ABSTRACT

The Effects of an Acute Flurothyl Seizure on Associative Learning and Memory

Andrew J. Holley, M.A.

Mentor: Joaquin N. Lugo Jr., Ph.D.

Past studies that utilized the flurothyl model of seizure induction have demonstrated impairment of spatial learning and memory in both neonatal and adult rats. However, most studies focused on inducing several seizures and much later examining learning and memory. The impact of a single acute seizure on learning and memory has not been investigated in mice. In the current study, the effects of a single acute flurothyl seizure were examined at different time points in relation to associative learning in adult mice. Results of memory tests carried out 24 h post-seizure revealed that only a pre-training seizure occurring an hour prior to learning affected what was later recalled during a conditioned stimulus presentation. These results suggest that an acute seizure occurring immediately before learning can have an effect on what is later remembered. Possible mechanisms for memory disruption and future directions are discussed.

The Effects of an Acute Flurothyl Seizure on Associative Learning and Memory

by

Andrew J. Holley B.A.

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Charles A. Weaver III, Ph.D., Chairperson

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Approved by the Thesis Committee

_____

Joaquin N. Lugo Jr., Ph.D., Chairperson

_____

Michael K. Scullin, Ph.D.

_____

Bessie W. Kebaara, Ph.D.

Accepted by the Graduate School

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J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School.

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CHAPTER ONE

Introduction

According to the World Health Organization approximately 50 million people worldwide suffer from epileptic disorders (WHO, 2015). In the United States alone epilepsy affects 2.3 million adults (Kobau, Luo, Zack, & Thurman, 2012). The conceptual definition of epilepsy was established in 2005, describing epilepsy as a disorder of the brain where there are at least two unprovoked seizures that occur more than 24 hours apart. However, there was immediate resistance to this definition because there are several instances where one seizure may be sufficient to classify the patient with epilepsy, and there are other instances where more than two seizures may be required to properly diagnose the patient with epilepsy. In 2014 a report from the International League Against Epilepsy altered the practical definition of epilepsy (Fisher et al., 2014). In the revised definition they stated that epilepsy can be considered to be present after one unprovoked seizure in individuals with other associated factors. The revised criterion has taken into account that a single seizure can have long-lasting and significant effects. Indeed, a single unprovoked seizure increases the risk for another seizure 40-52% (Berg & Shinnar, 1991).

In addition to increased susceptibility to additional seizures, damage and reactive plasticity can also have effects on a number of other processes, which lead to comorbidities. Individuals suffering from epileptic seizure disorders are more likely to also suffer from psychiatric disorders such as anxiety, depression, bipolar, and attention

deficit hyperactivity disorder (ADHD); and neurological conditions including sleep and movement disorders (Ottman et al, 2011). Pain disorders and asthma also show high comorbidity with epilepsy (Ottman et al., 2011). Another common comorbidity of epilepsy is cognitive impairment, such as memory deficiency. Memory problems can persist even when seizures are under control with antiepileptic medication (Titiz, Mahoney, Testorf, Holmes, & Scott, 2014). Persistent cognitive impairment could have significant effects on everyday functioning in affected individuals who may be unable to reliably form new memories or clearly recall old memories. Learning and memory deficits could therefore represent a significant effect that results from recurrent seizures.

Research utilizing clinical data and animal models has shown that prolonged or recurring seizures can impair different types of learning and memory including spatial, episodic, and emotional learning and memory (Inostroza, Brotons-Mas, Laurent, Cid, & de la Prida, 2013; Kemppainen, Nissinen, & Pitkanen, 2006; Lesting, Geiger, Narayanan, Pape, & Seidenbecher, 2011; Pearson, Schulz, & Patel, 2014). Notably, there has been much research using animal models of epilepsy to investigate the chronic effects of regular seizures on cognition, however, little research has investigated the effects of a single or acute seizure on this same subject. Therefore, while we may have some idea of the degree of long-term cognitive impairments that result from an epileptic condition, it is still important to separate out the effects each seizure may have on the processes of learning and memory. Multiple seizures can not only result in extensive cell loss and other damage that can affect learning and memory, the application of antiepileptic drugs (AEDs) to combat seizures has also been implicated in contributing to such cognitive impairment (Becker, Grecksch, & Brosz, 1995; Liu et al, 2003). It is important for these

reasons to examine the effects a single seizure may have on the brain and how such effects may translate into possible impairments on learning and memory in isolation from the effects of AEDs and damage from sustained seizures in status epilepticus (SE).

CHAPTER TWO

Literature Review

Fundamental Assumptions Concerning Epilepsy

Epilepsy is a complex and poorly understood disorder of the brain. The causes of epilepsy are numerous and individual cases of epilepsy are heterogeneous in their development, severity, and associated comorbidities. Fundamentally, seizures and epilepsy are thought to result from an imbalance between excitation and inhibition in the brain (Ben-Ari & Dudek, 2010). Such an imbalance can result from any of a variety of factors. Acquired types of epilepsy result from an initial insult that damages the brain such as a traumatic brain injury, stroke, infection, brain tumor, hypoxia, or status epilepticus (Sierra, Gröhn, & Pitkänen, 2015). A precipitating insult such as this can lead to significant neuropathological changes in limbic structures such as the hippocampus, a highly epileptogenic structure. Such changes are involved in a process referred to as epileptogenesis which involves changes in structural and functional connectivity of tissue necessary for the generation of the spontaneous seizures that characterize an epileptic brain (Sierra et al., 2015).

During the latent period of months to years (days to weeks in animal models) following an insult to the brain a variety of changes occur that predispose a brain to spontaneous recurrent seizures (SRSs). In the hippocampus, a common area of seizure initiation, there is often significant loss of excitatory and pyramidal neurons as well as inhibitory interneurons (Ben-Ari & Dudek, 2010). The loss of these types of cells can

lead to axonal and synaptic plasticity in surviving cells that are thought to underlie the formation of epileptogenic networks that are susceptible to seizure generation. A good example of altered connectivity often occurs in the mossy fiber pathway during epileptogenesis. Normally, mossy fibers project along the mossy fiber pathway from the dentate gyrus to the CA3 region of the hippocampus. In epileptic brains this connectivity is altered such that axons can form recurrent excitatory circuits with other granule cells in the molecular layer of the dentate gyrus (Malmgren & Thom, 2012). This type of change can occur in response to seizures and could potentially lead to spontaneous seizures as a result of the increased excitation that these recurrent circuits produce. At least one study has, however, shown that mossy fiber sprouting is not always required to produce further seizures, although it is associated with the extent of neuronal damage (Nissinen, Lukasiuk, & Pitkanen 2001).

Despite our knowledge that changes such as those described above occur during epileptogenesis, the specific roles that these alterations to the brain serve in the development of SRSs and various comorbidities is not fully understood. It is important to take into account the many changes such as cell loss, altered connectivity, gliosis, and neuroinflammation that can occur as the result of a brain insult (Jefferys, Steinhäuser, & Bedner, 2015). Because we are limited in our abilities to monitor such changes in humans as they are occurring, the use of animal models of seizures and epilepsy affords investigators the opportunities to reproduce many of these changes typically found in epileptic brains as well as the behavioral and cognitive alterations that consequently arise. Some of the more commonly used animal models used to study epilepsy will be reviewed next.

Seizure Induction Methods

Several animal models of epilepsy have been used for decades to examine the effects of repeated seizures on behavior and cognition. Two of the most widely used models of epilepsy are the kainic acid (KA) and pilocarpine/lithium pilocarpine (Pilo/Li-Pilo) models (Ben-Ari, Lagowska, Tremblay, & Le Gal La Salle, 1979; Turski et al., 1984). The KA model exerts its effects primarily on kainate receptors, GluK4 and GluK5, which are highly expressed in the hippocampus (reviewed in Lévesque & Avoli, 2013). Pilocarpine is a muscarinic agonist, acting on M1 muscarinic receptors to produce seizure activity (Turski et al., 1983a; Turski, Czuczwar, Kleinrok, & Turski, 1983b; Turski et al., 1984; Hamilton et al., 1997). Models of epilepsy such as KA and Pilo/Li-Pilo induce limbic motor seizures that progress into status epilepticus (SE) which is defined as a single or an uninterrupted string of seizures lasting at least 30 min (Ben-Ari, Tremblay, Riche, Ghilini, & Nacquet, 1981; Turski et al., 1984; Scott, 2014). Systemic kainate administration produces epileptoform activity that begins in the hippocampus and subsequently propagates to the amygdala, thalamus, and pre-frontal cortex. Postictal alterations to electrographic activity continue in the hippocampus and rhythmic spikes persist in the amygdala for 3-6 days post-status epilepticus (Ben-Ari et al., 1981). Similarly, pilocarpine produces epileptoform activity that begins in the hippocampus and then spreads to other cortical areas. Pilpocarpine-induced electrographic activity consists of ictal spikes accompanied by interictal depressions of activity that eventually develops into SE if left untreated (Turski et al., 1984).

Two other common methods of seizure induction make use of the chemoconvulsants flurothyl and pentylenetetrazol (PTZ). PTZ is an antagonist of

$GABA_A$ receptors that acts primarily on the picrotoxin site (Macdonald & Barker, 1978; Squires, Saederup, Crawley, Skolnick, & Paul, 1984). The exact mechanism of action for flurothyl is not currently known, however, evidence indicates that flurothyl acts on the benzodiazepine site as opposed to the picrotoxin site of $GABA_A$ receptors (Krasowski, 2000; Hashimoto, Araki, Suemaru, & Gomita, 2006). Prolonged or repeated administration of flurothly and PTZ can result in SE (Reddy, & Kuruba, 2013). There are additional methods of seizure induction such as an injection of NMDA that have similar experimental results (Stafstrom & Sasaki-Adams, 2003).

Numerous studies have focused on the effects of SE on cognitive function, specifically learning and memory. The majority of such work has looked at the effects on mature animals, however in more recent years there has been increased interest in the effects of early life seizures (ELS) occurring prior to maturity. I will begin by discussing seizure in adult animals and follow up with a review of research pertaining to ELS.

Adult Seizures

Spatial memory is most commonly tested using the Morris Water Maze (MWM) and the Radial Arm Maze (RAM). The MWM, developed by Richard Morris (1981, 1984), involves placing an animal into a large circular pool of opaque water that contains a submerged platform placed just below the water. The most basic procedure used for this test involves placing the hidden platform in a fixed location and training a rodent to find the platform. Over several learning trials, rodents learn to use distal cues to find and escape onto the hidden platform. Spatial memory is assessed by comparing the latency and the route used to reach this submerged platform over multiple days of testing.

Learning is inferred from progressive decreases in latency to reach the hidden platform as well as the use of increasingly more direct routes to the platform. A probe or transfer test is also commonly used in this task and involves the removal of the platform and measurement of the duration of time an animal spends swimming in each of four quadrants (NW, NE, SW, and SE) that the pool is divided into (Morris, 1984). The RAM task also measures spatial memory (Olton & Samuelson, 1976). It is considered to be more sensitive than the MWM and is capable of detecting differences in spatial working and reference memory (Karnam, Zhao, Shatskikh, & Holmes, 2009a). It uses a maze with multiple arms, typically eight, in which food baits are placed. Investigators typically measure how well animals learn which arms of the apparatus contain the baits and reference memory errors can be measured by the number of instances that an animal reenters an arm where a bait has already been consumed.

In one study a group of rats were given pilocarpine, along with scopolamine to limit peripheral effects of pilocarpine, and a separate set were given kainate. After 90 minutes of SE the pilocarpine-induced seizures were terminated with diazepam to reduce mortality. The kainite group did not receive diazepam. Following a three day recovery period an investigator began examining their spatial learning and memory with the MWM and a novel placement recognition task. Recognition memory was also evaluated using the novel object recognition task. In the MWM, experimental animals required more time to find a hidden platform and during the probe trial spent significantly less time in the target quadrant than saline controls (Pearson, Schulz, & Patel, 2014). The study revealed additional evidence that spatial memory was impaired using a spatial variant of the novel object recognition task. The impairment was found after KA but not

Pilo administration. The novel object recognition task involves first presenting a subject with two identical objects and recording their baseline interaction with each of the objects. Later one of the objects is replaced by a novel object. Interaction of subjects with the novel and familiar objects can then be compared as a measure of recognition memory. Impairment on the task is measured by non-preference for the novel object compared to the familiar object may represent a failure of recognition memory. Using this NOR task, Pearson and colleagues (2014) found long term recognition memory to be significantly impaired after KA- and Pilo-induced SE. These deficits were present one week after experiencing SE (Pearson et al., 2014). It was shown in another study that utilized the Li-Pilo model that rats that later display SRSs have deficits in spatial working memory when tested 5 months later (Detour, Schroeder, Desor, & Nehlig, 2005). This was indicated by a lack of improvement over five days in the RAM (Detour et al., 2005).

Inostroza et al. (2011) examined the effects on cognition of both KA and Li-Pilo administration in Wistar and Sprague-Dawley rats. They found similar severe deficits in the acquisition and long-term retention of spatial memory in both strains after Li-Pilo-induced SE. The deficits were suggested to be likely due to the extensive lesions in the brains of these animals. The Wistar rats injected with KA performed comparably to controls in the water maze in both acquisition of spatial memory and a probe trial in the MWM, in comparison the KA-treated Sprague-Dawley rats had difficulty with the task. Additionally, the KA-treated Sprague-Dawley but not Wistar rats had significantly reduced volume of parts of both the extended hippocampus and neocortex. Analysis of data from the elevated plus maze (EPM) showed that all but the KA-treated Wistar rats had reductions in anxiety. Altered anxiety and the greater extent of brain lesions in the

animals that displayed spatial impairments led Inostroza and colleagues (2011) to suggest that the spatial deficit may have had less to do with the seizures, as both groups of rats experienced SE of similar severity, and were more related to altered anxiety and damage.

The learning and memory deficits after seizures extend beyond spatial learning and memory. Multiple additional tests that examine learning and memory such as fear conditioning have also demonstrated that there are other memory deficits after an episode or episodes of SE. Fear memory is commonly assessed in rodents using auditory fear conditioning. Auditory fear conditioning involves pairing a tone stimulus, with the presentation of an aversive stimulus, typically a mild shock (Herry & Johansen, 2014). The pairing of the two stimuli creates an association that can then be tested by presenting the tone and measuring freezing behavior. The amygdala is the primary structure essential for creating such an association in fear learning during delay fear conditioning (Herry & Johansen, 2014). Adjusting the interval between the presentations of the two stimuli can differentially activate other brain structure such as the hippocampus and prefrontal cortex, such as what occurs in trace fear conditioning (Raybuck & Lattal, 2011). Increased levels of freezing during the presentation of a tone (or trace interval) are used to indicate that an association was made between the tone and shock that now causes the tone to elicit a conditioned fear response. Another measure of fear learning and memory, fear extinction refers to the decrease in performance of a conditioned response that occurs after repeated presentations of a conditioned stimulus in the absence of a previously associated unconditioned stimulus (Orsini & Maren, 2012).

Studies using fear conditioning have provided evidence that contextual memory is severely impaired in rats that had previously been injected with KA or subjected to

electrical stimulation of the amygdala (Kemppainen, Nissinen, & Pitkänen, 2006). There were no deficits in responses to basic tone cues that had been previously associated with an aversive stimulus, suggesting that memory for hippocampus-based but not amygdala-based learning may be differently affected. In another study that examined extinction in C57BL/6 mice following Pilo-induced SE and later display of SRSs, investigators observed a lack of decrease in freezing behavior indicating impairment in fear extinction (Lesting, Geiger, Narayanan, Pape, & Seidenbecher, 2011). Lesting and colleagues (2011) also reported differences associated with this impaired extinction on a neurophysiological level reporting that there was maintained amygdala-hippocampal theta frequency synchronization in Pilo-treated animals but not controls. Presentation of a fear-associated stimulus has been previously shown to results in amygdalar and/or hippocampal theta activity in each of these areas as well as synchrony between them during fear-memory retrieval (Paré, Collins, & Pelletier, 2002; Moita, Rosis, Zhou, LeDoux, & Blair, 2003; Seidenbecher, Laxmi, Stork, & Pape, 2003). Thus, theta oscillations represent contributions from different structures in the amygdala-hippocampal network that work together during fear to give an organism information about various aspect of the stimuli and context associated with a fear-producing event or situation. The lack of reduction of these theta oscillations further reflects the impaired extinction observed in the mice that experienced pilocarpine-induced SE.

Using the What-where-when task, a version of the NOR task that is adapted to measure episodic-like memory, investigators have shown that rats have a selective impairment in episodic-like memory following multiple episodes of KA-induced SE (Inostroza, Brotons-Mas, Laurent, Cid, & de la Prida, 2013). The NOR task was used to

assess the “what” aspect of memory and the novel placement recognition task was used to assess the “where” aspect. The “when” aspect of episodic memory was tested by first presenting a sample phase with two identical objects, then after an interval of 50 minutes, a second sample with two different identical objects was presented. After another 50 minute resting period, the animals were presented with one of each of the two objects presented in the previous two sampling phases. Thus, the task allowed the assessment of spatial memory for old and recent familiar objects. The investigators did not find impairments in what, where, and when memory when each was tested separately. When the investigators presented the modified What-where-when task that evaluated all three of these aspects of episodic memory simultaneously, however, there was impairment. The impairment for episodic-like memory was associated with an impairment of hippocampal theta activity as measured with electrophysiological recordings which was suggested may be central to the observed dysfunction.

Another notable study found that rats that had experienced KA-induced SE and later developed SRSs perform poorer in the MWM than rats that experienced SE but did not develop SRSs and controls (Yin et al., 2005). Yin and colleagues also found a downregulation in expression of Epilepsy Related Gene 1 (ERG1) in hippocampal field CA1 and dorsal dentate gyrus cells of the animals displaying SRSs using immunohistochemistry and in situ hybridization, which is believed to be related to SRSs. ERG1 encodes a rodent homologue of the ATPase N-ethylmaleimide-sensitive fusion protein (NSF) which is involved in fusion of vesicles during exocytosis by disassembling SNARE complexes. NSF may also be involved in long-term potentiation (LTP) on

postsynaptic cells through an interaction with GluR2 receptors which regulates AMPA receptor function at synapses (Yin et al., 2005).

The studies described above clearly indicate that there are significant alterations to both brain activity and behavior which occur after SE in adult rodents. Given the spatial deficits reported in many of these studies, it seems evident that the hippocampus, a vital structure involved in memory, is notably affected after SE in adult animals. Severity in many of these impairments and alterations are associated with the damage caused by status and structural and connectivity changes that occur as a result of that damage. It is tempting to conclude that such changes are the culprit also underlying spontaneous seizures seen in adult animals. Such an assumption would likely be incorrect. Letty et al. (1995) compared spatial and social deficits between KA and amygdala kindling, the latter of which does not produce significant damage, and found that although both treatments led to rats later developing spontaneous seizures, only the KA-treated animals showed spatial memory and social deficits.

One of the most consistent findings with the use of KA and Pilo has been that in adult rodents there is a significant deficit in spatial memory following an episode of SE (Pearson et al., 2014; Yin et al., 2005; Inostroza et al., 2011; Detour et al., 2005). In addition to spatial deficits, there are also alterations to recognition memory, episodic memory, and emotional memory that have been reported following SE (Pearson et al., 2014; Inostroza et al., 2013; Kemppainen et al., 2006). Additionally, KA and Pilo studies have shown that SE can result in altered social behavior, anxiety, and depression-like behavior (Letty, Lerner-Natoli, & Rondouin, 1995; Oliveira et al., 2015). Often the levels of these types of impairments and deficits can be related to the severity of damage

induced by SE (Letty et al., 1995). One important difference between SE induced via KA and Pilo is that of the extent of damage each method typically results in. Generally there is greater damage for those subjects in which SE was induced using Pilo (Covolan & Mello, 2000). To complicate things further, studies examining SE in younger animals have produced similar deficits in spatial memory, often without the types of overt damage or structural alterations seen in adult animals. It is therefore important to compare studies examining early life seizures with those of adult seizures when attempting to ascertain how epilepsy affects behavior and the brain and further how difference in age affects the underlying processes.

Early Life Seizures

Until recently, not as much was known about the effects of SE on immature and adolescent subjects. In a study examining spatial memory in young rats, SE was induced using KA injections on postnatal days P1, P7, P14, or P24 (Sayin, Sutula, & Stafstrom, 2004). These animals were then tested on P90-P100 for short term memory in the RAM, long term memory (LTM) in the MWM, and for anxiety in the elevated plus maze (EPM). The EPM consists of a raised platform with four arms, two of which are closed. Typically, rodents spend more time in the closed arms in this test. More time spent in the open arms is indicative of lower levels of anxiety. Investigators found that animals that had KA seizures had both longer latency to find and consume four food baits and more reference errors (entries into unbaited arms) in the RAM which indicated that ELS results in impaired short-term memory. Investigators also found deficits in long-term memory as indicated by longer escape latencies to the submerged platform over five days of testing

in the MWM. The escape latency was found to be highest for the P24 group followed by P7 then P1. Post hoc analysis showed only P24 and P7 latencies were significantly different from controls. As the age of animals increased they also showed progressively less time spent in the target quadrant of the maze (the quadrant where the submerged platform was located) during a probe trial after the platform was removed. This could be interpreted as impaired retrieval or failure to learn. Additionally, less time spent in the open arms of the EPM indicated greater levels of anxiety in the mice that experienced KA seizures. The reduction in anxiety was greatest for the P1 group followed by P7, P24, and finally P14.

Other methods of ELS induction have provided additional evidence that seizures prior to maturity can affect cognitive function. Stafstrom and Sasaki-Adams (2003) used intraperitoneal injections of N-methyl-D-aspartate (NMDA) to produce episodes of SE in young rats. Rat pups that experienced NMDA-induced SE at age P12-P20 showed severe impairments of long term spatial memory when tested in the MWM starting on P85 (Stafstrom & Sasaki-Adams, 2003). These animals also showed increased susceptibility to seizures when tested with PTZ, displaying both reduced latency to behavioral seizure and longer duration of seizures compared to controls. Finally, in a recent study that examined ELSs in C57BL/6J mice, investigators showed that seizures on P7-P11 (3 per day) induced via the inhalant flurothyl lead to deficits in hippocampal-dependent learning and memory in both fear conditioning and MWM (Lugo, Swann, & Anderson, 2014).

Examining rat pups that experienced 5 consecutive days of PTZ-induced seizures starting on P10 has revealed similar results in the MWM in groups tested on either P35 or P60 (Huang et al., 2002). There was also a significantly greater amount of mossy fiber

sprouting in hippocampal field CA3 that was found using Timm staining. The sprouting indicates structural reorganization can occur in immature brains following seizures. In another study that looked at ELSs induced with Li-Pilo in P14 rat pups, investigators found spatial memory deficits in both MWM and RAM when the animals were tested on P60 (Wu et al., 2001). Additionally, Wu and colleagues found that a third of their sample had developed lesions in the hippocampal CA1 region that could have contributed to the spatial impairments.

In a study comparing the effects of SE on immature and adult rats, investigators began by first training the animals in the MWM (P16-P19 and P56-P59, respectively) (Sarkisian et al., 1997). Then, at two day intervals, starting on P20 for the immature group and P60 for the mature group, they injected both groups of animals with KA or saline four times. Doses for the two age groups were adjusted to produce severe seizures while maintaining a low mortality rate. Sarkisian and colleagues then began MWM testing on P60 for the immature group and P100 for the mature group. After the second experience in the MWM, the animals were sacrificed and their brains removed for subsequent histological analysis. The rats that had been injected with KA starting on P60 showed a significant impairment in re-learning the submerged platform location on P100. The animals that were injected starting on P20 and saline controls for both ages seemed to retain their memory of the platform location from their first training experiences in the MWM. The histological analysis revealed significant cell loss in the CA1, CA3, CA4, and hilus of the hippocampus in only the mature group while no major loss was observed in the immature animals or controls at either age. It seemed as if the older animals failed to retain their spatial memory and that the failure might have been associated with the

damage that resulted from SE. There were no impairments found in the immature animals after SE. Other studies using younger animals have found spatial learning and memory impairments following SE.

In the last several years new data has been published which may explain why some experiments that did not result in cell loss still found impairments in learning and memory. Nishimura, Gu, & Swann (2011) induced three 3-min seizures per day for five days in C57BL/6 mice from P7-P11 and P30-P34. They found deficits in spatial learning and long-term spatial memory in the MWM for the younger group, but not the older group. Subsequent morphological analysis of Thy1GFP-M transgenic mice that expressed green fluorescent protein in hippocampal pyramidal cells showed that flurothyl seizures during P7-P11 resulted in significant decreases in dendritic length, branching, and volume of dendritic fields of basilar dendrites in CA1 pyramidal neurons compared to controls at P30. A separate set of Thy1GFP-M mice that received seizures from P30-P34 showed no impairments to spatial learning and memory, nor did they show alterations in dendritic arbor complexity.

These studies that examined ELS indicate that the overt damage more commonly seen as a result of adult seizures is not an imperative prerequisite for later cognitive deficits. It seems that younger brains are in some cases better able to handle seizure activity without suffering the major cell death that is common in mature brains. Studies have, however, shown that even though overt damage is avoided in some cases in younger subjects, there are more subtle changes to dendritic growth and complexity that may be linked to the learning and memory deficits present after ELS. For ELS studies it appears that a myriad of factors can affect whether or not impairments arise. There is a

growing body of literature on this subject and we now know with some certainty that it is important to consider the future repercussions that seizures may have on a developing brain. The continuing study of ELS will undoubtedly reveal more of the specific mechanisms that lead to cognitive impairments in later life.

Acute Seizures

As opposed to studies that examine the effects of SE, studies utilizing acute seizures are concerned with relatively shorter convulsive episodes. The typical experimental design for such studies involves inducing several distinct seizures over a period of days until some predetermined number of seizures has been experienced. Such a design is in contrast to studies that induce a single episode of SE. High quantities of acute seizures can lead to similar impairments of learning and memory as those produced by SE (Holmes, Gairsa, Chavassus-Au-Louis, & Ben-Ari, 1998; Sogawa et al., 2001; Zhao, Hu & Holmes, 2005). Use of acute seizures allows for testing of altered seizure susceptibility and is more time and cost effective for the testing of new antiepileptic compounds.

Studies examining acute seizures induced during early life have shown that acute seizures can result in memory impairment and changes to the brain. One method of acute seizure induction is via the volatile inhalant flurothyl which leads to behavioral seizures and eventually tonic extension with extended exposure in rodents. A sufficient infusion rate of flurothyl can lead to seizures within minutes which last until an animal is removed from an inhalation chamber (Holmes et al., 1998). Huang et al. (1999) found that 50 flurothyl seizures induced from P0-P9 in male Sprague-Dawley rats lead to changes in

neuronal connectivity characterized by mossy fiber sprouting in the CA3 field and the molecular layer of the dentate gyrus. The changes were accompanied by a "modest" impairment in spatial learning and memory as measured in the MWM (Huang et al., 1999). Isaeva and colleagues (2006) found that 25 early life acute seizures induced with flurothyl starting on P1 leads to decreased amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) in slices taken from these animals on P15-P17. The decrease was attributed to downregulation of GABAergic synaptic transmission possibly by a downregulation of $GABA_A$ receptors or their inhibitory activity (Isaeva, Isaev, Khazipov, & Holmes, 2006).

Alterations in spatial memory in the MWM have also been shown to be accompanied by changes in single cell firing and long-term potentiation (LTP). Adult Sprague-Dawley rats that experienced 10 seizures over five days showed alterations in hippocampal place cell firing patterns between 24 and 72 or more hours following their last seizure (Zhou, Shatskikh, Liu, & Holmes, 2007). Additionally, LTP could not be induced in these animals. Along this line, Karnam et al. (2009b) found similar alteration to hippocampal place cells between P100-P140. The alterations to place cells were following 100 flurothyl seizures induced between P15 and P37 that accompanied MWM impairments on P42-P46 and radial-arm water maze on P60-P80 (Karnam et al. 2009b).

Studies that have examined the effects of multiple repeated seizures cannot separate the immediate effects of each seizure from the damage that contributes to observed impairments and epileptogenesis. In one notable study that attempted to tease out some of these differences, Mao and colleagues (2009) tested the effect of single, short seizures on spatial and contextual learning. In the study investigators induced single

seizures in rats 30 minutes prior to or immediately subsequent to MWM training and contextual fear conditioning and then examined both short-term memory (STM) and LTM afterwards. A single pre-training convulsive dose of PTZ, a $GABA_A$ antagonist, resulted 24 hours afterwards in the impaired recall of spatial memory and fear memory in the MWM and contextual fear conditioning, respectively. The investigators found no impairment of memory after a post-training PTZ-induced seizure in the MWM. On the other hand, a post-training seizure after contextual fear conditioning resulted in an impairment of STM, although LTM was left unaffected 24 hours later. Additionally, it was shown that a pre-retrieval seizure transiently impairs recall of fear memory at a 24 hour LTM test; there is no impairment at 48 hours (Mao et al., 2009).

In the current study we investigated the effect of a single acute seizure induced in a mouse by the inhalant flurothyl at various time points before or after associative fear conditioning. Using a delay fear conditioning paradigm in the experiments, we examined the effect of a flurothyl seizure on fear learning and memory. We intended to first confirm that the impairments to contextual fear memory previously reported in rats could be extended to associative fear memory in mice. The step is a necessary prerequisite for later investigation utilizing transgenic mouse models to examine potential underlying mechanisms for any observed changes. Next we wanted to build upon the experimental design of Mao et al, (2009) by focusing more intensely in particular on fear memory. To this end, we also looked at a 6 hour pre-training seizure to examine whether a single seizure can affect learning and memory at a more distant time point.

CHAPTER THREE

Methods and Materials

Subjects

We used adult male and female 129SvEvTac mice that were generated and housed at Baylor University. The home colony room was maintained at an ambient temperature of 22 °C, with a 14-h light and 10-h dark (20:00 to 6:00h) diurnal cycle. The mice were group housed in standard cages and were allowed *ad libitum* access to food and water. The animal protocol was approved by Baylor University Animal Care and Use Committee and all procedures performed were in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Apparatus

Fear Conditioning

Fear conditioning and testing was performed using two Coulbourn Habitest® fear conditioning chambers (26 cm x 22 cm x 18 cm) placed inside of sound dampening, isolation cubicles. The chambers were configured with two acrylic and two metal sides as well as metal grid floors which delivered mild footshocks. The footshocks were delivered by Coulbourn Precision Animal Shockers which were manually calibrated using an ENV 420 Amp-meter (Med Associates inc) prior to testing on each day shocks were to be delivered. A white noise tone was delivered by an external PYLE® PRO PCA2 stereo amplifier that played though speakers mounted on the rear of the isolation cubicles.

Shocks were delivered and freezing behavior was recorded and measured for each testing day using FreezeFrame 3 software (Coulbourn, Ohio, USA).

Open Field

Two Fusion Nodes equipped with multiple Fusion Sensors measured activity level in the open field. The testing arena was a clear acrylic (40 cm X 40 cm X 40 cm) box. The automated measurements by the sensors were recorded using the Fusion software (Omnitech electronics, OH, USA) for the 10 minute trial.

Seizure Induction Method

Flurothyl Induction

All seizures were induced under a Supreme Air Laboratory Fume Hood from the Kewaunee ® Scientific Corporation inside a clear acrylic (29 cm x 16 cm x 15 cm) inhalation chamber. Flurothyl (bis-2,2,2-trifluroethyl ether), obtained from Sigma-Aldrich (St Louis, MO, USA), was pumped into the chamber using a Harvard Apparatus model 11 Plus syringe pump at a rate of 50 μL per minute.

Fear Conditioning Protocol

Delay Fear Conditioning

We used a delay fear conditioning protocol in which a mouse was placed into the chamber and exposed to the conditioning stimuli. There was a 2-minute baseline period, which was followed by the presentation of the conditioned stimulus (CS) for 20 seconds (80-dB white noise tone). The white noise was immediately followed by a mild footshock

(2 second 0.7-mA) that served as the unconditioned stimulus (US). The US was followed by an inter-trial interval of 1 minute, followed by another tone and shock pairing. There was a 20-second interval period following the second pairing. Mice received a total of 2 CS-US pairings. The FreezeFrame monitor system was used to control the timing of the CS and US presentation. After each testing session the chambers were cleaned with 30% isopropyl alcohol.

Memory Test

Mice were tested for cued fear conditioning after a seizure. We used the same protocol to measure both short- and long-term memory. The fear learning chambers were altered prior to testing in order to present a novel context. The texture, color, and shape of the chambers were altered using acrylic inserts and a novel odor (vanilla extract; Adam's Extracts, USA) was placed under the floor. We monitored freezing behavior for two 3-minute periods inside the chamber. The first period consisted of the new context for 3 minutes. During the second period, we presented the auditory CS for 3 minutes and measured freezing.

Experiment 1: One Hour Pre-training Seizure

In our first experiment we wanted to examine the effect of a single acute seizure induced one hour prior to fear conditioning on subsequent fear learning and memory (See Figure 1A). The mice were transported to a holding room where they were allowed to acclimate for 30 minutes prior to seizure induction. A single mouse was placed into a clean transfer cage and transported from a holding room to the main lab. The animal was then placed into an inhalation chamber under a chemical vent hood where it was exposed

to flurothyl until a behavioral seizure (wild running with tonic-clonic seizure) was observed. A control animal was also placed into a second inhalation chamber for the same amount of time in parallel to the experimental animal. After a behavioral seizure was induced, both experimental and control animals were removed from the inhalation chambers and returned to the transfer cages to recover. The mice were transported back to the holding room after a brief recovery period to await the next phase of the experiment.

Delay Fear Conditioning

One hour after a seizure was induced, mice were transported to a testing room where they underwent delay fear conditioning. The mouse was placed into the chamber for fear conditioning using the protocol that was described above. After the protocol ended, the animal was removed from the chamber and returned to the holding room.

Memory Test

Short-term memory, defined for the purposes of this study as memory that has been encoded but not consolidated into permanent long-term memory, of the associative learning was measured 1 hour following fear conditioning. We also tested the animal in the same conditions 24 hours after the seizure was induced to examine LTM. The protocol and measurement parameters were identical to those used in the STM test. After the completion of testing for all animals, the mice were returned to their home cages and transported to their home colony.

Experiment 2: Six Hour Pre-training Seizure

In our second experiment we examined whether animals that experienced an acute seizure 6 hours prior to delay fear conditioning would show a learning and memory deficit compared to control animals. A single pre-training acute flurothyl seizure was induced inside an inhalation chamber as described in experiment 1. Control animals were again placed into a second inhalation chamber concurrent with animals assigned to the experimental condition.

Delay Fear Conditioning

Animals were trained using delay fear conditioning 6 hours after they received a seizure.

Memory Test

In order to examine LTM, we tested the mice 24 hours post-seizure. We tested the mice in the same new context and conditions as previously described.

Experiment 3: Open Field

A separate set of mice were examined in a 10 minute open field test to evaluate whether the results of the STM test in experiment 1 might have been influenced by a decrease in locomotor activity level following a flurothyl seizure. We also wanted to verify that their baseline activity levels were not different than controls 24 hours after a seizure. A single acute seizure was induced using flurothyl and control mice were used as previously described.

Two hours after experiencing a seizure, a mouse was placed into the open field for a 10-minute period. We recorded general activity level and several other measures of activity. The mouse was removed and returned to its home cage after the 10-minute test. The open field arena was cleaned with 30% isopropanol and prepared for the next mouse. All mice were returned to their home colony after testing was completed. The mice were tested again in the open field at a 24 hours post-seizure time point to assess their activity levels in another 10-minute trial.

Experiment 4: One Hour Post-training Seizure

The previous experiments were conducted to investigate whether an acute seizure impairs the acquisition of a new memory. For our final experiment, we wanted to examine whether an acute seizure impairs the retention of a newly acquired memory (See Figure 1B).

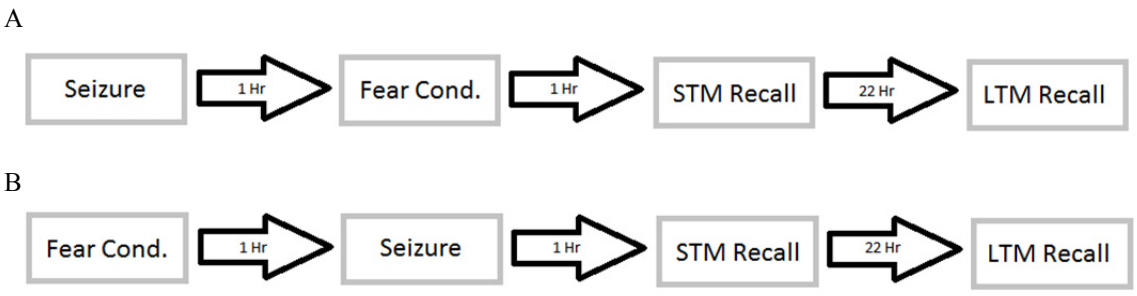

Figure 1. Timelines for 1 h pre- and 1 h post-training seizure experiments. A. 1 h pre-training group, B. 1 h post-training group.

Delay Fear Conditioning

The mice were first trained using the delay fear conditioning protocol.

Seizure Induction

One hour after the training phase of delay fear conditioning, we induced a flurothyl seizure in the mice.

Memory Test

One hour following the seizure we tested the STM of mice who had experienced a post-training seizure. On the next day (24 hours following the seizure) the mice were transported to the testing room where they were again placed into the chamber and LTM was examined.

Data Analysis

All data were analyzed using Prism 6 (GraphPad Software, Inc., La Jolla, CA). For all comparisons, the level of significance was set at $p < 0.05$. Males and females were combined per group since no statistically significant differences were found between them. Animals were monitored throughout the experiments for changes in weight and no significant differences were found.

CHAPTER FOUR

Results

Experiment 1: One Hour Pre-training Seizure

The results of memory tests revealed that long-term memory for a conditioned stimulus was impaired a day later following a 1 hour pre-training seizure. Mice that were given a seizure and then examined in delay fear conditioning showed an increase in freezing during all aspects of the training phase. There was a main effect of group during the training phase of delay fear conditioning $F(1, 13)= 46.19$, $p < 0.0001$ (Figure 2). The STM test was performed one hour after delay fear conditioning. The seizure group had a significant increase in freezing in the new context $t(1, 12) = 2.82$, $p < 0.05$ during the context phase but did not show a significant difference compared to control mice when the CS was presented $t(1, 12) = 1.79$, $p = 0.09$ (Figure 3: left two graphs). Twenty four hours after we induced seizures we tested the mice for alterations in LTM. We found that mice that had experienced a single seizure 1 hour prior to training showed a significant impairment in associative conditioning 24 hours later compared to controls when presented with the conditioned stimulus $t(1, 12) = 3.48$, $p < 0.05$. However, the seizure mice were no different in their freezing behavior in the new context compared to controls $t(1, 12) = 1.85$, $p = 0.08$ (Figure 3: right two graphs).

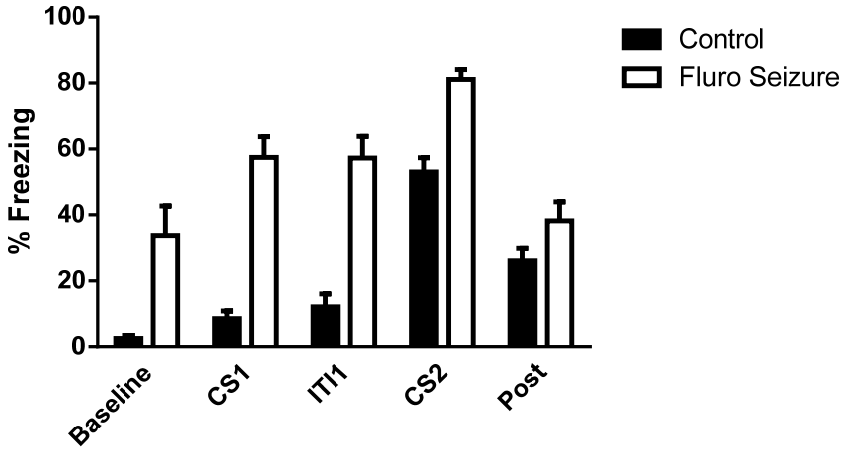

Figure 2. Freezing during training 1 h after an acute seizure. Freezing during fear conditioning in the 1 h pre-training group as compared to controls that did not experience seizures. Mice in the 1 h pre-training seizure group showed a main effect of group $F(1, 13) = 46.19$, $p < 0.0001$.

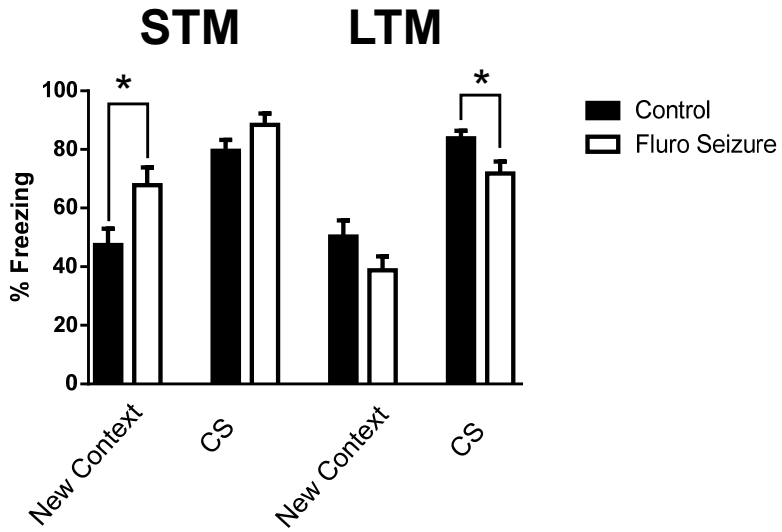

Figure 3. An acute seizure 1 h prior to fear conditioning impairs long-term memory. Mice that had experienced a single seizure 1 h prior to training showed a significant impairment in associative conditioning 24 h later compared to controls when presented with the white noise $t(1, 12) = 3.48$, $p < 0.05$. However, the seizure mice were no different in their freezing behavior in the new context compared to controls $t(1, 12) = 1.85$, $p = 0.08$. Error bars denote SEM. * = $p < .05$

Experiment 2: Six Hour Pre-training Seizure

A 6 hour pre-training seizure did not affect associative memory one day later. Mice that had experienced a seizure 6 hours prior to training did not show any significant difference in freezing behavior compared to controls during delay fear conditioning training $F(1, 9) = 3.1$, $p = 0.11$ (Figure 4). There was also no difference 24 hours after a seizure when presented with the conditioned stimulus $t(1, 9) = 0.81$, $p = 0.43$ or in the new context $t(1, 9) = 0.76$, $p = 0.46$ (Figure 5). However, both groups did demonstrate an increase in freezing when presented with the CS compared to the new context condition. A paired t-test revealed an increase in freezing for the control mice across the new context compared to CS condition $t(1, 5) = 3.9$, $p < 0.05$. Similar results were found in the seizure group $t(1, 4) = 4.3$, $p < 0.05$.

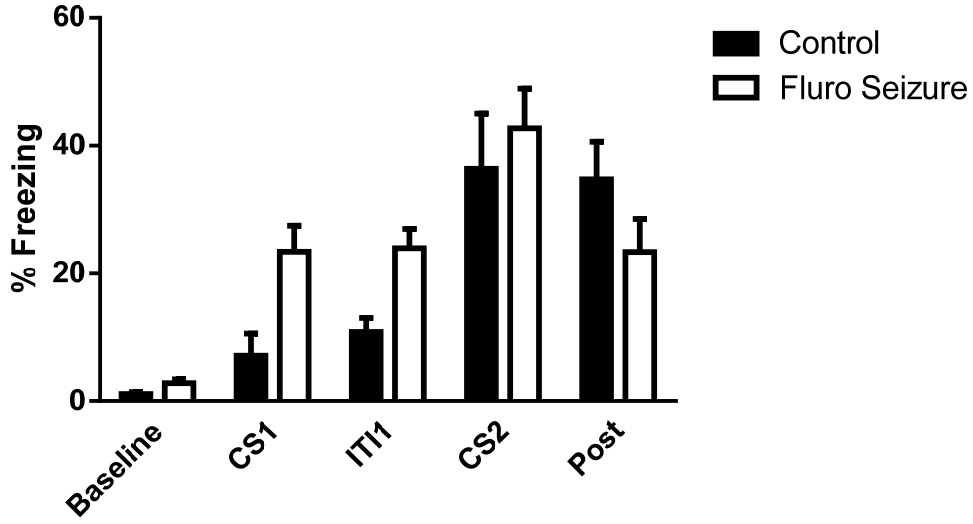

Figure 4. Freezing during training 6 h after an acute seizure. There was no difference in training from controls for mice that experienced a seizure 6 h prior to delay fear conditioning $F(1, 9) = 3.1$, $p = 0.11$.

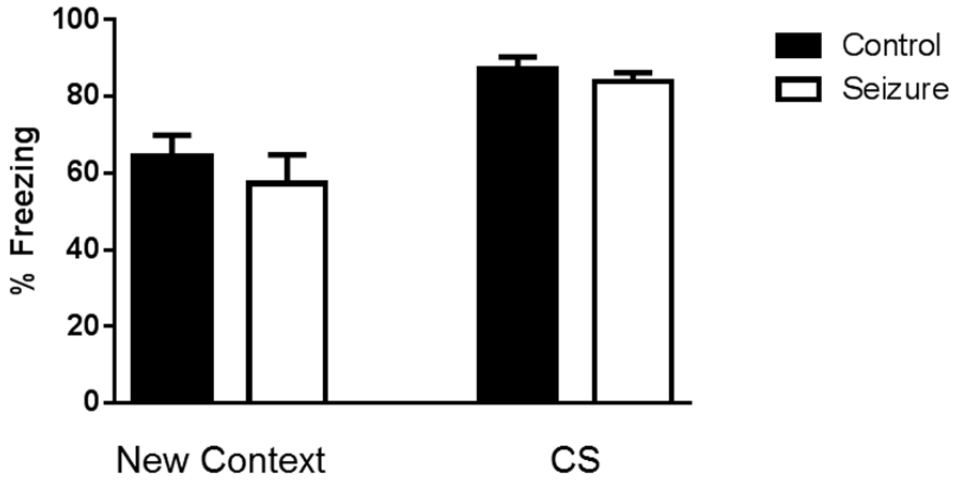

Figure 5. An acute seizure 6 h prior to fear conditioning does not affect long-term memory. In the 6 h pre-training seizure group there was no difference 24 h after a seizure when presented with the conditioned stimulus $t(1,9) = 0.81$, $p = 0.43$ or in the new context $t(1,9) = 0.76$, $p = 0.46$ in comparison to controls. Error bars denote SEM.

Experiment 3: Open Field

For experiment 3 we wanted to determine if a single seizure affected the activity levels of our mice during the short- and long-term memory tests. We found that an acute flurothyl seizure resulted in a significant decrease in activity 2 hours after the seizure was induced but the effect was not long-lasting. For the 2 hour analysis we used the Mann-Whitney U test because the variance of the data violated the assumption of homogeneity. The F test for the analysis was $F(7, 6) = 423$, $p < 0.0001$, $F(7, 6) = 42.31$, $p < 0.001$. We found a significant reduction in locomotor activity at the 2 hour time point $U = 1$, $p < 0.001$ (Figure 6A) and a significant reduction in movement frequency $U = 4.5$, $p < 0.01$ (Figure 6B). The effect on activity was temporary. When tested at 24 hours after seizure induction there was no difference in total activity or in movement frequency in mice that experienced a single flurothyl seizure compared to control mice in the open field test 24 hours after a seizure $t(1, 13) = 0.24$, $p = 0.81$; $t(1, 13) = 0.18$, $p = 0.86$.

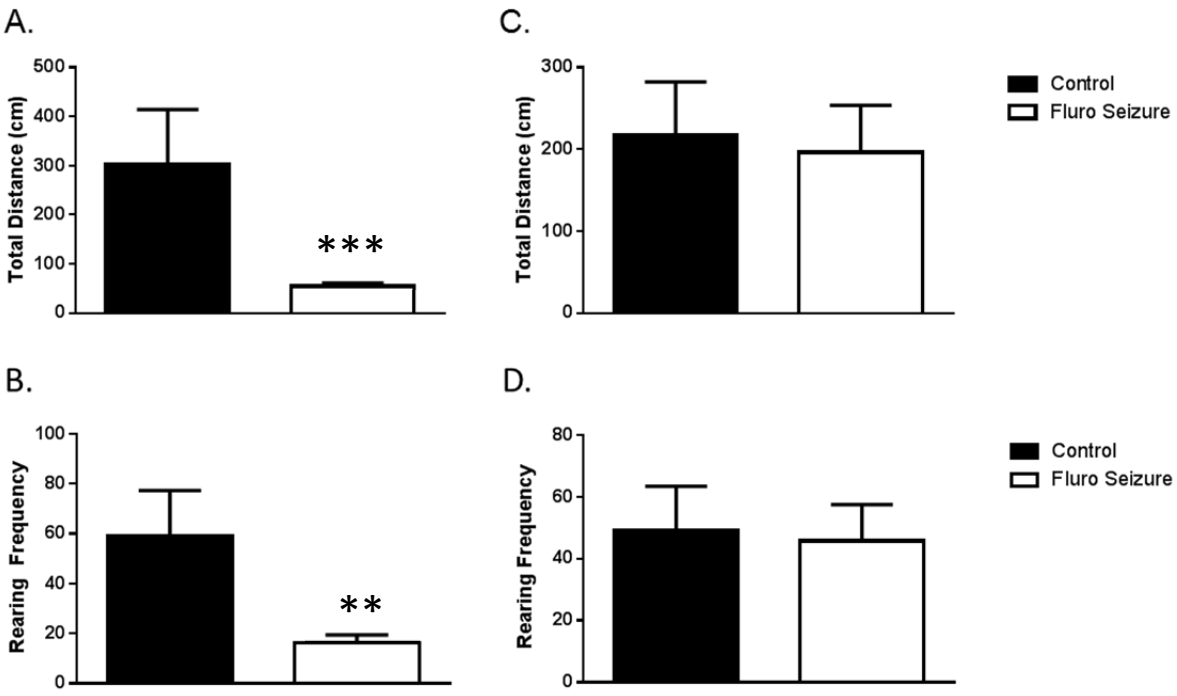

Figure 6. A flurothyl seizure transiently affects activity levels. We found A. a significant reduction in locomotor activity at the 2 h time point $U = 1$, $p < 0.001$ and B. a significant reduction in movement frequency $U = 4.5$, $p < 0.01$. However, we did not find a difference in C. total activity or in D. movement frequency in mice that had a single flurothyl seizure compared to control mice in the open field test 24 h after a seizure $t(1,13) = 0.24$, $p = 0.81$; $t(1,13) = 0.18$, $p = 0.86$. Error bars denote SEM. ** = $p < .01$ *** = $p < .001$

Experiment 4: One Hour Post-training Seizure

We found that a single post-training seizure did not affect associative memory when recalled a day later. There was no difference in freezing during training for mice that had experienced a seizure 1 hour after training $F(1, 30) = 0.39$, $p = 0.53$ (Figure 7). We found that the seizure group had an increase in freezing in the New Context testing phase during the STM test $t(1, 19) = 4.9$, $p < 0.0001$. A similar significant increase in freezing was found in the 3 minute period when the CS was presented $t(1, 19) = 2.34$, $p < 0.05$ 1 hour after a seizure (Figure 8). The effect was not long-lasting. At the 24 hour time point after the seizure was induced there was no difference between the groups in the new

context t(1, 19) = 0.66, p = 0.51 or in the CS condition t(1, 19) = 0.12, p = 0.90 (Figure 8).

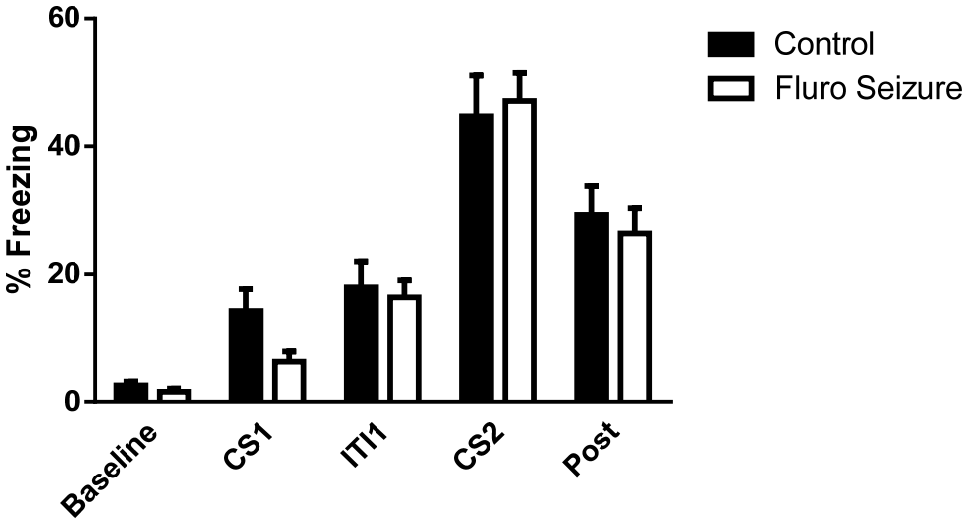

Figure 7. Freezing during training 1 h before an acute seizure. There was no difference in freezing during training prior to a 1 h post-training seizure F(1, 30) = 0.39, p = 0.53.

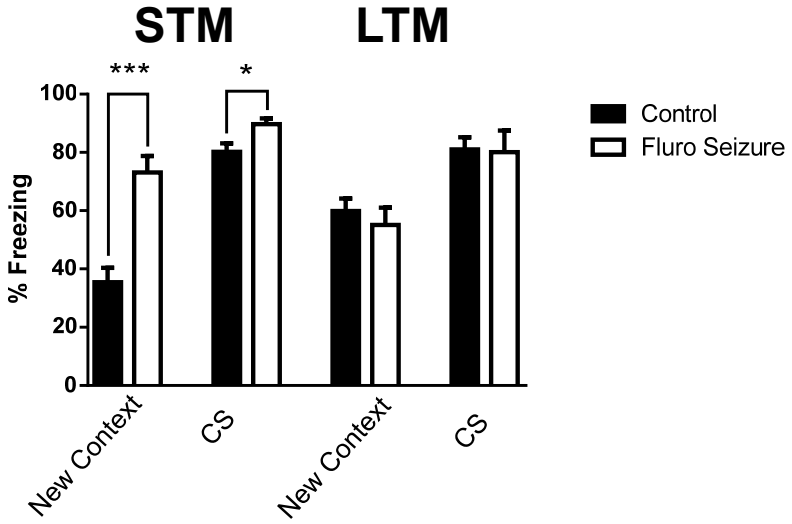

Figure 8. An acute seizure 1 h after fear conditioning does not affect long-term memory. Mice that experienced a single seizure 1 h after training had an increase in freezing in the New Context t(1,19) = 4.9, p < 0.0001 and during the CS presentation t(1,19) = 2.34, p < 0.05 1 h after the seizure. At 24 h after a seizure mice showed no difference in freezing in the new context t(1,19) = 0.66, p = 0.51 or in the CS condition t(1,19) = 0.12, p = 0.90 Error bars denote SEM. * = p < .05 ** = p < .01 *** = p < .001

CHAPTER FIVE

Discussion

In 2010, Dr. McAuley and colleagues conducted a study with the Comprehensive Epilepsy Program at Ohio State to examine what the highest concerns were for individuals with epilepsy. The second most important concern for those with epilepsy was their memory and it ranked as their third most frequent concern (McAuley et al., 2010). The patient report is in contrast to the report from practitioners where they ranked memory concerns as number 12. The contrast demonstrates the discrepancy in the concerns of patients with epilepsy compared to practitioners. There is accumulating evidence that cognitive impairments are an important consideration in epilepsy. In our study we induced seizures 1 hour prior to associative fear conditioning and observed associative learning deficits when we examined their memory 24 hours later. We did not observe deficits in associative memory when the seizure was induced after associative conditioning. The results from our studies provide evidence that the ability to form memories may be more sensitive to seizure disruption compared to memories acquired before seizure induction.

Our results confirm and extend those from a similar study that examined the influence of acute seizures on spatial and associative learning (Mao, et al., 2009). They induced seizures by an intraperitoneal injection of PTZ in adult rats. In one set of experiments they induced the seizure 1 hour prior to trials in the Morris Water Maze. In another experiment they induced the PTZ seizure 1 hour prior to fear conditioning. In

both tests they found deficits in the acquisition of learning. They also induced a seizure 1 hour after the animals were tested in the MWM and fear conditioning test and found no significant memory impairment 24 hours later.

One difference between our study and the Mao et al (2009) study is that they found that a seizure induced before fear conditioning results in a learning deficit when the animal is tested 1 hour after fear conditioning training. We did not observe such a difference in our study. One reason may be the PTZ seizure induction method produces more severe seizures. They report that the PTZ seizure duration is 60-70 seconds. The flurothyl seizure induction method we used rapidly results in a seizure within minutes of exposure (Holmes et al., 1998). The behavioral seizures in our study lasted for 20-30 seconds after the animals were removed from the inhalation chamber. Another concern with the use of flurothyl seizures is that there is a significant reduction in locomotor activity in the mice. We observed an increased level of freezing when we examined the freezing behavior of the seizure group 2 hours after seizures. Because freezing is the measurement of learning in the fear conditioning test, it appeared as though the animals had an enhancement of learning. However, we repeatedly observed increased freezing whenever we examined the freezing behavior of the animals within 2 hours after seizures. Therefore, we examined the locomotor behavior of another cohort of mice in an open field test. We administered a flurothyl seizure to the mice and observed their locomotor activity 2 hours and 24 hours later in an open field test. We found a significant reduction in activity at the 2 hour level. Locomotor reduction may explain why the animals had more freezing after seizure induction. It is important to note that the decrease in activity

was not present at the 24 hour time period. Therefore, any change of freezing at the 24 hour time point does not appear to be due to a change in activity levels.

The influence of PTZ on activity levels may help to explain another difference between the Mao et al (2009) paper and our study. They report short-term memory deficits in both retrieval and consolidation of a fear memory, which is shown through a reduction in freezing behavior in fear conditioning. It is not clear whether PTZ induces acute hyperactivity in the rodents. It is possible that the reduction in freezing after seizures could be due to a change in locomotor activity, which would then influence freezing behavior in the animals at this time point. Future studies examining learning and memory after seizures should also include measures of locomotion to rule out the influence on activity levels.

Our data differed from classical studies that used electroconvulsive shocks administered to rats after learning to examine memory consolidation. Specifically, these studies found that animals suffered from retroactive amnesia for events that occurred prior to the convulsive shock when tested later. Our data did not indicate a post-training seizure has this effect. Many of these studies found this result using various one-trial aversive learning designs (Madsen & McGaugh, 1961; Chevalier, 1965; Chorover & Schiller, 1965; Quartermain, Paolino, & Miller, 1965). Another study produced similar results in a one-trial appetitive learning task (Tenen, 1965). The particular type of disruption of memory was also shown to be greater the shorter the interval was between learning and electroconvulsive shock (Heriot & Coleman, 1962; Chorover & Schiller, 1965; King, 1965).

Another study that examined memory following post-training treatment with PTZ at different intervals that ranged from 10 seconds to 4 days also found impairment of memory for aversive learning (Pearlman, Sharpless, & Jarvik, 1961). This is again at odds with our data. It is puzzling that the seizures produced by these shocks and PTZ after learning produced memory impairments while those seizures in the present study using flurothyl did not. Interestingly, Mao et al. (2009) also used PTZ to induce seizures in rats and found only that a post-training seizure may have impaired STM, but not LTM. The difference between these two studies using the same convulsant may be explained by seizure duration or PTZ dosage as Pearlman et al. (1961) reported that clonus activity lasted around 4 minutes whereas Mao and colleagues (2009) reported that the duration of seizures in their study was 72.7 ± 12 seconds. It is possible that a more sustained acute seizure may affect memory differently than a relatively shorter seizure. Alternatively, the severity of seizures in the two studies may have also differed.

Our observations on the influence of seizures on memory are in line with our understanding of the consolidation of memories. In order for a short-term memory to become a long-term memory there is a necessary period of consolidation. Consolidation is believed to require a few hours to complete and requires protein synthesis. These assumptions are due to experiments where the drug anisomycin, which blocks protein synthesis, is given prior to fear conditioning and results in memory deficits (Schafe & LeDoux, 2000). Similar results are found if the drug is given 1-2 hours after fear conditioning, but little impairment occurs if the drug is given 4-6 hours after training. We included a group that received a seizure 6 hours prior to fear conditioning and found no impairment in memory. It may be that seizures are acting similar to anisomycin and are

preventing/disrupting protein synthesis but the effect is short-term. Future studies could address this process using western blot analysis or other assays and could lead to further understanding of the role of protein synthesis in memory consolidation.

One future direction will be to examine why a seizure prior to acquisition has an impact on learning whereas a seizure during the consolidation phase has no impact on learning. One possibility is that flurothyl seizures may have selectively impacted the lateral amygdala. In a previous study, investigators infused the $GABA_A$ agonist muscimol into the lateral amygdala to temporarily inhibit this region (Wilensky, Schafe, & LeDoux, 2000). They found that inactivation here blocked the acquisition of fear conditioning but not when it was given after fear conditioning. Therefore, the lateral amygdala plays a strong role in the acquisition of memories. In addition, the role of NMDA receptors could be investigated within the amygdala. It has previously been shown that the NMDA antagonist MK-801 given before fear conditioning disrupts contextual fear conditioning, but does not disrupt contextual fear conditioning after the training session (Gould, McCarthy, & Keith, 2002). Additional studies could examine whether seizures induce changes in specific areas of the amygdala and determine the role of NMDA receptors within the different regions of the amygdala and hippocampus.

Our research contributes to a large body of evidence that seizures have long-lasting impacts on learning and behavior. One of the issues with the previous studies that examined learning and memory in young and adult subjects is that there are numerous structural and biochemical changes that can occur between the induction of seizures and the examination of learning and memory deficits. There is an increase in cell death, mossy fiber sprouting in the hippocampus, and gliosis (de Lanerolle, Kim, Robbins, &

Spencer, 1989; Henshall & Meldrum, 2012; Wolf, Aliashkevich, Blumcke, Wiestler, & Zentner, 1997). The presence of these additional alterations makes it difficult to tease out the impacts on learning and memory of seizures themselves.

In addition to cell death, another concern is the impact of interictal spikes on cognitive deficits. One theory on how the initial seizure insult results in later spontaneous seizure involves the presence of interictal spikes. This theory is based on the observation that approximately 20% of the civilians who survive severe head injuries develop epilepsy (Annegers, Hauser, Coan, & Rocca, 1998). It is postulated that the injury leads to cell loss which leads to disinhibition then interictal spikes. The presence of spikes in the EEGs of epileptic patients suggests a high probability for spontaneous seizures (Sundaram, Sadler, Young, & Pillay, 1999; Verrotti, Morresi, Cutarella, Morgese, & Chiarelli, 2000). The correlation of spikes and spontaneous seizures is so high that often the diagnosis is made on the presence of these spikes. However, the role of the impact of interictal spikes on epileptogenesis and cognition is strongly debated (Staley, Hellier, & Dudek, 2005) and the impact of interictal seizures has largely been unknown until recently.

In order to separate out the impact of interictal spikes on cognition, investigators administered a low dose of flurothyl at a slow rate in postnatal day 12 rats for several days (Khan, Zhao, Miller, & Holmes, 2010). The procedure resulted in interictal spikes without seizures. When the animals were tested in adulthood they had memory impairments in the radial arm maze and Morris water maze. There was also significant cell loss in subregions of the hippocampus. The Khan et al (2010) study is important because it highlights the impact of interictal spikes. After the administration of

chemoconvulsants there are interictal spikes, learning deficits, and cell loss. It is not clear what the contribution is of the interictal spikes but they may have an additive impact on cognition. These spikes are a concern when testing the animal after the induction of seizures but before the presence of spontaneous seizures.

The difficulty of investigating the time after seizures where interictal spikes may be found or when there are spontaneous seizures makes it clear that it is important to examine what the effects are of a single acute seizure on the acquisition and consolidation of memories. Later studies could examine the effect of inducing a seizure in rodents that have spontaneous seizures and other studies could measure the impact of a spontaneous seizure on acquisition and consolidation of memories. Furthermore, by examining the biochemical changes that occur after a single seizure new therapeutics could be generated that could impact the effect of seizures on learning, and perhaps influence epileptogenesis.

Conclusion

Besides increasing the risk of future seizures, a single short seizure can also acutely affect cognitive processes such as memory when that seizure occurs in relatively close temporal relation to learning. Evidence from the current study further suggests that such immediate effects of a seizure on cognition are not permanent, and later fade after seizure activity subsides. Learning occurring more remotely after a seizure, 6 hours in the current study, appears to be immune to impairment. The impairment of memory occurring in mice was measurable within 24 hours after a seizure which suggests that the deficits were not likely due to structural changes or other compensatory processes. This

information should be taken into account when considering how epileptic conditions might affect the quality of life and ability to live independently for an individual diagnosed with epilepsy.

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