Spatial cognition during the active avoidance task: The role of the prefrontal cortex and preempting impairment following febrile status epilepticus

Matias V. Page

University of Vermont

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Spatial cognition during the active avoidance task:

The role of the prefrontal cortex and preempting impairment following febrile status epilepticus

Matias Page

University of Vermont
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Abstract

The active place avoidance task is a dynamic spatial cognition task that has been used to study spatial memory impairment in animal models of epilepsy in order to better understand how prolonged early-life seizures affect cognition. To determine whether the prefrontal cortex is necessary for this task, the performance of adult male rats (n = 3) was assessed before and after bilateral injections of muscimol or PBS in the medial prefrontal cortex (mPFC). Both muscimol and PBS impaired task performance, though only muscimol altered hippocampal oscillations in the theta and gamma ranges. Due to small sample size and potential confounds, these results do not strongly indicate the necessity of the mPFC in this task. However, muscimol had more profound effects on behavior and network activity than PBS, suggesting that with a bigger sample size the involvement of the mPFC could be demonstrated. In addition, the role of neuron-restrictive silencer factor (NRSF), a protein that is overexpressed after prolonged seizures, was investigated with regard to prolonged seizure-related cognitive deficits. Rats induced with febrile status epilepticus (FSE) and given intracerebral injections of neuron-restrictive silencer element (NRSE), which has been shown to decrease NRSF levels, performed as well as controls, measured in terms of shock zone entrances, shocks, and time spent opposite the shock zone. Untreated FSE rats were impaired in each of these measurements. These results provide strong evidence that NRSF overexpression mediates FSE-induced cognitive impairment.

Important abbreviations

<table>
<thead>
<tr>
<th>CA: cornu ammonis</th>
<th>NRSF: neuron-restrictive silencer factor</th>
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<tr>
<td>dHPC: dorsal hippocampus</td>
<td>ODN: oligodeoxynucleotide</td>
</tr>
<tr>
<td>FS: febrile seizure</td>
<td>PBS: phosphate-buffered saline</td>
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<tr>
<td>FSE: febrile status epilepticus</td>
<td>tCW: time spent in quadrant clockwise to shock zone</td>
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<tr>
<td>mPFC: medial prefrontal cortex</td>
<td>tOPP: time spent in quadrant opposite shock zone</td>
</tr>
<tr>
<td>NRSE: neuron-restrictive silencer element</td>
<td>tTARG: time spent in shock zone</td>
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1.0 Introduction

1.1 Early-life seizures and cognition

Epilepsy is one of the most common neurological diseases known to cause long-term alterations in neuronal networks that underpin cognition. It is often the case that the comorbidities associated with epilepsy have a greater impact on quality of life than seizures, and cognitive impairment is one of the most common among them (Holmes, 2015). It has been extensively documented that children with epilepsy tend to have lower IQ scores and experience greater difficulty in school due to learning disabilities than children without epilepsy (Bailet & Turk, 2000; Buelow et al., 2012; Prasad, Corbett, & Prasad, 2014; Reilly et al., 2014; Wakamoto, Nagao, Hayashi, & Morimoto, 2000). Bridging the gap between the psychological level of cognitive deficits and the physiological level of the underlying mechanisms has been a major challenge in the fields of neurology and cognitive neuroscience.

Animal models of epilepsy, particularly the febrile status epilepticus (FSE) model, have been instrumental in studying the putative causal link between seizure-induced physiological changes and altered cognition. By better understanding the biological factors that underpin normal and abnormal cognitive processes, new avenues for intervention and therapy open up for investigation. The general aim of this study is to further explore FSE-induced cognitive deficits that have recently been described by Barry et al. (2015, 2016) in a subset of FSE animals in the active place avoidance task, and how the associated seizure-induced physiological changes may be prevented. Furthermore, while the temporal coordination of neurons within and between CA1 and CA3 components of the hippocampal circuit has been shown to be necessary for performance of the active avoidance task (Barry et al., 2016), it is not yet known if task performance also necessitates coordination between the hippocampus and the medial prefrontal
cortex (mPFC). To this end, a potential role for the mPFC in the performance of the active avoidance task was investigated.

1.2 Cognitive outcomes post febrile status epilepticus

Febrile seizures (FS) are seizures associated with a fever above 38°C and are the most common type of seizure in early childhood, occurring in 2-5% of children under five years old (Huang et al., 1999; Knudsen, 1996; Shinnar & Pellock, 2002). Most FSs last less than 15 minutes and are associated with a low risk of recurrence, as well as generally favorable neurological and cognitive development (Ellenberg & Nelson, 1978; Knudsen, 1996; Smith & Wallace, 1982). However, when these seizures last over 30 minutes, continuously and without recovery of consciousness, the patient is said to be in FSE (Marr & Okada, 2008). Only about 5% of FSs result in FSE, but FSE is more likely to have negative cognitive and neurological outcomes than a simple FS (Berg & Shinnar, 1996; Hesdorffer et al., 2011).

Evidence suggests that prolonged FSs, which may be severe enough to be considered FSE, have a detrimental effect on cognition compared to healthy controls and children with simple FSs. Several studies report that patients with prolonged FSs have a greater risk of developing mild to moderate cognitive impairments, such as deficits in hippocampal-dependent memory, language ability, psychomotor functions, and non-verbal intelligence (Kölfen, Pehle, & König, 1998; Martinos et al., 2012; Martinos et al., 2013; Van Esch, Ramlal, van Steensel-Moll, Steyerberg, & Derksen-Lubsen, 1996). Tsai and colleagues (2015) recently reported that FSs lasting at least 15 minutes are associated with lower scores on a full-scale intelligence quotient test as well as decreases in perceptual reasoning and working memory.

Rodent studies also indicate that prolonged and repetitive FSs impair hippocampal-dependent memory. Rats that experienced hyperthermia-induced seizures for at least 20 minutes
performed significantly worse than controls on the Morris water maze, a hippocampal-dependent task that assesses spatial working memory and reference memory (Dube et al., 2009). The hippocampus is widely understood to be critical for the formation of declarative memories in humans (as reviewed by Scoville & Milner, 1957). There is also evidence that the hippocampus plays a crucial role in spatial memory, enabling animals to encode spatial relationships between non-egocentric environmental cues in order to guide behavior. Animal can use an internal representation of space as a cognitive map when navigating the environment and learning spatial associations (O’Keefe & Nadel, 1979). Hippocampal “place cells” located in the first subdivision of cornu ammonis (CA1) increase their firing rate specifically when an animal steps into a distinct region of space relative to its surroundings—a place field. Place cells are considered to be neural correlates of the cognitive map described by O’Keefe and Nadel (O’Keefe & Nadel, 1979). Several recent studies have demonstrated that the memory impairment seen in rats with prolonged FSs corresponds to larger, less specific place fields and higher firing rates of place cells in CA1 (Barry et al., 2015; Dube et al., 2009). This suggests that the network function of the hippocampus is altered in these animals, and that FSE may impair memory by altering the function of cells within hippocampal networks.

1.3 Assessing post-FSE cognitive outcome with the active avoidance task

This study will focus on how the active place avoidance task can be used to study treatment and prevention of cognitive deficits following FSE. This relatively new active avoidance task is designed to assess spatial memory in a context where the animals must regularly update their ever-changing position on a rotating arena. It therefore differs from other spatial tasks in that the conditions are not static. The task requires an animal to remember the location of an unmarked, aversive area of the arena in order to avoid a mild electrical shock...
paired with that location by using cues outside the arena. The arena rotates at a constant speed in one direction, but the shock zone does not rotate, and remains in a fixed position relative to the room frame. The rat must frequently move against the rotation of the arena to avoid being pulled into the shock zone (Cimadevilla, Kaminsky, Fenton, & Bures, 2000). The task was initially used to dissociate long-term reference memory from working memory and to study consolidation of short-term memory into long-term memory (Cimadevilla et al., 2000; Pastalkova, et al., 2006). It has also been used to study cognitive impairment in disease models.

Lee and colleagues (2012) first used the active avoidance task in a disease model where an early-life insult affects the development of brain circuits that support cognition. They studied the long-term effects of neonatal ventral hippocampal lesions in an established model of schizophrenia. Rat pups were injected with ibotenic acid in both ventral hippocampi on postnatal day 7 (P7). Rats lesioned in the ventral hippocampi showed greater difficulty performing the task when they had to differentiate between relevant and irrelevant cues. Concurrent electroencephalograph (EEG) recording also revealed that during task performance there were NVHL-dependent alterations in neural synchrony between the mPFC and the VH, which projects directly to the mPFC (Jay & Witter, 1991), suggesting abnormal communication between the two regions. This study was the first to employ the active avoidance task to relate hippocampal-prefrontal network changes to impaired cognition in a disease model.

Early life neurological insult caused by FSE can also alter the development of key circuits that are necessary for cognition. Barry and colleagues (2015) used the active avoidance task to detect spatial cognition impairment in an animal model of FSE. This study showed that FSE animals fit a bimodal distribution of learners and non-learners, where non-learners were defined as animals that could not meet the criterion of five or fewer shocks in two consecutive
sessions within 15 training sessions. These bimodal outcomes match what is observed in clinical populations, that a subpopulation of individuals responds poorly to FSE while others do not (Chang, Guo, Wang, Huang, & Tsai, 2001; Hesdorffer et al., 2011; Martinos et al., 2012). The authors suggest that this discrepancy in performance of FSE animals was due to physiological differences between FSE learners and non-learners.

Barry and colleagues (2015) were able to identify biomarkers that predicted performance on the active avoidance task following FSE. MRI T2 relaxation times, which are sensitive to deoxyhemoglobin levels, were measured two hours post-FSE and compared between normothermic control animals, FSE learners, and FSE non-learners. Lower T2 relaxation times have been associated with higher deoxyhemoglobin levels and are indicative of greater metabolic demand (Choy et al., 2014). T2 relaxation times in FSE animals were found to be significantly lower than those of control animals in the whole brain, as well as in specific regions such as the basolateral amygdala and dorsal hippocampus (dHC). Moreover, FSE learners had significantly lower T2 relaxation times than FSE non-learners in the whole brain ($\text{mean}_L = 54.98 \pm 0.21 \text{ ms}$; $\text{mean}_{NL} = 56.11 \pm 0.23 \text{ ms}$), basolateral amygdala ($\text{mean}_L = 53.39 \pm 0.25 \text{ ms}$; $\text{mean}_{NL} = 54.53 \pm 0.31 \text{ ms}$), and dHC ($\text{mean}_L = 55.72 \pm 0.33 \text{ ms}$; $\text{mean}_{NL} = 56.91 \pm 0.23 \text{ ms}$). There was no correlation between T2 relaxation times and task performance in control animals, suggesting that the detectable differences in the brains of FSE learners and non-learners arise after the prolonged seizures, and may reflect differential metabolic activity in brain areas important for the task (Barry et al., 2015). In an earlier study, place field stability, a measurement of retention of precise spatial information over time, was significantly lower in rats with higher T2 relaxation times following prolonged FSs (Dube et al., 2009). This implies that elevated T2 relaxation times in FSE animals serve as a biomarker for long-term spatial memory impairment. The reason for
this association between cognition and T2 relaxation time in FSE animals is not well understood, but it is indicative of lasting changes that occur in important brain areas immediately after FSE that separate learners from non-learners.

To further implicate the hippocampus in mediating cognitive outcome following FSE, the same study also demonstrated that the FSE non-learners had aberrant place cell activity compared to both the FSE learners and the controls. The place cells in FSE non-learners had significantly higher firing rates and larger, less specific place fields than FSE learners and controls (Barry et al., 2015). Prolonged FSs have also been shown to increase the overall excitability of the hippocampus, lowering the threshold for seizures in response to excitatory input from both kainic acid injections in vivo and electrical stimulation in vitro (Dube et al., 2000). This is in agreement with studies showing that the incidence of epilepsy is elevated in patients with prolonged FSs, ranging from 6 to 9% (Annegers, Hauser, Shirts, & Kurland, 1987; Nelson & Ellenberg, 1976). These results indicate that following prolonged seizures, which include FSE, the balance between excitation and inhibition is altered in the hippocampus, and individuals with poorer cognitive outcomes have more hyperactive place cells (Barry et al., 2015). The diverging cognitive outcomes in individuals with FSE are therefore linked to observable physiological differences. This then poses a new question: What are the specific network alterations occurring after FSE that contribute to cognitive impairment?

1.4 Hippocampal network function

A mechanistic theory has been developed asserting that cognitive impairment in mental disorders is caused in part by aberrant dynamic coordination of neuronal firing within and between neural circuits, specifically in relation to theta oscillations (Barry et al., 2016; Fenton, 2015). Synchronized activity of populations of neurons gives rise to local field potentials of
various frequencies that can be measured using EEG. Theta rhythms are oscillations in the 5-12 Hz range that reflect periodic, coordinated activity of excitatory neurons, and are thought to play an important role in mnemonic processes such as spatial memory (Jones & Wilson, 2005). The theta rhythm is thought to coordinate the activity of groups of hippocampal pyramidal cells so that they fire together in time (Jones and Wilson, 2005; Singer, 1999; Varela, Lachaux, Rodriguez, & Martinerie, 2001). Theta oscillations in CA1 arise from rhythmic fluctuations of excitatory inputs, which include projections from the CA3 region of the hippocampus and the entorhinal cortex, and inhibitory inputs from interneurons that are driven by the medial septum (Brankack, Stewart, & Fox, 1993; Stewart & Fox, 1990). Excitatory input from CA3 is thought to carry information about previously encoded spatial associations, whereas input from the entorhinal cortex is thought to carry information from the sensory pathways about the current spatial relationships of features in the environment (Brun et al., 2002; Steffenach, Sloviter, Moser, & Moser, 2002).

Hasselmo and colleagues (2002) developed a hypothesis describing the relationship between the phase preference of place cells relative to theta at the fissure and the encoding or retrieval of spatial information. During epochs of encoding novel spatial maps, as place fields begin to form, pyramidal cells in CA1 tend to fire at the troughs of local theta oscillations, which correspond to strong synaptic currents from the entorhinal cortex and weak synaptic currents from CA3 pyramidal cells (Brankack et al., 1993; Wyble, Linster, & Hasselmo, 2000). During epochs of retrieval, such as when a rat has learned a spatial task and uses encoded spatial information to guide its behavior, place cells in CA1 tend to fire at the peaks of local theta oscillations, which correspond to strong synaptic currents from CA3 pyramidal cells and weak synaptic currents from the entorhinal cortex (Brankack et al., 1993). Thus phase preference of
place cells relative to theta is crucial for organized communication between different functional structures within the hippocampus during behaviors that involve spatial cognition.

A recent study has shown that cognitive impairment following FSE can be linked to a loss of temporal coordination of place cell firing relative to the theta rhythm in CA1 (Barry et al., 2016). Barry and colleagues found that in control rats, place cells in CA1 have a significant phase preference for firing at the descending phase of theta, approximately 260° relative to local theta oscillations, during performance of the active avoidance task. Likewise, CA1 place cells in FSE learners have a significant phase preference for firing at 235° relative to local theta oscillations during performance of the active avoidance task. On the other hand, CA1 place cells in FSE non-learners do not have a significant phase preference during performance of the active avoidance task, indicating that their place cells fire more randomly in time. Another important finding in this study was that in control rats and FSE learners, pyramidal cells in CA1 shifted their phase preference during performance of the task to fire in register with static CA3 phase preference. The FSE non-learners, however, did not show aligned phase preference between the two structures. Performance on this task requires retrieval of encoded spatial associations to guide behavior, so this suggests that the temporal discoordination between CA1 and CA3 may be causative of poor spatial memory retrieval.

1.5 First objective: determine the role of the prefrontal cortex in active avoidance

Temporal coordination between CA1 and CA3 appears to be important for performance on the active avoidance task. However, spatial cognition involves other brain areas that work in concert with the hippocampus, such as the prefrontal cortex (Hok, Save, Lenck-Santini, & Poucet, 2005; Jones & Wilson, 2005; Kim & Shadlen, 1999). While the hippocampus codes for space, the medial prefrontal cortex (mPFC) is necessary for attention and planning with respect
to goals (Birrell & Brown, 2000; Granon, Hardouin, Courtièr, & Poucet, 1998; Yu & Frank, 2015). While unproven, the dynamic nature of the active avoidance task implies that hippocampal-prefrontal interaction is necessary for performance. If a rat does not continuously attend to its position relative to external cues, it will be rotated into the shock zone (Kelemen & Fenton, 2010). It is therefore possible that discoordination between mPFC and hippocampal neurons may also lead to some of the cognitive impairment associated with FSE. In addition, epochs of deliberation during spatial working-memory tasks are associated with more synchronous neuronal firing between the mPFC and the hippocampus (Jones & Wilson, 2005), so it is possible that disrupted communication between these areas could impede decision making during the active avoidance task. However, it is not currently known whether the mPFC is necessary for performance on the active avoidance task. The dHC and basolateral amygdala have both been shown to be necessary for the task (Cimadevilla, Fenton, & Bures, 2000; Vafaei, Jezek, Bures, Fenton, & Rashidy-Pour, 2007), but the role of the mPFC is unclear. Before the active avoidance task can be used to assess whether temporal discoordination between the hippocampus and the mPFC contributes to cognitive impairment in FSE non-learners, the importance of the mPFC in performing this task must be determined. We attempted this using temporary pharmacological inactivation of the mPFC.

1.6 Second objective: blocking post-FSE changes in NRSF expression

One approach to improving the likelihood of good cognitive outcomes of FSE patients is to prevent the cascade of physiological changes that occur following FSE that may eventually lead to temporal discoordination. FSs lasting more than 20 minutes have been shown to cause a number of long-term alterations in the molecular, cellular, and network properties of the hippocampus. FSs have not been linked to neuronal death in the hippocampus, nor do they affect
neurogenesis in the dentate gyrus (Bender et al., 2003; Dube, Yu, Nalcioglu, & Baram, 2004; Toth, Yan, Haftoglou, Ribak, & Baram, 1998). However, Toth and colleagues (1998) showed that in animals with prolonged FSs, distinct populations of neurons in CA1 and CA3 of the hippocampus, as well as the lateral division of the central nucleus of the amygdala, were morphologically different from normal neurons. On a network level, prolonged FSs can lower the threshold for seizures in the hippocampus, suggesting increased excitability (Dube et al., 2000). Paradoxically, a study by Chen, Baram, and Soltesz (1999) showed that hyperthermia-induced seizures cause an increase in GABA\textsubscript{A} receptor-mediated inhibition of pyramidal cells in CA1. Chen and colleagues (2001) helped explain these seemingly contradictory findings by showing that a single FS can cause an increase in CA1 pyramidal cell h-channel activation in rats. H-channels are hyperpolarization-activated Na\textsuperscript{+} channels that generate a rebounding depolarizing current ($I_h$), converting inhibitory post-synaptic potentials to depolarizing currents (Siegelbaum, 2000). With elevated h-channel activity, GABA-mediated inhibitory currents could theoretically result in greater excitation of pyramidal cells. These findings illustrate how molecular changes can result in complex network modifications that may disrupt normal network functioning.

The second part of this study examined the role of a protein called neuron-restrictive silencer factor (NRSF) in producing FSE-related cognitive deficits. NRSF is a transcription factor that modulates the expression of many genes that influence neuronal functioning (McClelland et al., 2014). It binds to a 21-bp DNA sequence called neuron-restrictive silencer element (NRSE), which is embedded in many neuronal genes (Roopra, Huang, & Dingledine, 2001). NRSF reduces the rate of transcription of genes containing NRSE by recruiting repressor molecules such as histone deacetylase to the genes containing NRSE (Naruse, Aoki, Kojima,
Mori, 1999; Roopra et al., 2000). Histone deacetylase removes acetyl groups from neighboring histones, causing the chromatin to condense (Grunstein, 1997; Hebbes, Thorne, & Crane-Robinson, 1988).

NRSF expression increases twofold to threefold in rat hippocampi following kainic acid-induced status epilepticus (SE). This has been shown to inhibit the expression of 28 different NRSE-containing genes that predominately express proteins for various neurotransmitters and channels, any one of which could have long-term effects on hippocampal network function (McClelland et al., 2014). McClelland and colleagues (2011) showed that it is possible to block FSE-induced NRSF overexpression by treating rats with NRSE oligodeoxynucleotides (ODN), which act as decoy NRSE sequences to competitively inhibit the binding of NRSF to neuronal genes. Blocking the cascade of molecular changes due to NRSF overexpression could potentially improve cognitive outcomes after FSE. The second objective of this study is to use the active avoidance task to test whether NRSF overexpression impairs cognition, and if so, whether stopping NRSF overexpression can attenuate this effect.

2.0 Methods

2.1 Apparatus

The active avoidance arena was located in a rectangular room where it was approximately 50 cm from black curtains on the south and east sides and 50 cm from white walls on the north and west sides. The north and west sides had visual cues including an 11 cm gray power-strip that formed a continuous line 50 cm above the floor of the arena, a red star centered 18 cm above the arena floor on the west wall, a black circle centered 18 cm above the arena floor on the north wall, and a 53 cm x 84 cm white sheet of paper with five 2.5 cm-wide black
diagonal stripes centered 5 cm above the gray power strip on the north wall. The arena (BioSignal Corp., Brooklyn, NY) was elevated 80 cm off the ground, and consisted of a steel disk 82 cm in diameter, transparent Plexiglas walls 31 cm tall, a motor that rotated the arena at 1 rpm, and an arm with a light emitting diode (LED) extending 8 cm from the edge of the arena. Light sources were positioned above and below the arena. The LED at the end of the arm rotated with the arena, and was used as a reference to calculate the position of the rats in the arena frame. The position of the rats in the arena was sampled at 60 Hz (BioSignal Group Corp., Brooklyn, NY) using a digital video camera that detected a second LED attached to the animal, as described in section 2.2.1.

A current generator (BioSignal Group Corp., Brooklyn, NY) was used to send mild alternating current discharges to the rat upon entry into the designated shock zone. An output cable connected to the current generator hung down from the scaffolding above the arena and was attached to a stainless steel swivel pinned to the skin on the back of the rat’s neck using an alligator clip. An LED was also attached to the alligator clip and used for tracking the rat’s position. The software program Tracker (BioSignal Group Corp., Brooklyn, NY) was used to control the arena parameters, rotational speed, and electrical current sent through the cable. It was also used to record behavioral data during the active avoidance task, such as rat’s position, number of shocks, and number of shock zone entrances. The shock zone was a wedge-shaped sector in the northwest quadrant with the origin in the center of the arena and the outer arc spanning 60°. The entrance latency of the electrical shock was 1 ms, the exit refractory time was 1.5 s, the duration of the shock was 0.5 ms, and the inter-shock latency was 2 s. Therefore it is important to note that if an animal fails to escape the shock zone within 2 s, it will receive multiple shocks in a single shock zone entrance.
2.2 Prefrontal inactivation experiment

2.2.1 Animals

Adult male Sprague Dawley rats (n = 5) were acquired from Charles River Laboratories and trained on the active avoidance task. One rat was never able to learn the task, and two other rats were able to learn the task, but later failed to relearn the task following surgical implantation. These rats were excluded from the study and euthanized by a lethal dose of isoflurane. New adult male Sprague Dawley rats (n = 5) were acquired from Charles River Laboratories and trained on the active avoidance task. In total, two of the original rats and one of the new rats (n = 3) were implanted and included in the study. The rats were housed separately in quiet facilities with controlled temperature and lighting. They were handled for approximately 7 minutes per day for 4 days before the initial training. Before performing the task, the rats were lightly anesthetized with inhaled isoflurane and implanted with a stainless steel barrel swivel (Eagle Claw Fishing Tackle Co., Denver, CO) in the skin between the shoulders, which would be connected to the shock cable and tracking LED. They were allowed to recover from the anesthesia for at least 24 hours before performing the active avoidance task.

2.2.2 Implants

mpFC: Custom implants were acquired from Plastics One Inc. (Roanoke, VA) that consisted of bilateral 26-gauge guide cannulae and EEG wires. The guide cannulae were 6 mm long from the base and 1 mm apart. Each EEG wire was adjacent to the guide cannula and extended 1 mm past the guide cannulae. Bilateral stainless steel stylets 0.2032 mm (0.008 in.) in diameter were initially inserted into the guide cannulae, and extended 1.5 mm past the guide cannulae. During injections, the stylets were removed and 33-gauge bilateral injection cannulae were inserted into the guide cannulae. These extended 1 mm farther than the guide cannulae.
CA1: Custom 16-channel EEG implants were built in the lab, consisting of a 9 x 2 milled millimax array (Neuralynx Inc., Bozeman, MT) to house electrode/pin connections. There were eight EEG wires 50 µm in diameter in each row, and the ninth was used for a stainless steel ground wire. Eight EEG wires on the left side of the implant were threaded through a 22-gauge metal cannula which was soldered to the base of the implant. Seven EEG wires on the right side of the implant were threaded through an identical cannula which was also soldered to the base of the implant. One of the EEG wires was used as a reference in the cerebellum. The EEG wires were cut so that they extended 2.5-3.5 mm from the metal cannula. Each EEG wire, as well as the ground wire and the cerebellar reference, was held in place in the base of the implant with a gold female pin.

2.2.3 Design

The rats were trained on the active avoidance task over the course of five to six days. They were first habituated to the arena and allowed to move about freely for 5 minutes. Next, a shock cable with a tracking LED was clipped to the swivel between their shoulders, and the animals were allowed to move about freely for 10 minutes while the arena rotated at 1 rpm without an active shock zone. After habituation, the rats were allowed to rest in their home cages for at least 10 minutes. They were then reintroduced to the arena, the shock cable/LED was reattached, and they were trained in 10-minute sessions with an active shock zone. During training sessions, the current generator was set to 0.4 mA and the arena rotation was set to 1 rpm. Between each session the rats were disconnected from the shock cable/LED and allowed to rest in their home cages. Training sessions were repeated until each rat received five or fewer shocks in consecutive sessions.
Once the rats were trained on the task, they were implanted bilaterally with guide cannulae and single EEG wires in the mPFC (Plastics One Inc., Roanoke, VA), as well as 16-channel EEG implants in the CA1 of the dHC. After recovering for at least one week following surgery, the rats were retrained on the task until they received five or fewer shocks in consecutive sessions. On day 1 of the experiment, two active avoidance sessions were recorded, followed by bilateral prefrontal injections of PBS. One hour after the injections, two post-injection sessions were recorded. On day 2 of the experiment, two additional sessions were recorded to determine if the PBS injections caused long-term impairment on the task. On day 3 of the experiment, two pre-injections sessions were recorded, followed by bilateral prefrontal injections of muscimol. One hour after the injection, two post-injection sessions were recorded. On day 4 of the experiment, two additional sessions were recorded to determine if the muscimol injections caused long-term impairment on the task. Each recording session was 10 minutes long. This procedure was repeated for each animal. The task performance and EEG signals were analyzed for the sessions immediately before and immediately after injection of either PBS or muscimol.

2.2.4 Surgical procedure

Five rats underwent implantation in the mPFC and the dHC, though two were later excluded from the study. The rats were anesthetized with inhaled isoflurane and placed in a stereotaxic frame. After injecting a local analgesic (0.3 ml of 0.25% bupivacaine) subdermally into the scalp, a midline incision was made in the scalp, exposing the skull. Four stainless steel screws were inserted onto the skull surface, two of which were anterior to the left and right ends of bregma and two of which were near the cerebellum on the left and right side. The right cerebellar screw was used for grounding. Holes were drilled in the skull above the left and right
mPFC, and the prefrontal cannulae were inserted at the following coordinates relative to bregma: AP + 3.5 mm, L ± 0.5 mm, DV – 3.0 mm (below the dura). A trephine was used to drill holes 2.5 mm in diameter above each dHC, and the tips of the hippocampal implant were inserted at the following coordinates relative to bregma: AP –3.8 mm, L ± 2.5 mm, DV – 2.5 mm. A hole was drilled approximately above the middle of the cerebellum, into which the cerebellar reference was inserted. The implants were then secured with dental cement (Dentsply Inc.) and the wound was closed with silk, non-absorbable sutures (Oasis, Mettawa, IL). After surgery, the rats received intraperitoneal injections of ketoprofen (5 mg/kg), and Neosporin was applied to the scalp. The rats were monitored for 24 hours following surgery and given further ketoprofen injections if they were determined to be in pain. Each rat was placed back in its home cage and allowed to recover for at least one week before retraining.

2.2.5 Muscimol and PBS injections

Bilateral intracranial injections of muscimol, a GABA<sub>A</sub> receptor agonist that inhibits local neuronal activity (Robbins et al., 2014), were used to temporarily silence the activity of the left and right mPFC. Muscimol starts taking effect within 5 minutes, and lasts several hours (Blanquat, Hok, Save, Poucet, & Chaillan, 2013). During the injections, rats were gently restrained while the stylets were removed and replaced with sterile injection cannulae. The injection cannulae were connected with PE 50 thin wall tubing (PlasticsOne Inc.) to a 10 µl Hamilton syringe connected to a BS-8000/9000 Multi-Phaser<sup>TM</sup> programmable syringe pump (Braintree Scientific Inc., Braintree, MA). The rats received 0.25 µl of muscimol at a concentration of 1.0 µg/µl in each side of the mPFC, injected at a rate of 0.20 µl/min. The radius of inactivated brain tissue with this volume and concentration has been estimated to be 1.5 – 2.0 mm, which is large enough to cover the target structure without affecting adjacent regions.
(Edeline, Hars, Hennevin, & Cotillon, 2002). The same volume and rate of injection was used for 1.0 \( \mu \text{g}/\mu\text{l} \) PBS. The injection cannulae were left in the guide cannulae for 1 minute after injection to allow for diffusion. The entire procedure took approximately 5 minutes per side, or 10 minutes in total. The rats were then allowed to rest in their cages. They were reintroduced to the arena and assessed in the active avoidance task approximately 1 hour after the injection procedure. All equipment and surfaces were sterilized with 70% ethanol before and after each injection procedure.

### 2.2.6 Behavioral analysis

The software program Track Analysis (BioSignal Group Corp., Brooklyn, NY) was used to analyze behavior during the active avoidance task and to generate figures showing the total path of each rat and polar histograms for time spent in each 10\(^{\circ}\) sector of the arena. Behavioral dependent variables included the number of shocks and entrances into the shock zone, time spent in the shock zone sector (tTARG), and time spent in equivalent-sized sectors in the quadrants clockwise (tCW) and opposite (tOPP) to the shock zone.

### 2.2.7 EEG recording

EEG signals were recorded using a Cheetah32 analog acquisition system (Neuralynx Inc., Bozeman, MT). During sessions, a custom cable with headstage preamplifiers was connected to the prefrontal and hippocampal recording electrodes. Amplification of analog signals was controlled by the Cheetah software program (Neuralynx Inc., Bozeman, MT) and digitized at 30 kHz. Signals were referenced to a 50 \( \mu \text{m} \)-diameter stainless steel wire placed over the cerebellum during surgery.

In order to have a common time reference for the electrophysiological data and behavioral data, frame pulses from the BioSignal firewire camera were captured and sent to the
Neuralynx digital I/O port. These pulses were recorded as events in the Cheetah program. Custom software (Mathworks Inc., Natick, MA) was then used to transform these events to common timestamps for both streams of behavioral and electrophysiological data and therefore synchronize the animal’s EEG signals with its position in space.

2.2.8 EEG analysis

EEG signals were analyzed using the Matlab Signal Processing Toolbox (Mathworks Inc., Natick, MA) and the Chronux toolbox (Mitra and Bokil, 2008). Neural synchrony between the dHC and mPFC were originally intended to be analyzed by measuring the frequency-specific phase locking value of both regions, as described by Lee et al. (2012). However, two of the three rats had low signal-noise ratios and continual artifact interruption in the mPFC, so they could not be analyzed. Other electrophysiological measurements related to spatial cognition were considered instead, including the mean frequencies of theta oscillations (5-12 Hz), low gamma oscillations (25-50 Hz), and high gamma oscillations (65-120 Hz). EEG oscillations were speed filtered such that they were only included when the animal was moving faster than 5 cm/s. The mean frequencies of each of these ranges was compared before and after injections of either muscimol or PBS.

2.2.9 Histology to confirm location of electrodes and cannulae

The rats were given a lethal dose of isoflurane and intracardially perfused with saline followed by 4% paraformaldehyde (PFA). The brains were then extracted and kept in 4% PFA. Coronal sectioning and cresyl violet staining to confirm the location of electrodes and cannulae are currently ongoing.
2.3 FSE/NRSE experiment

2.3.1 Animals

This experiment was done in collaboration with the Baram lab at the University of California, Irvine School of Medicine. Male Sprague Dawley rats (n = 20) were acquired from the Baram lab and split into four treatment groups. The CTRL-SCRAM group (n = 4) consisted of rats without FSE that had been treated with scrambled NRSE. The CTRL-NRSE group (n = 4) consisted of rats without FSE that had been treated with NRSE. The FSE-SCRAM group (n = 6) consisted of rats induced with FSE that had been treated with scrambled NRSE. The FSE-NRSE group (n = 6) consisted of rats induced with FSE that had been treated with NRSE. The induction of FSE and treatment of NRSE or scrambled NRSE are described in sections 2.3.3 and 2.3.4, respectively. While at the University of Vermont, the rats were housed separately in quiet facilities with controlled temperature and lighting. They were handled for approximately 7 minutes per day for 4 days before training. Before performing the task, the rats were lightly anesthetized with inhaled isoflurane and implanted with a stainless steel barrel swivel, as described earlier. They were allowed to recover from the anesthesia for at least 24 hours before performing the active avoidance task. The procedures and behavioral tasks involving these rats are in compliance with the Institutional Animal Care and Use Committee (IACUC) of the University of Vermont, and all experiments followed the guidelines for the humane treatment of animals as provided by the National Institute of Health and the University of Vermont.

2.3.2 Design

The rats were trained on the active avoidance task over the course of eight sessions per day for 2 days. Before the initial training session, the rats were first habituated to the arena and allowed to move freely for 5 minutes. The shock cable/LED was then clipped to the swivel
between their shoulders and the arena was set to 1 rpm for 10 minutes without an active shock zone. The rats were placed back in their home cages to rest while another rat was habituated to the arena. After habituation, the rats were reintroduced to the arena, the shock cable/LED was reattached, the current generator was set to 0.4 mA, and the arena was set to 1 rpm. They then completed eight 10-minute sessions each with an active shock zone, switching rats between each session. The arena was cleaned in between sessions.

The rats were randomly assigned to four squads of five and the experimenter was blind as to which treatment group each animal belonged to. One squad underwent its first eight sessions on the first day. On the second day, the first squad underwent its second day of training and a new squad underwent its first day of training. This procedure was repeated until each squad underwent two days of training. The number of shocks and entrances and time spent in each quadrant were then compared between groups.

2.3.3 Induction of FSE

The FSE induction procedure was carried out by the Baram lab at the University of California, Irvine School of Medicine. Using a procedure described by Chen, Baram, and Soltesz (1999), 12 rat pups (P10) were placed in a glass container and their core temperatures were monitored. The rats’ core temperatures were elevated with warm air to approximately 40.5°C, resulting in hyperthermia-induced seizures, characterized by sudden freezing, oral automatisms, forelimb clonus, and body flexion. Hyperthermia was maintained for up to 38 minutes, such that the seizures lasted at least 30 minutes per rat. Rats in the control group (n = 8) were kept at normal temperatures, but were removed from their cages for the same duration as the FSE rats to control for potential stress from handling and separation from the dam.
2.3.4 Treatment with NRSE and scrambled NRSE

Treatment with NRSE and scrambled NRSE ODNs was carried out by the Baram lab at the University of California, Irvine School of Medicine. Intact NRSE or scrambled NRSE ODN sequences were injected into the lateral cerebral ventricles 3 hours after the end of FSE. A 30-gauge hypodermic needle was used as a cannula, with the depth marked for the ventricles of a P10 rat pup (3 mm) by a small amount of solder applied to the needle. Microtubing was used to connect the cannula to a 10 ml Hamilton syringe. The cannula was then loaded with either NRSE or scrambled NRSE ODNs and placed into a syringe pump (KD Scientific Inc., Holliston, MA). Injections were done free-hand using bregma demarcations, which are visible through the skin of a P10 rat pup. The cannula was inserted directly through the skin and skull and into the brain until the solder mark was reached. 2.5 ml of either NRSE or scrambled NRSE ODNs (2.5 nmol) were injected into each ventricle at a rate of 0.5 ml/min. Injections were later repeated exactly as described 24 hours after FSE to ensure reduction of NRSF during noted times of overexpression following prolonged seizures.

2.3.5 Behavioral analysis

Behavior during the active avoidance task was analyzed using Track Analysis, as previously described. The number of shocks and entrances were measured, as well as tOPP.

2.4 Statistical methods

The software program SPSS (SPSS, Armonk, NY, version 22) was used to analyze the statistical significance of behavioral and electrophysiological data. Since pre-injection and post-injection sessions were from the same animal, independence of observations cannot be assumed. Therefore, general estimating equations (GEE), a class of regression marginal model for exploring multivariable relationships in repeated measurements, were used to test for differences
in behavioral and electrophysiological variables between pre- and post-injection sessions. The difference scores between behavioral measurements (entrances, shocks, tTARG, and tCW) in muscimol and PBS sessions were also evaluated using this method. The absolute values of the difference scores between tOPP in muscimol and PBS sessions, which were all negative values, were also analyzed.

3.0 Results

3.1 Prefrontal inactivation experiment

3.1.1 Number of entrances

Comparisons using the GEE showed that the number of entrances into the shock zone was significantly greater after muscimol injections (mean = 12.67 ± 2.60) than before muscimol injections (mean = 3.00 ± 1.53, p = 0.01), as shown in figure 1. However, there was no difference between the number of entrances before (mean = 3.00 ± 1.53) and after PBS injections (mean = 6.33 ± 1.76, p = 0.16). The difference scores for the number of entrances before and after muscimol (mean = 9.00 ± 2.83) were statistically the same as the difference scores for the number of entrances before and after PBS (mean = 2.67 ± 1.91, p = 0.109).

3.1.2 Number of shocks

Comparisons using the GEE showed that injecting muscimol had an effect on the number of shocks received, but there was a PBS effect as well. The number of shocks was significantly greater after muscimol injections (mean = 16.33 ± 3.53) than before muscimol injections (mean = 4.33 ± 2.33, p < 0.001), as shown in figure 2. The number of shocks was also significantly greater after PBS injections (mean = 6.00 ± 2.52) than before PBS injections (mean = 3.67 ± 1.33, p = 0.007). The difference scores for the number of shocks before and after muscimol
(mean = 12.67 ± 2.76) were significantly greater than the difference scores for the number of shocks before and after PBS (mean = 3.00 ± 1.24, p = 0.001).

3.1.3 Time spent in each quadrant

Comparisons using the GEE showed that injecting muscimol had an effect on tTARG, tCW, and tOPP, but there was also an effect from PBS injections for all three variables. tTARG was significantly greater after muscimol injections (mean = 32.14 ± 12.54 s) than before muscimol injections (mean = 7.88 ± 5.75 s, p < 0.001), as shown in figure 3. tTARG was also significantly greater after PBS injections (mean = 13.68 ± 5.97 s) than before PBS injections (mean = 7.90 ± 4.42 s, p = 0.013). tCW was significantly greater after muscimol injections (mean = 276.42 ± 31.16 s) than before muscimol injections (mean = 45.58 ± 21.11 s, p < 0.001), as shown in figure 4. tCW was also significantly greater after PBS injections (mean = 228.28 ± 20.11 s) than before PBS injections (mean = 105.46 ± 39.43 s, p = 0.001). Lastly, tOPP was significantly smaller after muscimol injections (mean = 229.46 ± 37.43 s) than before muscimol injections (mean = 439.40 ± 75.89 s, p = 0.017), as shown in figure 5. tOPP was also significantly smaller after PBS injections (mean = 304.24 ± 32.05 s) than before PBS injections (mean = 455.72 ± 49.88 s, p = 0.015).

Though both muscimol and PBS both had significant effects on these variables, when the difference scores, which are shown in table 1, were compared using the GEE, muscimol had greater effects than PBS. The difference scores for tTARG before and after muscimol (mean = 24.26 ± 5.68 s) were significantly greater than the difference scores for tTARG before and after PBS (mean = 5.78 ± 2.42 s, p = 0.002). The difference scores for tCW before and after muscimol (mean = 228.84 ± 29.26 s) were also significantly greater than the difference score for tCW before and after PBS (mean = 122.82 ± 18.44 s, p = 0.007). The difference scores for tOPP
before and after muscimol (mean = –209.94 ± 92.24 s) were, however, statistically the same as the difference scores for tOPP before and after PBS (mean = –151.48 ± 63.74 s, p = 0.524).

3.1.4 EEG Analysis

As shown in table 2, muscimol injections had an effect on the mean theta, low gamma, and high gamma frequencies in the dHC, whereas PBS injections had no effect. The mean theta frequency was significantly lower following muscimol injections (p < 0.001), and was not significantly different following PBS injections (p = 0.95). The mean low gamma frequency was significantly lower following muscimol injections (p < 0.001), and was not significantly different following PBS injections (p = 0.24). The mean high gamma frequency was significantly higher following muscimol injections (p = 0.002), and was not significantly different following PBS injections (p = 0.62).

3.1.5 Histology to confirm location of electrodes and cannulae

Histology is currently ongoing.

3.2 NRSF experiment

3.2.1 Number of entrances

A significant main effect for cumulative number of entrances into the shock zone was found across training days with regard to treatment group (p < 0.001). The FSE-NRSE group (mean = 16.62 ± 3.17) made fewer cumulative entrances than both CTRL-NRSE (mean = 43.04 ± 10.05, p = 0.002) and FSE-SCRAM groups (mean = 39.58 ± 7.54, p < 0.001), yet exhibited similar results as the CTRL-SCRAM group (mean = 15.93 ± 3.72, p = 0.683), shown in figures 6 and 7.
### 3.2.2 Number of shocks

A significant main effect for cumulative number of shocks was found across training days with regard to treatment group (p < 0.001). The FSE-NRSE group (mean = 20.27 ± 4.61) received fewer cumulative shocks than both CTRL-NRSE (mean = 71.24 ± 19.84, p = 0.001) and FSE-SCRAM groups (mean = 80.37 ± 18.28, p < 0.001), yet exhibited similar results as the CTRL-SCRAM group (mean = 23.28 ± 4.61, p = 0.72), as shown in figures 8 and 9.

### 3.2.3 Time spent in opposite quadrant

A significant main effect for cumulative time spent in the quadrant opposite the shock zone was found across training days with regard to treatment group (p < 0.001). The FSE-NRSE group (mean = 2463.76 ± 229.69 s) cumulatively spent more time opposite the shock zone than the FSE-SCRAM group (mean = 1747.82 ± 162.95 s, p = 0.027), yet exhibited similar results as both CTRL-SCRAM (mean = 2459.03 ± 280.77 s, p = 0.717) and CTRL-NRSE groups (mean = 2458.94 ± 280.76 s, p = 0.894), as shown in figures 10 and 11.

### 4.0 Discussion

#### 4.1 Muscimol and PBS both affect behavior on the active avoidance task

The first objective of this study was to determine whether normal functioning of the mPFC is necessary for performance on the active avoidance task. In order to temporarily diminish the activity of the mPFC, the GABA<sub>A</sub> agonist muscimol was injected bilaterally through cannulae that were implanted in the prelimbic cortex on either side of the brain. Behavior and electrophysiology in the session before and the session after injection of either muscimol or PBS were compared.
The results do not conclusively demonstrate that prefrontal inactivation impaired performance on the active avoidance task. As shown in figures 1 and 3, injecting muscimol into the mPFC significantly increased the number of entrances into the shock zone and the number of shocks, but PBS injections also increased the number of shocks. Since performance was impaired by PBS as well as muscimol, the effects could be due to extraneous variables, and cannot necessarily be attributed to prefrontal inactivation. However, the results suggest that muscimol had a greater effect on performance than PBS. The number of entrances increased only after muscimol injections. An increase in the number of entrances reflects a diminished ability to avoid the shock zone, suggesting that the rat either does not remember the location of the shock zone as well or is unable to use spatial information as efficiently to guide its behavior. The rats injected with PBS were able to avoid the shock zone just as well, but were unable to escape the shock zone fast as quickly as before they were injected, resulting in significantly more shocks. Also, when the difference scores were compared between muscimol and PBS, muscimol had a greater effect on the number of shocks than PBS, as shown in table 1. Although both injections impaired performance, muscimol had more of an impact than PBS.

The amount of time spent in each quadrant of the arena is useful for comparing the avoidance strategy a rat uses. The most effective strategy for avoiding the shock zone is to stay in the opposite quadrant, which is farthest from the aversive region, as shown in figure 15. When rats do not actively maintain their position in this safe region, they tend to get pulled by the rotation of the arena toward the clockwise quadrant and eventually the shock zone, as shown in figure 16. Barry et al. (2016) showed that FSE non-learners spent less time opposite the shock zone and more time in the clockwise quadrant than control animals, which was indicative of impaired spatial cognition.
The muscimol injections had an effect on avoidance strategy. The rats spent significantly less time opposite the shock zone and more time in the shock zone and clockwise to the shock zone post-muscimol, as shown in figures 5, 7, and 9. However, injections of PBS had the same effects. This shows that although muscimol injections altered the strategy of rats during active avoidance, other uncontrolled factors could have also caused these changes. Nevertheless, muscimol had more pronounced effects on strategy than PBS, since the difference scores for the time spent in the shock zone and clockwise to the shock zone were greater for muscimol than for PBS, as shown in table 1.

It is possible that PBS altered neuronal functioning due to the fact that PBS and cerebrospinal fluid (CSF) have different ionic concentrations (Cold Spring Harbor, 2006; Shiobara, Ohira, Doi, Nishimura, & Kawase, 2013). In the future, artificial CSF might be considered instead of PBS. It is also possible that the change in intracranial pressure when removing the stylets and inserting the injection cannulae affected neuronal functioning in the mPFC, or that restraining the rats during injections increased their stress levels, which could have impaired performance.

This experiment only included three animals, so although most of the results are significant, additional rats would be necessary to make conclusive statements about the effects of prefrontal inactivation, especially given the effects of PBS. However, the results point toward the possibility that muscimol in particular has a negative effect on performance. Although there was an effect from control injections, roughly doubling the number of entrances, muscimol injections were much more detrimental to performance, resulting in four times as many entrances and shocks, and altering avoidance strategy to a greater degree than PBS. These findings therefore
call for more animals to be added to the experiment so as to more definitively reveal the effect of inactivating the mPFC.

4.2 Muscimol affects theta and gamma oscillations

To examine the potential changes in hippocampal-prefrontal communication, the initial intention was to compare hippocampal-prefrontal neural synchrony during task performance before and after muscimol or PBS injection, using methods described by Lee and colleagues (2012). However, because the prefrontal signals were poor in two of the rats included in the present study, neural synchrony between the mPFC and dHC was not analyzed. Instead, the mean theta, low gamma, and high gamma frequencies in the dHC were examined to see if there were any changes in network activity following muscimol or PBS injection. Theta oscillations coordinate hippocampal place cell firing and are crucial for learning and memory processes (Jones and Wilson, 2005; Singer, 1999; Varela, Lachaux, Rodriguez, & Martinerie, 2001). They also appear to be important for communication with the mPFC during mnemonic tasks (Colgin, 2011), so changes in theta frequencies may reflect altered communication between the hippocampus and the mPFC. Gamma oscillations are also indicative of synchronization between structures. Low gamma oscillations (25-50 Hz) are thought to coordinate activity between CA1 and CA3, whereas high gamma oscillations (65-120 Hz) are thought to coordinate activity between CA1 and the entorhinal cortex (Colgin et al., 2009). As shown in table 2, the mean theta, low gamma, and high gamma frequencies all shifted following muscimol injections, but not PBS injections, suggesting that only muscimol altered the network activity of the hippocampus.

Two recent studies have examined how disrupted hippocampal-prefrontal communication affects performance of spatial memory tasks such as the continuous place navigation task and the
continuous alternation task on a modified T-maze (Blanquat et al., 2013; Ito, Zhang, Witter, Moser, & Moser, 2015). Neither study showed immediate changes in performance from disrupting hippocampal-prefrontal communication, possibly because these tasks are more static than the active avoidance task. The active avoidance task is unique in that it requires the animal to constantly update its position relative to spatial cues, and it must pay attention to only the cues outside the arena, ignoring local cues. This study provides preliminary evidence that communication between the hippocampus and the PFC might be important for performance on the active avoidance task, but a larger sample size is needed in order to show that there were no effects from confounding variables.

**4.3 NRSE treatment improves FSE-induced spatial memory deficits**

NRSF is a protein that increases twofold to threefold in the hippocampus shortly after prolonged seizures, silencing a number of important genes involved in normal neuronal functioning (McClelland et al., 2011, 2014). The second objective of this study was to test whether NRSF overexpression contributes to cognitive impairment following FSE and whether it would be a good target for pharmacological intervention. NRSE ODNs were injected into the ventricles of rats shortly after they were induced with FSE. The Baram lab has shown that intraventricular NRSE ODN injections decrease NRSF levels in rats induced with FSE (personal communication, May 10, 2016). The present study used the active avoidance task to assess cognitive outcomes of FSE, and showed that FSE rats treated with NRSE performed just as well as controls in terms of the number of entrances into the shock zone, the number of shocks received, and the amount of time spent opposite the shock zone, as shown in figures 12-14. On the other hand, FSE rats treated with scrambled NRSE, which does not bind to NRSF, performed significantly worse on all three behavioral measures than the FSE-NRSE group and the controls.
This indicates that FSE causes impairment in spatial cognition that is dependent on NRSF expression.

These results provide strong evidence that NRSF overexpression is a key factor contributing to the spatial memory deficits that develop in some rats with FSE. Moreover, treating FSE animals with NRSE soon after prolonged seizures was shown to improve cognitive outcome. This has potential implications for future research into developing new forms of prophylaxis for children that experience FSs early in life, especially those with FSE. Treatments that attenuate NRSF expression could possibly be used to prevent cognitive impairment associated with FSE. Because NRSF modulates a multitude of genes that are important for neuronal functioning (McClelland et al., 2014), more research is needed to uncover the mechanism by which NRSF mediates cognitive impairment. In addition, it is not yet understood how NRSF expression affects place cell activity or coordination between neural structures within the hippocampus and communicating neocortical areas. These are all questions that warrant future application of the active avoidance task in the FSE model.

4.4 Conclusion

The dynamic nature of the active avoidance task makes it a unique tool for assessing systems-level cognition in animal disease models that involve cognitive impairment. In conjunction with electrophysiological methods for recording hippocampal EEG and place cell activity, it has been used to associate spatial memory impairment in a subset of FSE rats with neural discoordination between different functional regions of the hippocampus (Barry et al., 2016). The findings of this study, though not yet conclusive, suggest that the mPFC may also be involved in the cognitive processes underlying performance in the active avoidance task. If the mPFC is indeed necessary for this task, then hippocampal-prefrontal coordination may be
another factor to explore in relation to spatial memory impairment following FSE. Furthermore, this study showed that NRSF, a protein that regulates gene expression, plays an important role in the cognitive deficits that accompany FSE, and could be a promising target for therapy by preventing a cascade of changes that occur immediately after prolonged seizures.

5.0 Acknowledgments

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Figure 1 shows the average number of entrances into the shock zone for all three rats before and after injections of muscimol (red) or PBS (blue), as well as the standard error.

Figure 2 shows the number of entrances into the shock zone for each rat during a single 10-minute session before and after injections of muscimol (red) or PBS (blue).
Figure 3 shows the average number of shocks received for all three rats before and after injections of muscimol (red) or PBS (blue), as well as the standard error.

Figure 4 shows the number of shocks each rat received during a single 10-minute session before and after injections of muscimol (red) or PBS (blue).
Figure 5 shows the average time (s) spent in the shock zone for all three rats before and after injections of muscimol (red) or PBS (blue), as well as the standard error.

Figure 6 shows the amount of time (s) each rat spent in the shock zone during a single 10-minute session before and after injections of muscimol (red) or PBS (blue).
**Figure 7** shows the average time (s) spent in the quadrant clockwise to the shock zone for all three rats before and after injections of muscimol (red) or PBS (blue), as well as the standard error.

**Figure 8** shows the amount of time (s) each rat spent in the quadrant clockwise to the shock zone during a single 10-minute session before and after injections of muscimol (red) or PBS (blue).
Figure 9 shows the average time (s) spent in the quadrant opposite the shock zone for all three rats before and after injections of muscimol (red) or PBS (blue), as well as the standard error.

Figure 10 shows the amount of time (s) each rat spent in the quadrant opposite the shock zone during a single 10-minute session before and after injections of muscimol (red) or PBS (blue).

Table 1 shows the individual difference scores for the behavioral variables between pre- and post-injection sessions for either PBS or muscimol (Cond. = condition; tTARG = time in shock zone; tCW = time in quadrant clockwise to shock zone; tOPP = time in quadrant opposite shock zone)
Figure 11 shows the relationship between animal speed and theta oscillation power. As an animal increases its speed, the power of theta increases, indicated by the dark band around 9 Hz that corresponds to spikes in speed. During analysis, all EEG oscillations were filtered for speed, such that they were only included when animal speed was $\geq 5$ cm/s.

Table 2 shows the mean frequencies for all three animals of theta oscillations (5-12 Hz), low-gamma oscillations (25-50 Hz), and high-gamma oscillations (65-140 Hz) in CA1 before and after injections of either muscimol or PBS. The p-values indicate whether the mean frequencies change significantly after each injection.
Figure 12 shows the average number of entrances into the shock zone per session for the CTRL-SCRAM group (blue, solid), CTRL-NRSE group (blue, dashed), FSE-SCRAM group (red, solid), and the FSE-NRSE group (red, dashed) on the active avoidance task, as well as the standard error.

Figure 13 shows the average number of shocks per session for the CTRL-SCRAM group (blue, solid), CTRL-NRSE group (blue, dashed), FSE-SCRAM group (red, solid), and the FSE-NRSE group (red, dashed) on the active avoidance task, as well as the standard error.
Figure 14 shows the average time spent in the quadrant opposite the shock zone per session for the CTRL-SCRAM group (blue, solid), CTRL-NRSE group (blue, dashed), FSE-SCRAM group (red, solid), and the FSE-NRSE group (red, dashed) on the active avoidance task, as well as the standard error.
Figure 15 shows the trajectory of a rat that avoided the shock zone effectively. The shock zone is the shaded sector of the arena. A polar histogram is overlaid onto the figure to show how much time the rat spent in each 10° sector of the arena.

Figure 16 shows the trajectory of a rat that did not avoid the shock zone effectively. The shock zone is the shaded sector of the arena. The red circles in the shock zone indicate the locations the rat received a shock. A polar histogram is overlaid onto the figure to show how much time the rat spends in each 10° sector of the arena.