We only see impairment in the gc's ability to pattern separate in the DG, since there is less observable network change when comparing the cell population activity between two different cues (Figure 8B and 10A), and no noticeable impairment in CA3.

Our preliminary data has led us to believe that the DG and CA3 process information differently despite the fact that they are anatomically connected. The dysfunction of the DG in MTLE appears to have no apparent effect on its only downstream target, CA3. We speculate that CA3 may not depend on the DG to properly function, which raises the question of what the purpose of the DG really is? Does it play a significant role in memory formation in the brain? Or

does CA3 rely on other inputs than the DG to function? Alternatively, we can also consider that the information that is being transmitted to CA3 is still precise enough to drive the process in CA3. Despite the small measured changes, the DG is still somewhat capable of rate remapping (Figure 8D) in Epileptic rats when compared to Controls. It also remains to be varied whether or not this task is a DG dependent paradigm. In order to address these questions a larger number of rats are needed to perceive a better effect. Further testing in a task that is known to be dentate dependent (e.g. morph sequence) (J. K. Leutgeb et al., 2007) will also help answer these questions. Perhaps with this task we may see a deficit in CA3 function as well, giving us more incite as to whether firing rate originates in the LEC or the DG. It may also be true that MTLE patients have memory impairments in some but not all types of memory formation and those impairments may not involve the function of the DG.

4.3 Correlation of Anatomical Reorganization and Pattern Separation

The amount of impairment seen in the DG ability to pattern separate seems to correlate with the amount of restructuring found in mf sprouting (Timm stains). The higher the Timm score of the epileptic rats correlated with the decreased ability to encode the differences between a square and circular environment (Figure 10B). This is shown by the higher mean PV correlations, which means there is a positive correlation of decreased pattern separation capability and increased mf sprouting (Figure 10B). This proposes the possibility that the recurrent excitatory circuit formed by the mf sprouting in the DG can contribute to the functional impairment of these animals. Previous research has shown that mf sprouting continues throughout the animals' life. Built on the relationship that the higher the Timm score, the more the DG is impaired in pattern separation we can hypothesize that as MTLE progresses the more the DG will be impaired.

Conclusion

Based on the connectivity of the DG and CA3 region, one would expect that functional impairments would arise in both areas. Surprisingly, we see a correlated functional deficit in the DG in relation to the amount of mf sprouting, but there is no such deficit in the CA3 region. There are several theories as to why CA3 is not recognizably effected: it relies on other inputs besides the DG, the DG is still capable of sending certain information the CA3 requires to function properly, the experimental paradigm used in our experiments were not DG dependent, or that the DG function is not as important as we previously expected in CA3 function. These preliminary findings have paved the way for added experiments in answering these hypotheses. It also allows for future studies where a comparison of the long term affects of MTLE and immediately after seizure effects can be made possible. Or whether or not we can predict seizures based on single cell activities leading up to a seizure.

By understanding the anatomical changes in relation to functional changes in rats we can relay our findings to human patients. This research may be beneficial to discovering the mechanisms behind the memory deficits seen in MTLE patients since many of the same anatomical changes occur. Therapeutic methods can be developed to prevent the further progression of reorganization (i.e. mf sprouting, inappropriate synapses, or inappropriate migration of neurons) in the hippocampus and hopefully seizure activity as well, once a patient has been diagnosed with MTLE. It would be difficult to prevent the initial development of MTLE since it is typically the result of head trauma. However, not all head traumas initiate the advancement of MTLE.

Tables

Table 1 The numerical values of the points seen in Figure 8B, C, and D are listed for control and epileptic groups of the DG analysis.

Dentate Gyrus Control

	Within Shape Comparison		Between Shape Comparison			
	$\boxed{1}\boxed{2}$	$\textcircled{1}\textcircled{2}$	$\boxed{1}\textcircled{1}$	$\boxed{2}\textcircled{2}$	$\boxed{1}\textcircled{2}$	$\boxed{2}\textcircled{1}$
Mean PV Correlation	0.65 ± 0.02	0.85 ± 0.01	0.46 ± 0.02	0.62 ± 0.02	0.43 ± 0.02	0.70 ± 0.02
Mean PF Correlation	0.64 ± 0.06	0.88 ± 0.04	0.45 ± 0.09	0.51 ± 0.08	0.36 ± 0.09	0.60 ± 0.07
Mean Overlap	0.62 ± 0.02	0.68 ± 0.01	0.50 ± 0.10	0.56 ± 0.12	0.37 ± 0.06	0.61 ± 0.05

Dentate Gyrus Epileptic

	Within Shape Comparison		Between Shape Comparison			
	$\boxed{1}\boxed{2}$	$\textcircled{1}\textcircled{2}$	$\boxed{1}\textcircled{1}$	$\boxed{2}\textcircled{2}$	$\boxed{1}\textcircled{2}$	$\boxed{2}\textcircled{1}$
Mean PV Correlation	0.84 ± 0.01	0.86 ± 0.01	0.77 ± 0.01	0.77 ± 0.01	0.69 ± 0.02	0.80 ± 0.01
Mean PF Correlation	0.57 ± 0.12	0.57 ± 0.13	0.33 ± 0.10	0.36 ± 0.10	0.26 ± 0.10	0.45 ± 0.10
Mean Overlap	0.75 ± 0.07	0.75 ± 0.05	0.71 ± 0.01	0.62 ± 0.01	0.69 ± 0.02	0.64 ± 0.01

Table 2 The numerical values of the points seen in Figure 9B, C, and D are listed for control and epileptic groups of the CA3 analysis.

CA3 Control

	Within Shape Comparison		Between Shape Comparison			
	$\boxed{1}\boxed{2}$	$\textcircled{1}\textcircled{2}$	$\boxed{1}\textcircled{1}$	$\boxed{2}\textcircled{2}$	$\boxed{1}\textcircled{2}$	$\boxed{2}\textcircled{1}$
Mean PV Correlation	0.90 ± 0.01	0.82 ± 0.02	0.60 ± 0.02	0.69 ± 0.02	0.64 ± 0.02	0.60 ± 0.02
Mean PF Correlation	0.94 ± 0.01	0.88 ± 0.05	0.77 ± 0.05	0.75 ± 0.09	0.73 ± 0.09	0.77 ± 0.06
Mean Overlap	0.72 ± 0.03	0.71 ± 0.08	0.44 ± 0.08	0.46 ± 0.08	0.51 ± 0.09	0.38 ± 0.07

CA3 Epileptic

	Within Shape Comparison		Between Shape Comparison			
	$\boxed{1}\boxed{2}$	$\textcircled{1}\textcircled{2}$	$\boxed{1}\textcircled{1}$	$\boxed{2}\textcircled{2}$	$\boxed{1}\textcircled{2}$	$\boxed{2}\textcircled{1}$
Mean PV Correlation	0.88 ± 0.01	0.85 ± 0.01	0.63 ± 0.01	0.65 ± 0.01	0.64 ± 0.01	0.62 ± 0.01
Mean PF Correlation	0.79 ± 0.03	0.68 ± 0.06	0.62 ± 0.04	0.62 ± 0.05	0.64 ± 0.04	0.57 ± 0.04
Mean Overlap	0.74 ± 0.03	0.64 ± 0.04	0.56 ± 0.05	0.49 ± 0.04	0.56 ± 0.04	0.54 ± 0.05

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