UNCOVERING THE ROLE OF THE HIPPOCAMPUS IN THE TRANSITIVE INFERENCE TASK UTILIZING PHARMACOLOGICAL AND GENETIC MANIPULATIONS: IMPLICATIONS FOR PATIENTS WITH SCHIZOPHRENIA

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ABSTRACT

Patients with schizophrenia show a number of cognitive deficits that may be related to abnormal hippocampal physiology and function. One such cognitive deficit is in transitive inference. Simply stated, transitive inference is the ability to infer A > C after directly learning A > B and B > C. The hippocampus has been implicated in transitive inference as lesions of the hippocampus in C57BL/6 mice after initial training and testing impairs transitive inference. Likewise, lesions of the hippocampus in rats prior to training also impair transitive inference. However, lesions of the whole hippocampus are not able to specifically examine the role of the dorsal versus ventral hippocampus in this task. This is important because studies suggest that the dorsal and ventral poles of the hippocampus may be functionally different. The present experiment used reversible inactivation of the dorsal and ventral hippocampus to examine the role of these structures in transitive inference. Mice were trained to learn that A>B, B>C, C>D, and D>E during training phases and then were tested to show if they learned that A>E (the novel control pairing) and that B>D (the novel pairing which requires transitive inference) during test sessions. Following these test sessions, cannulae were inserted into the hippocampus and the mice were allowed 5 days to recover. After the recovery period, mice underwent 4 more test sessions. The $GABA_A$ agonist muscimol or saline was infused into the dorsal or ventral hippocampus thirty minutes before each test session. The mice which received muscimol infusion into the dorsal hippocampus performed similarly to controls on the novel control pairing (A>E) but were significantly impaired on the novel pairing (B>D) which required transitive inference.

The DBA/2 strain of mice have altered hippocampal function and has been used to model schizophrenia. The study also compared performance of DBA/2J and C57BL/6J inbred mice in TI, and foreground and background fear conditioning, which both involve the hippocampus. Separate mice were then trained with two different fear conditioning paradigms. For background fear conditioning, mice are trained with two paired presentations of a conditioned stimulus (CS, 30 second, 85 dB white noise) and an unconditioned stimulus (US, 2 second, 0.57 mA foot shock). Mice are then tested the next day for both freezing to the training context. Foreground fear conditioning differed in that the mice were presented with only the shocks during training. DBA/2J mice performed significantly worse than the C57BL/6J in both foreground and background fear conditioning and transitive inference. These results provide further support for the role of the dorsal hippocampus in transitive inference. Moreover, these results may help provide a better understanding of the cognitive deficits associated with schizophrenia.

Keywords: hippocampus, learning, transitive inference, fear conditioning, schizophrenia

Abbreviations: nAChR-nicotinic acetylcholinergic receptor; PPI: Pre-Pulse Inhibition

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CHAPTER ONE

INTRODUCTION

Schizophrenia is a cognitive disorder with multiple symptoms including attention and learning deficits (Gold and Harvey, 1993; Lewis and Lieberman, 2000). The clinical presentation of schizophrenia varies both between individuals and within the same individual at different stages of the illness (Holden, 2003). Approximately 3 million adults in the United States, or 1.1 percent of the general population, are affected with this disease (Sawa and Snyder, 2002). Significant advances have been made in developing therapies for treating the specific positive and negative symptoms of schizophrenia. However, approximately 85 percent of patients exhibit what has been termed a "generalized deficit" that remains largely untreated (Green and Braff, 2001). This generalized deficit means that patients with schizophrenia perform at levels below normal controls across a wide distribution of cognitive tests.

One of the most robust findings of cognitive function in patients with schizophrenia is that episodic memory is significantly impaired (Heimrich et al., 1985; Saykin et al., 1991; Aleman et al., 1999). Episodic memory is memory for personal events and this form of memory depends upon autonoetic awareness - the awareness of one's past, present, and future (Tulving, 1985; Wheeler et al., 1997). The impaired episodic memory in patients with schizophrenia is mediated through altering its critical feature - autonoetic awareness (Huron et al., 1995). Whereas some studies report no significant differences between schizophrenia and control groups in tasks of recall and recollection (Bauman and Murray, 1968; Koh and Peterson, 1978; Goldberg et al., 1989;

Beatty et al., 1993; Nathaniel-James et al., 1996) other studies describe a severe memory deficit in these same tasks (Gold et al., 1992; Stirling et al., 1997; Danion et al., 1999). Several experiments and meta-analyses uncovered the variability in memory performance in patients with schizophrenia. The manner in which the different cognitive tests are administered leads to a focus on different aspects of learning and memory and therefore may account for the variability in memory performance in patients with schizophrenia (Aleman et al., 1999; Danion et al., 1999; Pelletier et al., 2005). Through examining these differences it appears as though the deficits are primarily due to a lack of relational binding; and it is the degree to which tests examine relational binding that contribute to differences in performance.

Relational binding is linking multiple memories to one another which allows one to consciously make inferences among indirectly related events. A lack of relational binding means that information regarding separate aspects of an event, such as content (what the event was) and source (where, when, and how the event occurred), are no longer linked (Johnson et al., 1993; Conway and Dewhurst, 1995; Donaldson, 1996; Danion et al., 1999). In schizophrenia, a piece of the deficit that does not allow for the "flexibility" of declarative memory expression might be a defect in this relational binding (Cohen, 1984; Danion et al., 1999). Two lines of evidence indirectly support this hypothesis. First, patients with schizophrenia perform poorly in tasks involving source information (Harvey, 1985; Goldberg et al., 1989; Bentall et al., 1991; Schwartz et al., 1991; Gold and Harvey, 1993; Gras-Vincendon et al., 1994; Vinogradov et al., 1997). The term source information refers to an assortment of features that together specify the circumstances in which a memory is acquired (Johnson et al., 1993). This deficit may not

be due to deficits of source memory exactly, but to defective links between content and source information (Rizzo et al., 1996). Therefore, a deficit in linking information may underlie a deficit during consolidation - the period in which recent memories are integrated into long-term memory. This may account for the learning and memory impairments in some patients with schizophrenia (Ribot, 1882; Burnham, 1903; McClelland et al., 1995), Based on the fact that recognition deficits exist in patients with schizophrenia (Schwartz et al., 1991; Rizzo et al., 1996) and recognition is based mainly on relational information (Danion et al., 1999), using the transitive inference task is one method to investigate relational memory and the impairment in this disease.

The transitive inference task investigates explicit, declarative memory in humans and laboratory animals. Transitivity is the ability to infer that A > C after learning that A > B and B > C. The basic methods of the transitive inference entail subjects inferring two novel relationships based on directly learned relationships. That is to say, subjects are trained that always selecting A yields reward; selecting B over any letter except A yields a reward; and so on…, where each letter represents an character or pattern for humans or an odor for rodents. Subjects are tested to determine if they learned that A>E (the novel control pairing; does not require transitive inference due to the fact that A is always rewarded and E is never rewarded) and that B>D (the novel pairing which requires transitive inference) by associating the premise pairs A > B, B > C, C > D, and D > E in an ordered hierarchy (A>B>C>D>E). Titone and colleagues (2004) found that patients with schizophrenia were able to correctly identify the novel control pairing (A>E) but were significantly impaired when choosing the B>D pairing which required transitive inference. This finding demonstrates impairment in the patients with schizophrenia to

perform transitivity and deficit in relational memory because they are unable to bind the individual components into the hierarchy.

Researchers are currently working to tease apart the neural substrates involved in transitivity. The strongest support thus far involves the hippocampus, parietal lobe, and prefrontal cortex (Dusek and Eichenbaum, 1997; Zalesak and Heckers, 2009; Devito et al., 2010a; DeVito et al., 2010b; Wendelken and Bunge, 2010). However, the role of the hippocampus is contentious. Studies have found that the hippocampus may be involved in the training of the premise pairs (e.g., A > B) in this task but not necessarily in the ability to demonstrate transitive inference (Van Elzakker et al., 2003; Frank et al., 2005; Van der Jeugd et al., 2009). Further investigation is therefore required to elucidate the specific role of the hippocampus in this task. The following set of studies examines one factor which may explain why patients with schizophrenia perform poorly in the transitive inference task. The first study examines the effect of dorsal versus ventral hippocampal disruption through temporary inactivation on performance in the transitive inference task. Next, performance in hippocampus-dependent contextual fear conditioning is compared to performance in the transitive inference task in the DBA/2 strain of mouse - an animal model of schizophrenia (Stevens et al., 1996; Stevens and Wear, 1997), versus the C57BL/6 strain. Being able to strongly link the physiological change to performance changes may help provide a better understanding of the cognitive deficits associated with schizophrenia.

CHAPTER TWO

THE EFFECT OF INACTIVATION OF THE DORSAL VERSUS VENTRAL HIPPOCAMPUS ON PERFORMANCE IN TRANSITIVE INFERENCE IN C57BL/6 MICE

Introduction

Transitivity, the ability to infer a relationship based upon knowledge from other learned relationships, is a form of learning and memory in which the neural substrates are just beginning to be studied. The hippocampus has been shown to be critical in many learning and memory tasks (Squire, 1992); therefore, the hippocampus may be essentially involved in transitivity. In one study, rats in which connections to and from the hippocampus were cut were trained alongside normal rats in the task (Dusek and Eichenbaum, 1997). The lesioned rats were unable to significantly perform beyond chance for the novel transitive inference pairing B>D. The authors suggest that this provides strong evidence for the role of the hippocampus in transitivity. Furthermore, Ongur and collaborators (2006) conducted a transitive inference experiment with patients with schizophrenia and healthy controls that examined brain activity while the task was being performed. In addition to supporting the finding that patients with schizophrenia were impaired when choosing between the novel transitive pair B>D, they found that this learning deficit was associated with decreased activity of the right parietal cortex and medial temporal lobe which includes the hippocampus. This evidence, combined with the animal evidence mentioned previously, appears to support a role for the hippocampus in

the transitivity deficits seen in patients with schizophrenia. Research from other learning and memory tasks demonstrates that the dorsal hippocampus is involved in the temporal and contextual aspects of event representation while the ventral hippocampus is more involved in fear and anxiety processes (Esclassan et al., 2009). This suggests that the dorsal hippocampus alone may also be the critical area involved in the transitive inference task due to the fact that there is a temporal aspect to learning ordered hierarchies.

Although the aforementioned evidence appears to suggest a role for the hippocampus in the transitive inference task, other researchers have suggested that the hippocampus is not necessary for this task (Van Elzakker et al., 2003; Frank et al., 2005). It has been argued that transitive inference is not mediated by declarative, relational learning processes, but instead is the result of making choices based on reinforcement values that are learned implicitly through the striatal-dopamine system. Specifically, A and B receive strong positive strengths while D and E receive strong negative strengths. Therefore, choosing A over E and B over D relies on the positive strengths attached to A and B during training rather than an associative hierarchical ordering. Furthermore, through behavioral and computational analysis, Frank and colleagues theorize that, when active, the hippocampal system competes with the striatal dopamine system to lower performance in transitive inference (Frank et al., 2003; Van Elzakker et al., 2003). These systems interact competitively (Packard and McGaugh, 1996; Poldrack et al., 2001; Poldrack and Packard, 2003; Atallah et al., 2004; Seger and Cincotta, 2006); thus, these data suggest performance of transitive inference is enhanced when the hippocampal system is disrupted.

To test the hypothesis that the hippocampus is not involved in transitive inference, Frank and colleagues (2006) examined performance in the transitive inference task in humans while their hippocampi were inactivated by systemic administration of the drug midazolam, a $GABA_A$ receptor agonist. GABA is generally an inhibitory neurotransmitter and therefore administration of an agonist causes disruption of excitatory synapses and subsequent inactivation. They found that performance during the testing of the novel pairs in the transitive inference task was enhanced relative to controls. However, midazolam was administered systemically and has been demonstrated to enhance striatal system activity (Rattan and Tejwani, 1997; Tejwani and Rattan, 1997) and could affect multiple regions. Thus, one cannot definitively state that any results are related to solely hippocampal inactivation. In order to clarify how the hippocampus may be involved in this task and if abnormal function of the hippocampus can explain the deficits seen in patients with schizophrenia, the present study examined the effect of dorsal and ventral hippocampal inactivation on performance in the transitive inference task. The C57BL/6 strain was trained and tested in the transitive inference task. Following this, surgeries were performed and their dorsal or ventral hippocampi were inactivated by the $GABA_A$ receptor agonist muscimol. Their performance in the trials that test their knowledge of the novel pairings was then re-tested.

Materials and Methods

Subjects:

Subjects were male C57BL/6 mice that were 8 weeks at the start of the study (Jackson Laboratory, Bar Harbor, ME). The C57BL/6 mice were selected because previous research has shown that this strain demonstrates transitive inference (Devito et

al., 2010a; DeVito et al., 2010b) and performs well in several other hippocampus-dependent tasks (Logue et al., 1997; Owen et al., 1997). Mice were housed in pairs with *ad libitum* access to food and water until free-feeding weights were established. Mice were then food restricted to 85% of their free-feeding weight. Mice were maintained on a 12:12 light/dark cycle (lights on at 7:00 am). Behavioral and surgical procedures occurred between 8:00 am and 5:00 pm. For identification purposes, the mice were either ear-punched or marked with a hydrogen peroxide marking solution. All behavioral and surgical procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus and materials:

The training and testing chamber was a clear Plexiglas box measuring 29.2 x 20.3 x 15.2 cm. The box was divided in the center by a piece of Plexiglas measuring the width and height of the box. There were two Plexiglas lids, one for each compartment of the box. The bottom of the box was lined with corncob bedding. Mice were trained to dig in small, circular cups (3.8 cm in diameter, 1.4 cm in height) filled with scented playground sand. The sand was scented with thyme, celery salt, paprika, coffee, basil, cumin, and cocoa at a 1% weight of the sand concentration. These scents were chosen to maintain continuity with previous experiments which examined transitive inference in rodents and allow comparison to them (Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003; Devito et al., 2010a). Digging behavior was rewarded with 45 mg chocolate precision pellets (Bio-Serv, Frenchtown, NJ).

Behavioral Procedures:

Shaping

Mice were trained as described in DeVito, Kanter, and Eichenbaum (2010a). The mice were trained to dig in cups of sand. Initially, there was only one cup. The mice were placed into one compartment then the divider was lifted allowing the mouse access to both compartments. The reward was placed on top of the sand in the cup so that the mice could easily find and consume the reward. This is the definition of one trial, and once a trial ended, mice were placed into the original compartment and the divider was lowered until the next trial began. As the time to find the reward decreased, the reward was subsequently buried under sand at steps of 10%, 25%, 50%, 75%, 90%, and finally fully covered. Once the mice were able to reliably recover the reward a second cup with a reward was introduced. Finally, 2 sessions or blocks of 10 trials between two differently scented cups of celery salt and thyme were used. There were two cups to choose from but only the cup with celery salt-scented sand had the pellet reward.

Training

The training phases began after the second session discriminating between celery salt and thyme. At each phase of training, mice were given 16 trials broken up into two sessions per day: 8 trials in the first session followed by at least a one hour break then 8 trials in the second session. Mice were trained to reach a criterion of 75% accuracy (6/8 trials) across four consecutive sessions in order to move on the next phase of training. The mice learned an ordered hierarchy among the scented cups A>B>C>D>E, where" >" means preferred over. A=Paprika, B=Coffee, C=Basil, D=Cumin, and E=Bitter-sweet cocoa.

Phase I consisted of 8 trials of A>B and 8 trials of B>C followed the next day by 8 trials of C>D and 8 trials of D>E. During Phase II, there were 4 trials of A>B and B>C in the first session and 4 trials of C>D and D>E in the second session. During Phase III, there were 2 trials each of A>B, B>C, C>D, and D>E in both the first and second sessions. Phase IV consisted of a pseudorandom presentation of all pairs intermixed with a total of 4 trials of each pair per day (Table One).

Table One: Organization for the training and testing sessions.

	Session One	**Session Two**
Phase I:		
Odd days	8 trials of A>B	8 trials of B>C
Even days	8 trials of C>D	8 trials of D>E
Phase II		
	4 trials of A>B straight followed by 4 trials of B>C	4 trials of C>D straight followed by 4 trials of D>E
Phase III		
	2 trials of A>B followed by 2 of B>C, followed by 2 of C>D followed by 2 of D>E	2 trials of A>B followed by 2 of B>C, followed by 2 of C>D followed by 2 of D>E
Phase IV:		
	8 pseudorandom presentation of all pairs, 2 trials of each pair	8 pseudorandom presentation of all pairs, 2 trials of each pair
Probe Tests		
Day 1	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings
Day 2	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings

Probe Re-test		
Day 1	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	
Day 2	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	
Day 3	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	
Day 4	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	

Probe Trial Tests

Probe trial sessions were identical to Phase IV of training except that the cups had no food reward and the animals were tested for transitive inference with B>D and non-transitive inference with A>E. The amount of time the mouse spent digging in each cup was recorded. A preference index was calculated using a preference index, shown as percent preference, developed by Bunsey and Eichenbaum (1996); for B>D ([B-D]/[B+D]) and A>E ([A-E]/[A+E]) where each letter corresponds to the amount of time digging in that cup. When digging in B over D was significantly greater than chance, this provided support for the use of transitive inference since B and D were both rewarded 50% of the time. As a comparison, digging in A over E could be guided by processes other than transitive inference because choices of A were always rewarded and choices of E were never rewarded. The probe trial sessions after cannulation surgery differed in that

30 minutes prior to the trials, the mice were infused with either muscimol or saline. Furthermore, only one session was performed per day over 4 consecutive days instead of two due to the length of time muscimol stayed in the system.

Surgical Procedures:

Surgeries were performed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Mice were anesthetized using isoflourane (5% induction, 2% maintenance) and surgeries occurred under aseptic conditions. Surgical instruments were sterilized via dry heat sterilization before and after each surgery. Following induction of anesthesia, the scalp was excised and stainless steel double guide cannulae (C232G, 22 gauge, Plastics one, Roanoke, VA) were inserted at the following stereotaxic coordinates: Dorsal hippocampus: -1.7 mm posterior to bregma, +/- 1.5 mm lateral to the midline, and -2.3 mm (injection depth) ventral to the skull surface. Ventral hippocampus: -2.8 mm posterior to bregma, +/- 3.0 mm lateral to the midline, and -4.0 mm (injection depth) ventral to the skull surface. The cannulae were fixed to the skull with dental cement. Ketophen (ketoprofen 2 mg/kg) was administered i.p. immediately after surgery to control for post-operative pain. Cannulae were fitted with double dummy cannulae (C232DC, Plastics One, Roanoke, VA) to prevent clogging. The mice then had 5 days to recover before any behavioral procedures.

Drugs and Infusions:

Muscimol (5-aminomethyl-3-hydroxy-isoxazole; Sigma, St. Louis, MO), a $GABA_A$ receptor agonist, was dissolved in saline (0.9%). For infusions, mice were gently restrained and dummy cannulae were removed. Dummy cannulae were replaced with injection cannulae attached to polyethylene tubing (PE50; Plastics One, Roanoke, VA).

The PE50 tubing was connected to a 10-µl Hamilton syringe and drug administration was controlled by a microinfusion pump (KDS 100; KD Scientific; New Hope, PA). Muscimol or saline was infused at a rate of 0.50 µl/min at an injection volume of 0.50 µl/side - resulting in a 1.0 µg dose of muscimol per mouse. Infusion cannulae were left in place for 1 minute after the infusion to allow for diffusion of the drug. Muscimol was infused 30 minutes prior to probe trial re-test sessions. This dose produces approximately 90% inactivation of tissue within 1-3 mm^3 of the injection site (Martin, 1991; Edeline et al., 2002).

Histological Verification:

Mice were euthanized immediately after the second probe trial session. Brains were removed and stored in a 10% formalin solution (Fisher Scientific, Pittsburgh, PA) for at least 24 hours before sectioning. A cryostat (-18 degrees C) was used to obtain 60 um coronal sections (taken proximal to cannulae and injection tracts). Slices were mounted on microscope slides and stained with cresyl violet. Cannulae and lesion placements were assessed and recorded on schematics of the mouse brain (Paxinos and Franklin, 2001). Data from animals with incorrect placements were excluded from statistical analyses which totaled 24 mice throughout all experiments.

Statistical Analyses:

Main effects for performance during probe trial sessions were analyzed using one sample and independent sample t-tests. One sample t-tests were used to find the difference from chance and independent sample t-tests were used to compare between strains. Any subjects with values more than 2.5 standard deviations from the mean were

deemed outliers and excluded from analysis, which totaled 5 mice throughout all experiments. All tests were performed at the $p < 0.05$ level using SPSS version 16.0.

Results

Dorsal Hippocampus:

Pre-operative performance on learned and novel pairings during probe trial sessions

Days to Complete Training

All mice successfully learned the four presented pairs over an average of 25.79 ± 2.64 days. Mice took longer for the first two stages (8.68 and 6.58 days) than the third and fourth stages (4.68 and 5.84 days); the time the muscimol mice took to complete Phase I was significantly different than the time to complete Phases III and IV ($t(9) = 3.92$, $p<0.05$ and $t(9) = 1.98$, $p<0.05$, respectively). There was no significant difference between muscimol and saline groups in the time to acquire the presented pairs ($p>0.05$) (Figure One). Muscimol was not administered at this stage.

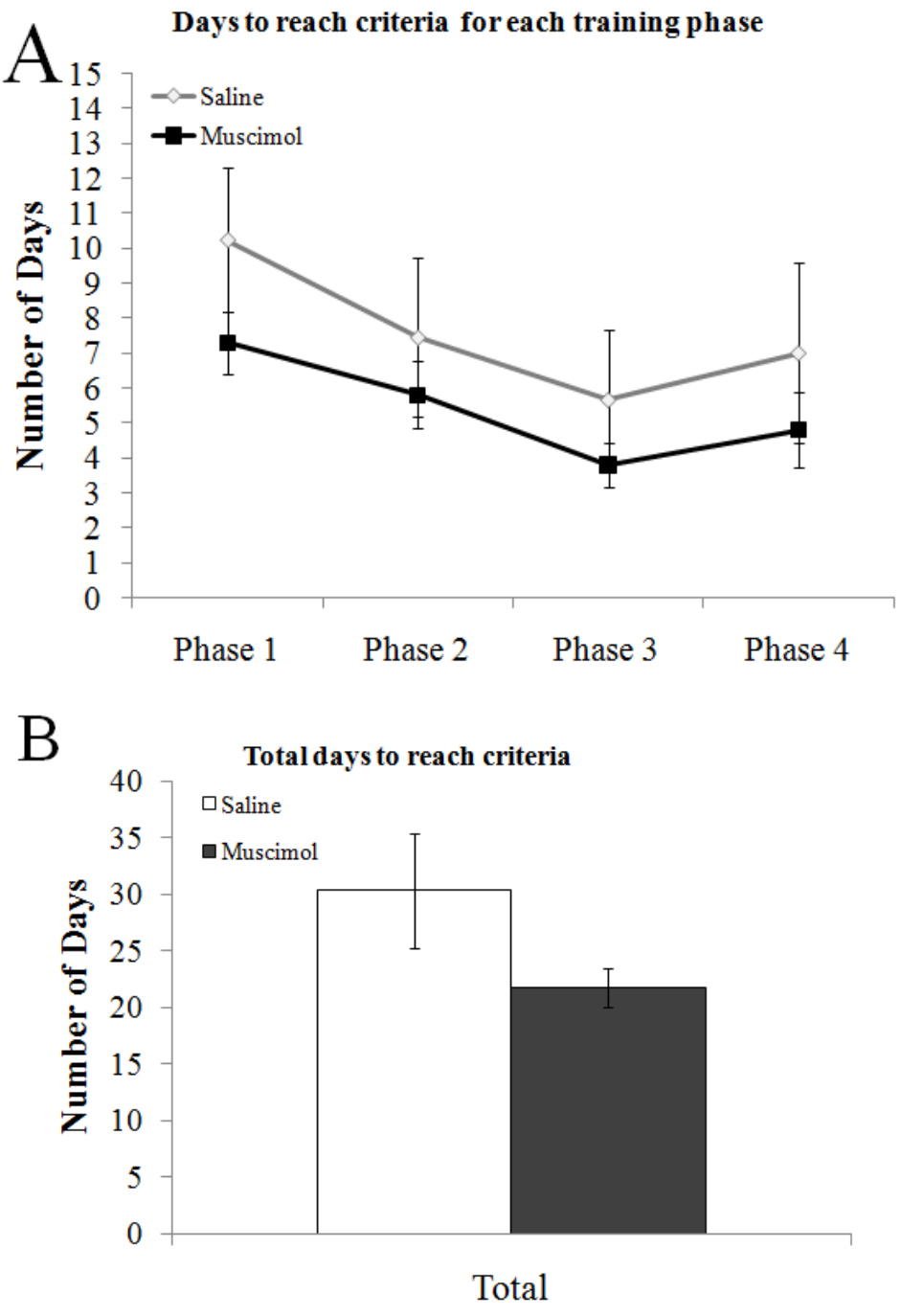

Figure One: A) Days to reach criteria for each phase of training. B) Saline mice took an average of 30.33 days and muscimol mice took an average of 21.70 days to reach criteria. Error bars represent ± the standard error of the mean.

Performance on learned pairings during pre-infusion probe trials: Both groups performed better on the D>E trials than on all other trials and there was a significant difference between D>E and C>D (saline: $t(16) = 2.44$, $p<0.05$; muscimol $t(18) = 3.23$, $p< 0.05$). Furthermore, saline mice performed significantly better on D>E than B>C ($t(16) = 3.17$, $p<0.05$; Figure Two).

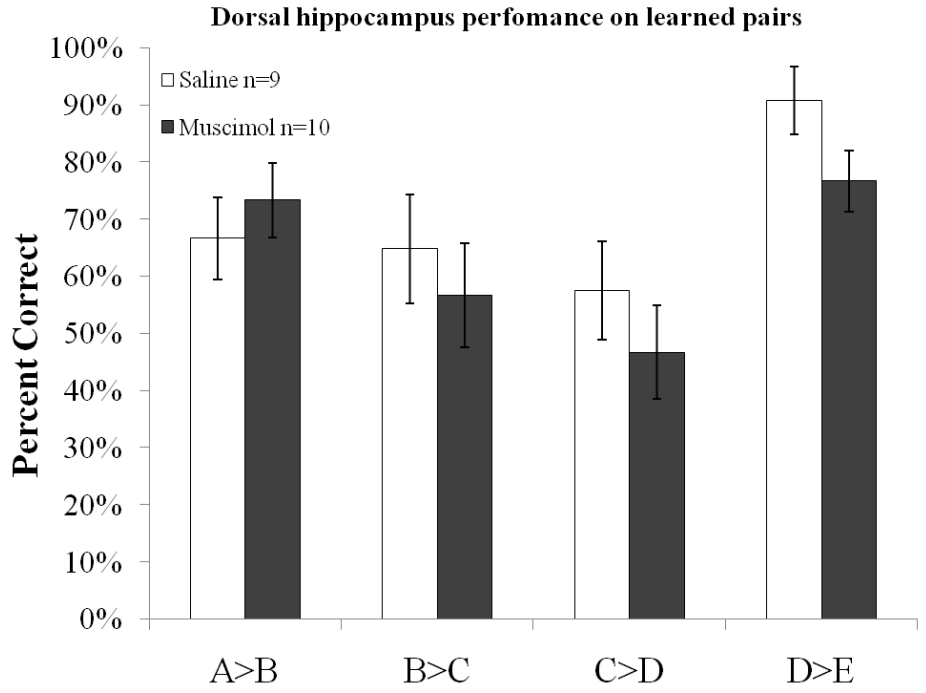

Figure Two: Performance on the learned pairs during pre-infusion probe trials. There were no differences between the saline and muscimol groups but there were differences between the learned pairs. Error bars represent ± the standard error of the mean.

Performance on novel pairings during pre-infusion probe trials: Performance on the novel transitive inference pairing (B>D) and the novel control pairing (A>E) was significantly different from chance for both the saline and muscimol groups (saline: $t(8) = 3.59$, $p<0.05$ ($t(8) =12.53$, $p <0.05$; muscimol: $t(9) =4.78$, $p<0.05$ and $t(9)= 23.10$, $p <0.05$ respectively). There were no significant differences between the saline and muscimol groups (Figure Three).

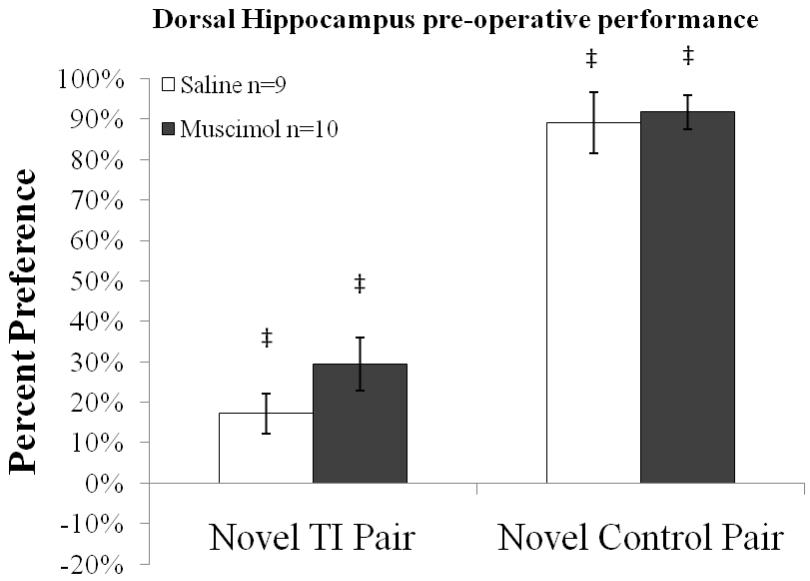

Figure Three: Performance on the novel pairs during pre-infusion probe trials. All groups performed significantly different from chance. There were no differences between groups. Error bars represent ± the standard error of the mean. ǂ represents significantly different from chance.

Post-operative performance on learned and novel pairings during probe trial sessions

Performance on learned pairings: Performance on the learned premise pairs during these sessions differed across the pairs. There was a significant difference for both groups in B>C and D>E (saline: $t(16)$-2.77, $p<0.05$; muscimol: $t(18)=2.21$, $p<0.05$) (Figure Four). Furthermore, mice infused with muscimol performed significantly better on the C>D pair compared to their pre-operative performance ($t(18)=2.44$, $p<0.05$)

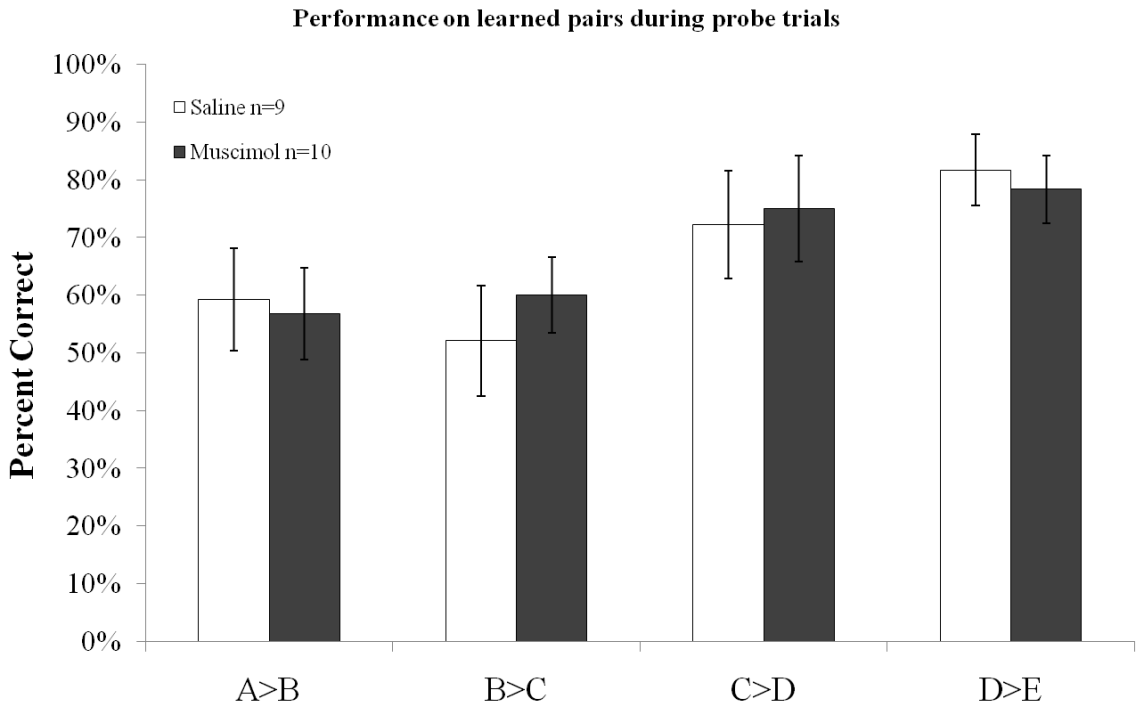

Figure Four: Performance on the learned pairs during post-infusion probe trials. There were no differences between the saline and muscimol groups but there were differences between the learned pairs. Error bars represent ± the standard error of the mean.

Performance on novel pairings: Mice that received saline before the probe trial sessions performed significantly different from chance on the novel transitive inference pairing B>D and the novel control pairing A>E ($t(8) = 2.29$, $p<0.05$ and $t(8)= 21.33$ $p<0.05$ respectively). Mice that received muscimol before the probe trial sessions performed greater than chance on the novel control pairing A>E ($t(9) = 23.59$, $p<0.05$) but not the novel transitive inference pairing B>D ($p>0.05$). Further analysis found that post-operative performance was significantly different than pre-operative testing ($t(18) =4.44$, $p<0.05$) and the performance of the mice that received saline pre- and post-operatively ($t(17) =3.29$, $p<0.05$ and $t(17) = 2.32$, $p<0.05$, respectively; Figure Five).

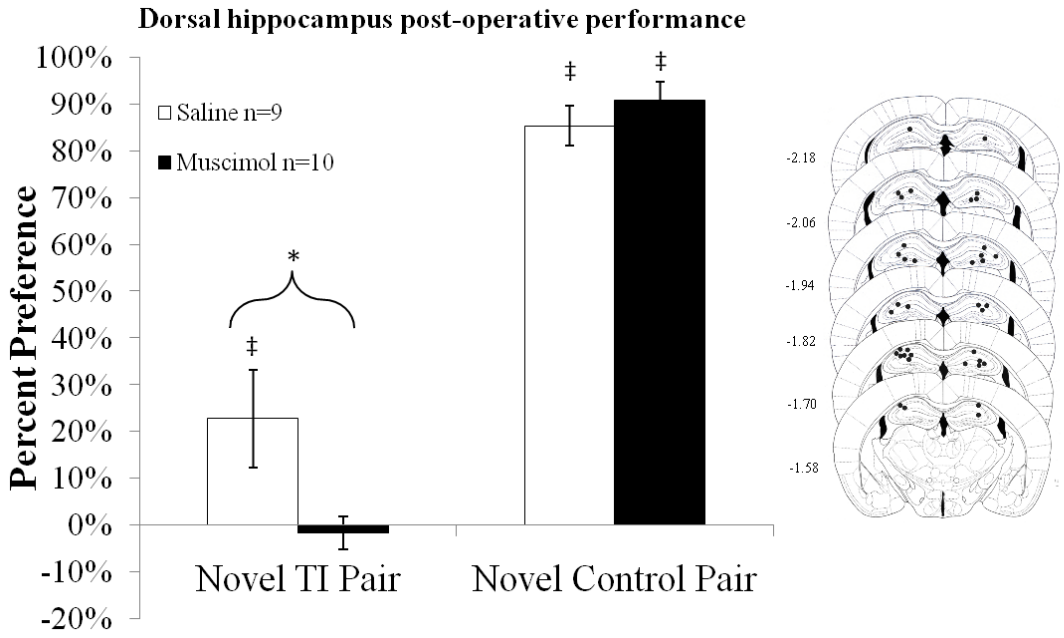

Figure Five: Performance on the novel pairs during post-infusion probe trials. Performance was significantly different from chance for the saline group in both the novel transitive inference and control pairs but was only greater than chance for the muscimol group in the novel control pair. There was a significant difference between the saline and muscimol groups for the novel transitive pair (B>D). Error bars represent ± the standard error of the mean. Asterisks represent significance between groups at $p<0.05$. ǂ represents significantly different from chance.

Ventral Hippocampus:

Pre-operative performance on learned and novel pairings during probe trial sessions

Days to Complete Training

All mice successfully acquired the four premise pairs over an average of 18.38 ± 0.64 days.. Mice took longer for the first two stages (7.89 and 4.63 days, respectively) than the third and fourth stages (3.05 and 2.58 days, respectively) but there were no significant differences. ($p>0.05$). There was no significant difference between the saline and muscimol groups in the time they took to learn the premise pairs ($p>0.05$; Figure Six).

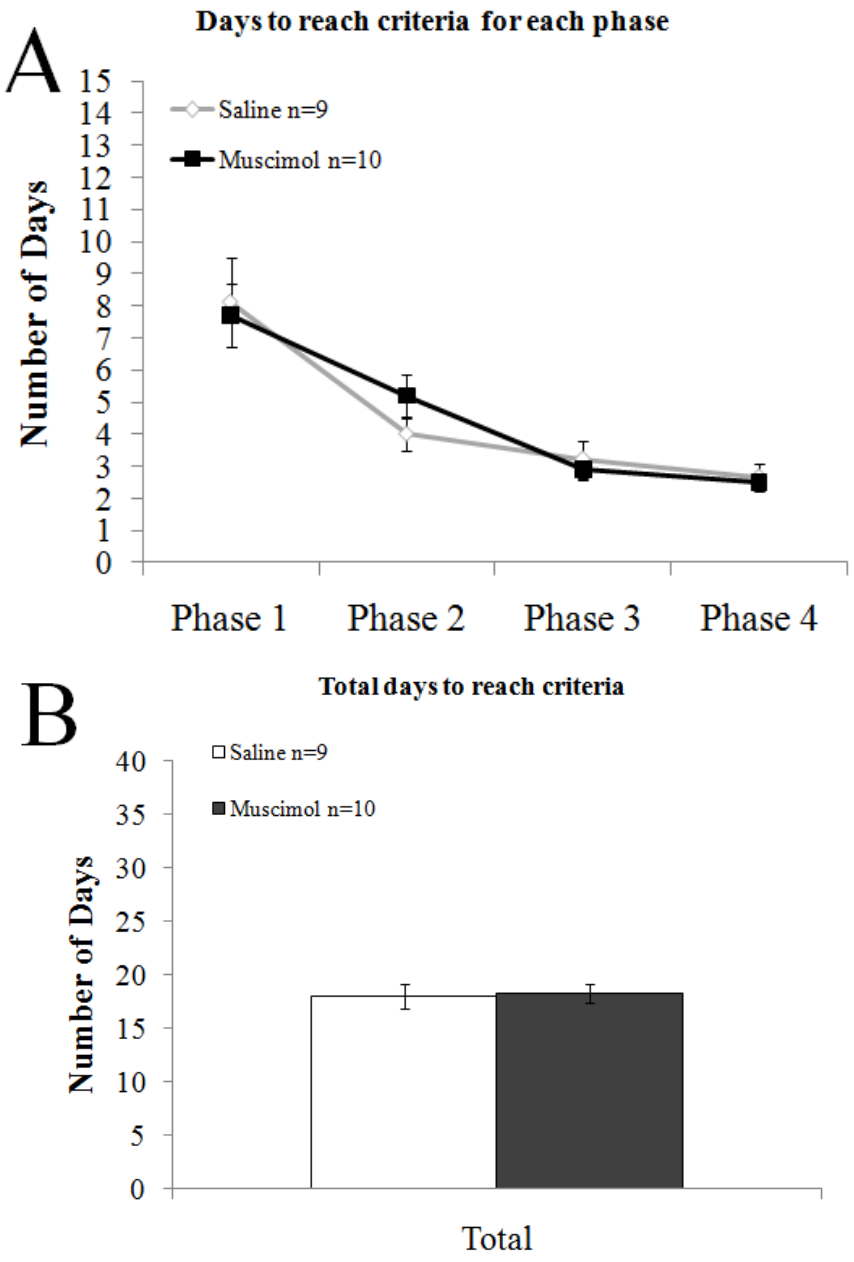

Figure Six: A) Days to reach criteria for each phase of training. B) Saline mice took an average of 18.00 days and the muscimol mice took an average of 18.30 days total. Error bars represent ± the standard error of the mean.

Performance on learned pairings: Performance on the learned premise pairs during the probe trial sessions differed across the pairs. Mice performed better on the A>B and D>E trials than on the B>C and C>D trials; however, the only significant differences

were between A>B and B>C and D>E and B>C in the muscimol group ($t(18)= 2.22$, $p<0.05$ and $t(18) = 3.48$, $p<0.05$, respectively). There were no significant differences between the two groups (Figure Seven).

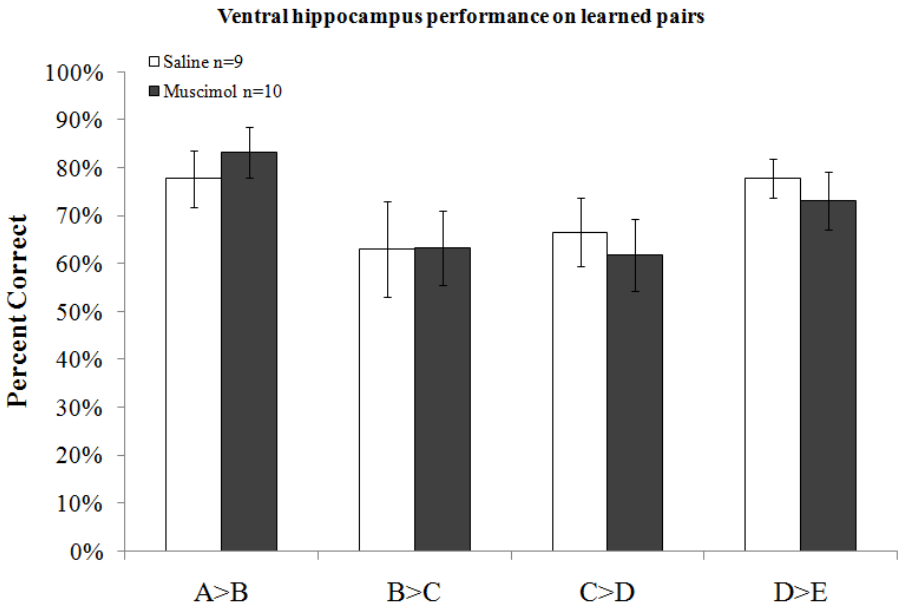

Figure Seven: Performance on the learned pairs during pre-infusion probe trials. There were no differences between the saline and muscimol groups but there were differences between the learned pairs. Error bars represent ± the standard error of the mean.

Performance on novel pairings: Performance on the novel transitive inference pairing B>D and the novel control pairing A>E was significantly different from chance for the muscimol group ($t(9) = 2.46$, $p<0.05$ and $t(9)= 13.23$, $p<0.05$ respectively). The saline group performed significantly different from chance on the novel control pairing A>E ($t(8)= 25.80$, $p< 0.05$) but not the novel pairing B>D ($p>0.05$). There were no significant differences between the groups ($p>0.05$; Figure Eight).

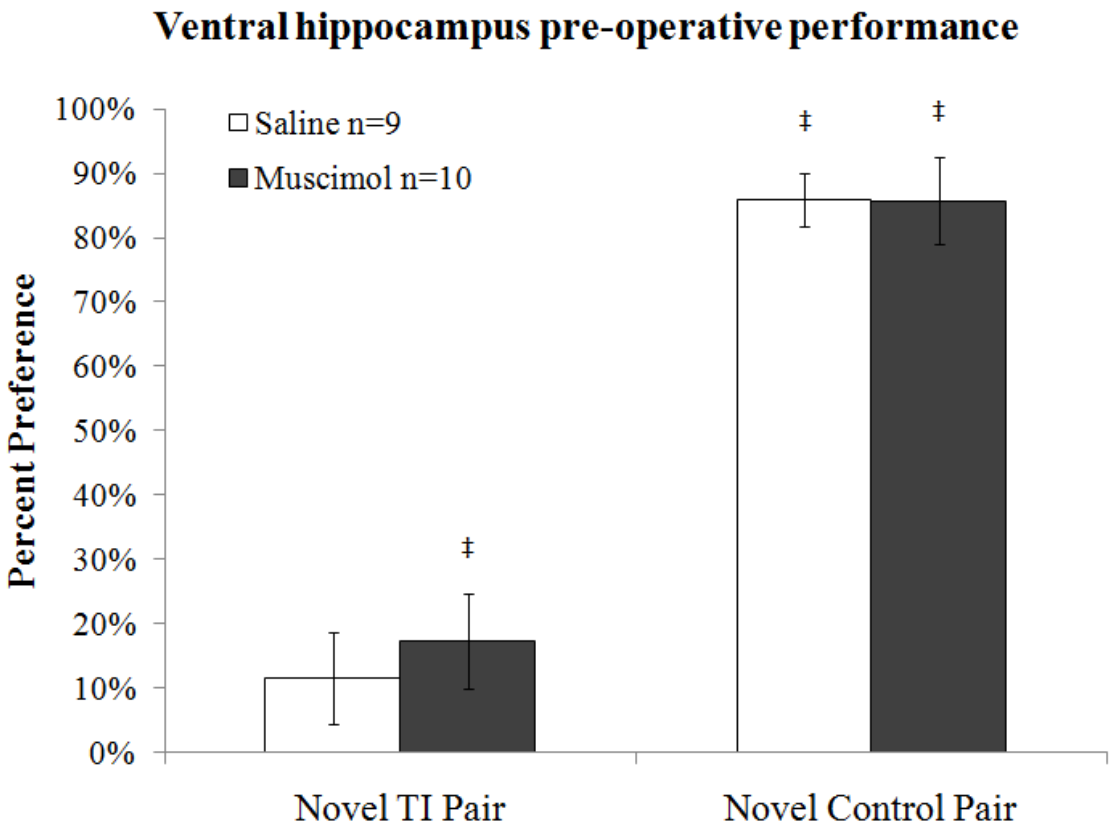

Figure Eight: Performance on the novel pairs during pre-infusion probe trials Error bars represent ± the standard error of the mean. Asterisks represent significance between groups at $p<0.05$. ‡ represents significantly different from chance.

Post-operative performance on learned and novel pairings during probe trial sessions

Performance on learned pairings: Performance on the learned premise pairs during these sessions again differed across the pairs. There was a significant difference for the muscimol group in the performance of A>B and B>C and B>C and D>E ($t(18)=2.22$, $p<0.05$; $t(18)=3.48$, $p<0.05$, respectively; Figure Nine).

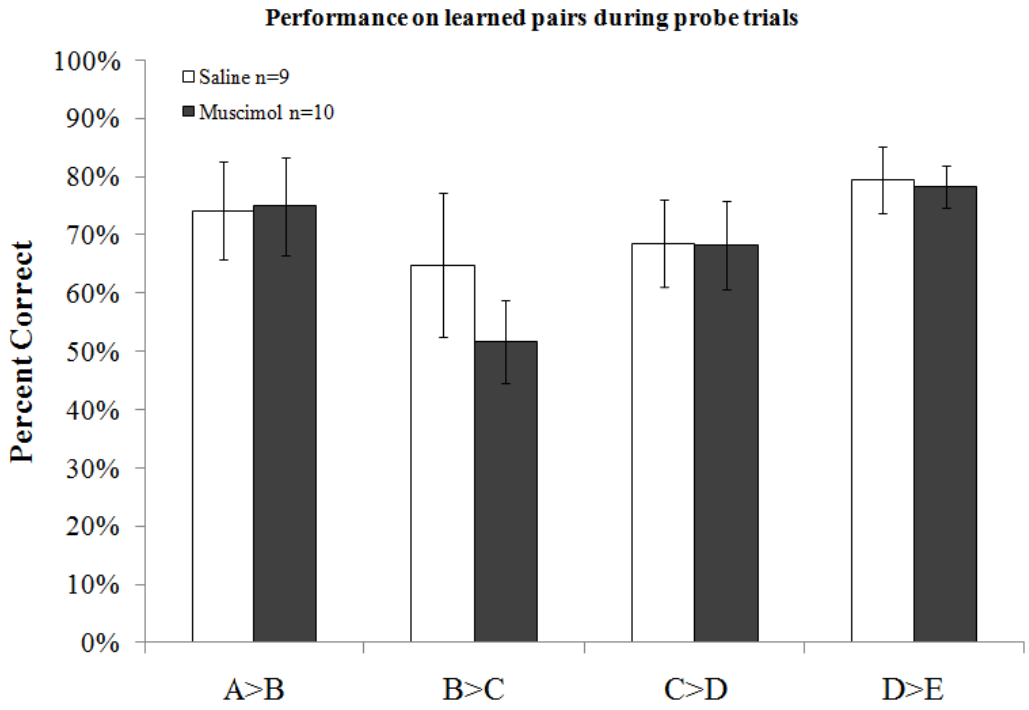

Figure Nine: Performance on the learned pairs during post-infusion probe trials. There were no differences between the saline and muscimol groups but there were differences between the learned pairs for the muscimol group. Error bars represent ± the standard error of the mean. Asterisks represent significance between groups at $p<0.05$

Performance on novel pairings: Mice which received saline before the probe trial sessions did not perform significantly difference from chance on the novel transitive inference pairing B>D ($p>0.05$) but did on the novel control pairing A>E ($t(8) = 16.35$, $p<0.05$). Mice which received muscimol before the probe trial sessions performed above chance on the novel transitive inference pair B>D and the novel control pairing A>E ($t(9) = 3.34$, $p<0.05$ and $t(9)=9.22$, $p<0.05$, respectively). Further analysis found that performance was significantly different between the mice which received saline before post-operative testing and muscimol before post-operative testing ($t(17) =2.21$, $p<0.05$; Figure Ten).

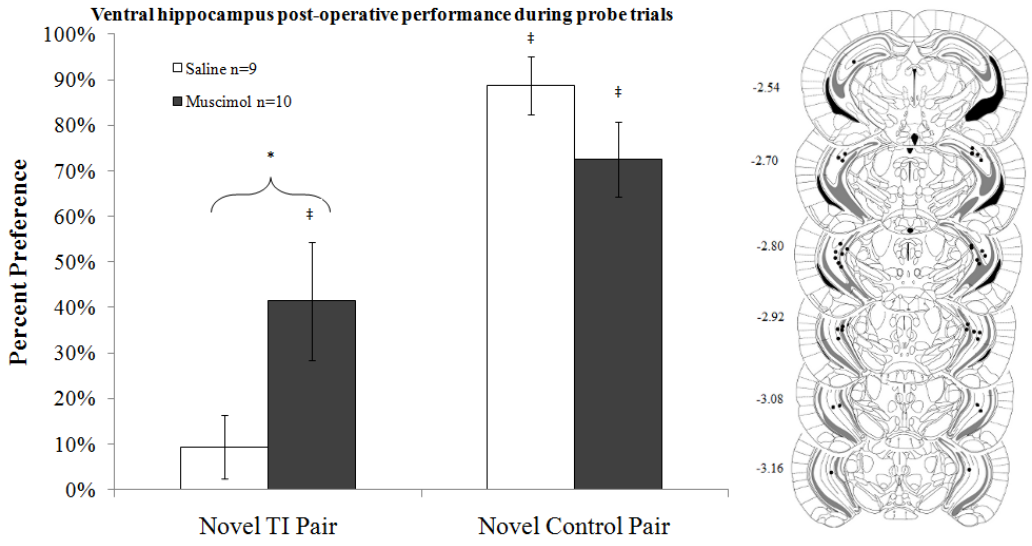

Figure Ten: Performance on the novel pairs during post-infusion probe trials. Performance was significantly different from chance for the muscimol group in both the novel transitive inference and control pairs but was only above chance for the saline group in the novel control pair. There were no differences between groups. Error bars represent ± the standard error of the mean. ǂ represents significantly different from chance.

Discussion

The present study found that inactivation of the dorsal hippocampus by muscimol disrupts transitive inference. Mice infused with muscimol before probe trials did not show any preference in the novel pairing B>D. On the other hand, inactivation of the ventral hippocampus with muscimol enhanced transitive inference. Mice infused with muscimol before probe trials showed a preference in the novel pairing B>D greater than mice in the saline group. The finding that inactivation of the dorsal but not ventral hippocampus disrupts performance demonstrates, for the first time, regional differences in hippocampal function for transitive inference. It has previously been demonstrated in rats that the hippocampus is involved in transitive inference (Dusek and Eichenbaum, 1997). Transecting the fornix or removing the perirhinal and entorhinal cortices impairs

the novel transitive inference pairing. These areas are adjacent to the hippocampus and are known to mediate hippocampal connections with the neocortex. Involvement of the hippocampus is further supported by a study from Eichenbaum and colleagues (2010a) in which mice received full lesions of the hippocampus including the CA fields, dentate gyrus, and subiculm. These mice were unable to perform properly in the novel transitive inference pairing. The lesioned mice's performance was also different from controls (Devito et al., 2010a). The present study adds to these findings by demonstrating that the dorsal hippocampus supports performance of this task while ventral hippocampal activity may decrease transitive inference.

Although support for hippocampal involvement in transitivity is strong, Frank and colleagues (2006) suggest otherwise. Performance during the testing of the novel pairs in the transitive inference task was enhanced in participants that received intravenous administration of midazolam, a $GABA_A$ receptor agonist, relative to controls (Frank et al., 2006). They interpret this finding as midazolam inactivating the hippocampus. However, midazolam was administered systemically in this study and therefore the results may not be only related to hippocampal inactivation. $GABA_A$ receptor agonists have been shown to enhance striatal system activity and thus the performance of the midazolam administered group may be more related to this enhancement than to impairment of the hippocampus (Rattan and Tejwani, 1997; Tejwani and Rattan, 1997). Furthermore, the present results show that inactivating the ventral hippocampus enhances performance in this task. Therefore, the results of Frank et al (2006) may be related to inactivating the ventral hippocampus rather than the hippocampus as a whole. Van der Jeugd and colleagues (2009) also found that lesions to the hippocampus post-training did not affect

performance in the novel transitive inference pairing. However, although the lesions in this study were intended to be specific to the dorsal hippocampus, they were made 2.5 mm posterior to bregma which is more posterior than any point in the present study that was considered dorsal hippocampus.

Until recently, the neuroanatomical basis for the functional differences found between the dorsal and ventral hippocampus were unclear. However, by screening the expression patterns of nearly 2,000 genes in the hippocampal region, Dong and colleagues (2009) found genes which display consistent regional specificities in field CA1. Using these genes as molecular spatial markers they divided field CA1 into three distinct domains along the dorsoventral or longitudinal axis: dorsal, intermediate, and ventral domains. These findings were extended to other layers of the hippocampus further demonstrating an anatomical difference in the dorsal and ventral poles (Christensen et al., 2010). Based on these data, it has become clear that the dorsal hippocampus is smaller than the ventral hippocampus, taking up less than half of the dorsal portion. Therefore, it is quite likely the lesions made in the Van Jeugd study were intermediate or ventral hippocampus and not dorsal hippocampus. Additionally, Van Jeugd and colleagues trained the mice using visual stimuli, unlike in the present study or the studies by Eichenbaum and colleagues (Dusek and Eichenbaum, 1997; Devito et al., 2010a; DeVito et al., 2010b). The neural connections in these systems differ in rodents; anatomical studies have confirmed that the hippocampal formation receives a strong olfactory input. This is most likely due to the olfactory system's close anatomical ties to the limbic system and hippocampus - areas of the brain that have long been known to be involved in emotion and place memory, respectively (for review see (Vanderwolf, 1992)). Visual

information, on the other hand, could not be found in a literature search to have a direct connection to the hippocampus. Thus, direct comparisons between findings in the visual transitive inference study and those based on olfactory stimuli may be difficult. Based on our results, it may be more likely that the degree of hippocampal inactivation and region specificity may determine behavioral effects

There is extensive literature supporting the idea that the dorsal hippocampus is specifically involved in memory function and the ventral hippocampus is involved in emotional and affective processes (for review see (Fanselow and Dong, 2010)). Lesioning more than 70% of the entire hippocampus, but leaving a small portion of dorsal hippocampus intact still allows for spatial learning in a water maze while lesioning only 40% of the hippocampus, including the dorsal hippocampus, disrupts learning of this task (Moser et al., 1995). On the other hand, lesions of the ventral hippocampus produce an increase in exploring open arms in the elevated plus maze, which measures anxiety, while lesions to the dorsal hippocampus have no effect (Kjelstrup et al., 2002). Esclassan and colleagues (2009) found that in trace conditioning- a task shown to be hippocampus dependant- the ventral and dorsal parts of the hippocampus process different information. The dorsal hippocampus processes the temporal and contextual information while the ventral hippocampus is involved in fear and anxiety. Inactivating the ventral hippocampus may disrupt affective processes allowing for greater involvement of the dorsal hippocampus in temporal and navigational aspects of the task. Enhanced learning after a ventral hippocampal lesion suggests amelioration of "normal" competition between the different poles of the hippocampus during learning.

The present study also found that all groups performed better on the A>B and D>E pairs than the B>C and C>D pairs. This may be due to the location of these stimuli in the ordered hierarchy. Because A is always rewarded, E is never rewarded, and B, C, and D are each rewarded 50% of the time, the distinction between the outer pairs of the hierarchy may be more salient for forming associations compared to the inner pairs. In addition, the findings are line with results from other transitive inference studies using both rodents (Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003; Devito et al., 2010a) and humans (Titone et al., 2004; Frank et al., 2006; Ongur et al., 2006).

In the present study, mice in the saline group in the ventral hippocampus experiment did not perform significantly different from chance before or after surgery. These mice did not differ in any manner from the mice which would receive muscimol before surgery. Therefore, the difference in these groups is likely due to a sampling error. However, the saline group performed worse after infusions and were significantly different from the mice infused with muscimol. This was not the case for infusions into the dorsal hippocampus. These findings suggest that the process of infusing into the ventral hippocampus may cause an increase in anxiety which affects transitive inference.

Together, the present study adds to the literature by suggesting that abnormalities in the hippocampus result in poor performance in transitive inference. It appears that disruption of neurons in the dorsal hippocampus is specifically involved deficits in transitive inference. The dorsal hippocampus has been shown to be involved in memory function; however, most studies have examined tasks which require contextual or spatial memory. The involvement of the dorsal hippocampus in transitive inference furthers our understanding of the role of hippocampus in cognition and the learning and memory

systems supported by it. Patients with schizophrenia perform poorly in the transitive inference task; this suggests that abnormal functioning of the hippocampus is a physiological symptom of this disease. Gaining a better understanding of the neural substrates involved in transitive inference may allow for the development of more effective treatments for patients with schizophrenia as these patients have deficits in transitive inference (Titone et al., 2004; Ongur et al., 2006).

CHAPTER THREE

COMPARISON OF THE PERFORMANCE OF DBA/2 AND C57BL/6 MICE IN TRANSITIVE INFERENCE AND FOREGROUND AND BACKGROUND CONTEXTUAL FEAR CONDITIONING

Introduction

The study of inbred mouse strains offers an important means of understanding the role of genetics in behavior. Similarities between mice and human genes range from about 70 to 90% (Church et al., 2009) and therefore are an appropriate model for the study of human diseases. Inbred strain comparisons completed previously for measures of learning and memory and numerous studies have demonstrated that the C57BL/6 and DBA/2 inbred strains of mice perform differently on several behavioral paradigms (Logue et al., 1997; Owen et al., 1997). Compared to the C57BL/6 strain of mice, the DBA/2 strain of mouse exhibits several brain abnormalities including reduced levels of hippocampal protein kinase C (PKC) activity (Wehner et al., 1990; Fordyce and Wehner, 1992) and altered mossy fiber projections (Heimrich et al., 1985; Crusio et al., 1986; Schopke et al., 1991). Moreover, hippocampal long-term potentiation (LTP), a cellular model of synaptic plasticity, is less persistent in DBA/2 mice than in C57BL/6 mice (Matsuyama et al., 1997; Nguyen et al., 2000). These genetic-based abnormalities in the hippocampus may be the basis for the poor performance of DBA/2 mice in many learning and memory tasks (Nie and Abel, 2001). For example, DBA/2 mice perform poorly in the Morris water maze (Upchurch and Wehner, 1988b, a, 1989; Logue et al., 1997; Owen et al., 1997) and lesions to the hippocampus disrupt performance (Morris et al., 1982;

Sutherland et al., 1982; Eichenbaum et al., 1990). Cued fear conditioning, on the other hand, is a task in which the DBA/2 strain performs similarly to the other strains such as the C57BL/6 strain (Paylor et al., 1994) and this task is hippocampus-independent (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Rudy, 1993; Phillips and LeDoux, 1994). These differences in performance support the proposal that genetic-based abnormalities within the hippocampus contribute to the poor performance of this strain. Examining how the DBA/2 strain performance in hippocampus-dependent tasks is essential to better understanding the role of the hippocampus in learning and memory. Therefore, the present study examined the performance of this strain in comparison to the C57BL/6 strain in three hippocampus-dependent tasks - transitive inference and background and foreground contextual fear conditioning.

Transitive inference is the ability to infer that A>C, knowing that A>B and B>C. Eichenbaum and Cohen (2001) have stated that transitive inference captures the relational processing requirements of many other tasks such as spatial navigation, configural learning, and explicit/declarative memory in humans. This task has also been suggested to be hippocampus-dependent through lesion studies in rodents (Dusek and Eichenbaum, 1997; Devito et al., 2010a; Wendelken and Bunge, 2010) and imaging studies in humans (Ongur et al., 2006; Zalesak and Heckers, 2009). Transitive inference has only recently been examined in mice and has not yet been examined in the DBA/2 strain. Because this strain is known to be deficient in performing hippocampus-dependent tasks, and transitive inference is suggested to be hippocampus-dependent, examining how DBA/2 mice perform in the task will help clarify its role.

A more extensively studied task shown to require the hippocampus for proper performance is contextual fear conditioning. During contextual fear conditioning, an animal is trained to produce a conditioned response (CR) by forming an association between a neutral conditioned stimulus (CS), with an unconditioned stimulus (US), such as a foot shock (Rescorla and Wagner, 1972). Previous studies examining the performance of the DBA/2 strain in contextual fear conditioning have primarily used background contextual fear conditioning in which an tone CS is paired with a foot shock US, reducing the context to a secondary, or background stimulus (Logue et al., 1997; Nie and Abel, 2001; Balogh et al., 2002). Following background contextual fear conditioning, the tone is believed to be a stronger predictor of the shock than the context due to the close temporal association between presentation of the tone and the shock compared to the context, which is ubiquitous throughout the training session (Odling-Smee, 1975); however, this overshadowing effect has not been observed in all studies (see (Lolordo et al., 2001). When foot shocks are administered without the tone, the context is more salient and becomes a foreground or primary stimulus (Odling-Smee, 1975, 1978). The DBA/2 mice perform poorly in background contextual fear conditioning but it may be that the context is not a salient cue and poor performance is due to a lack of attention rather than an inability to form the association. If this is the case, the DBA/2 strain should perform better in foreground contextual fear conditioning compared to background contextual fear conditioning. I argue that it is the inability to form an association between the context and the foot shock because of hippocampal abnormalities that is the basis of poor performance. Thus, the DBA/2 strain should perform poorly in both forms of the task. Taken together, the results of the transitive inference and both

foreground and background contextual fear conditioning experiments may offer more insight into how abnormalities in the hippocampus affect learning and memory. Moreover, the results may clarify the role of the hippocampus in the transitive inference task and how it may differ from other hippocampus dependent tasks such as contextual fear conditioning.

Materials and Methods

Subjects:

Subjects were male C57BL/6 and DBA/2 mice that were 8 weeks at the start of each experiment (Jackson Laboratory, Bar Harbor, ME). Mice in the fear conditioning paradigm were housed four to a cage with *ad libitum* access to food and water throughout the study. Mice in the transitive inference experiment were housed in pairs with *ad libitum* access to food and water until base weights were established then food restricted to 85% of their free fed weight. Mice were maintained on a 12:12 light/dark cycle (lights on at 7:00 am) with behavioral procedures occurring between 8:00 am and 5:00 pm. All procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus and materials:

Fear Conditioning: Training and testing of fear conditioning took place in four identical conditioning chambers (18 X 19 X 38 cm) housed in sound-attenuating boxes (MED Associates, St. Albans, VT, USA). Ventilation fans at the back of the boxes provided air exchange and background noise (69 dB). A speaker mounted to the outside right wall of each chamber produced an 85-dB white noise CS. The conditioning chambers were assembled out of clear Plexiglas walls in the front and back and stainless steel on the

sides. The grid floors were connected to a shock scrambler and generator. The shock US was a 0.57 mA footshock for 2 seconds. An IBM PC-compatible computer running MED-PC software interfaced with the conditioning chamber to control stimuli administration. All chambers were cleaned with 70% ethanol before and after each use.

Transitive Inference: The training and testing chamber was a clear Plexiglas box measuring 29.2 x 20.3 x 15.2 cm. The box was divided in the center by a piece of Plexiglas measuring the width and height of the box. There were two Plexiglas lids, one for each compartment of the box. The bottom of the box was lined with corncob bedding. Mice were trained to dig in small, circular cups (3.8 cm in diameter, 1.4 cm in height) filled with scented playground sand. The sand was scented with thyme, celery salt, paprika, coffee, basil, cumin, and cocoa at a 1% weight of the sand concentration. These scents were chosen to maintain continuity with previous experiments which examined transitive inference in rodents and allow comparison to them (Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003; Devito et al., 2010a). Digging behavior was rewarded with 45 mg chocolate precision pellets (Bio-Serv, Frenchtown, NJ).

Behavioral Procedures:

Fear Conditioning: Methods for training and testing mice in contextual fear conditioning were based on previous studies (Gould and Higgins, 2003; Davis et al., 2006; Andre et al., 2008).

Background Training: During training mice were placed into conditioning chambers for 5 minutes and 30 seconds and freezing was used as the behavioral measure of learning. Freezing was defined as the absence of movement except for respiration during a 1 second period assessed every 10 seconds (Blanchard and Blanchard, 1969). At the start of

training, baseline freezing behavior was recorded for 120 seconds. Next, the CS (85 dB white noise) was presented for 30 seconds and co-terminated with a 2-second 0.57 mA US foot shock. A second CS-US pairing was presented at 270 seconds. The mice remained in the chamber 30 seconds after the second CS-US presentation. Twenty-four hours after training, freezing to the context was assessed by placing mice in the training chamber for 5 minutes freezing was scored.

Foreground Training: During training mice were placed in the conditioning chambers for 5 minutes and 30 seconds. Baseline freezing behavior was recorded during the first 120 seconds of the session. At 148 seconds, a 2-second 0.57 mA foot shock US was presented. At 298 seconds, an additional 2-second foot shock US was presented. The mice remained in the chamber 30 seconds after the second US presentation. Twenty-four hours after training, freezing to the context was assessed by placing the mice in the training chamber for 5 minutes during which freezing behavior was recorded.

Transitive Inference:

Shaping

Mice were trained as described in DeVito, Kanter, and Eichenbaum (2010a). The mice were trained to dig in cups of sand. Initially, there was only one cup. The mice were placed into one compartment then the divider was lifted allowing the mouse access to both compartments. The reward was placed on top of the sand in the cup so that the mice could easily find and consume the reward. This is the definition of one trial, and once a trial ended, mice were placed into the original compartment and the divider was lowered until the next trial began. As the time to find the reward decreased, the reward was subsequently buried under sand at steps of 10%, 25%, 50%, 75%, 90%, and finally

fully covered. Once the mice were able to reliably recover the reward a second cup with a reward was introduced. Finally, 2 sessions or blocks of 10 trials between two differently scented cups of celery salt and thyme were used. There were two cups to choose from but only the cup with celery salt-scented sand had the pellet reward.

Training

The training phases began after the second session discriminating between celery salt and thyme. At each phase of training, mice were given 16 trials broken up into two sessions per day: 8 trials in the first session followed by at least a one hour break then 8 trials in the second session. Mice were trained to reach a criterion of 75% accuracy (6/8 trials) across four consecutive sessions in order to move on the next phase of training. The mice learned an ordered hierarchy among the scented cups A>B>C>D>E, where" >" means preferred over. A=Paprika, B=Coffee, C=Basil, D=Cumin, and E=Bitter-sweet cocoa.

Phase I consisted of 8 trials of A>B and 8 trials of B>C followed the next day by 8 trials of C>D and 8 trials of D>E. During Phase II, there were 4 trials of A>B and B>C in the first session and 4 trials of C>D and D>E in the second session. During Phase III, there were 2 trials each of A>B, B>C, C>D, and D>E in both the first and second sessions. Phase IV consisted of a pseudorandom presentation of all pairs intermixed with a total of 4 trials of each pair per day (Table Two).

Table Two: Organization for the training and testing sessions.

	Session One	**Session Two**
Phase I:		
Odd days	8 trials of A>B	8 trials of B>C
Even days	8 trials of C>D	8 trials of D>E
Phase II		
	4 trials of A>B straight followed by 4 trials of B>C	4 trials of C>D straight followed by 4 trials of D>E
Phase III		
	2 trials of A>B followed by 2 of B>C, followed by 2 of C>D followed by 2 of D>E	2 trials of A>B followed by 2 of B>C, followed by 2 of C>D followed by 2 of D>E
Phase IV:		
	8 pseudorandom presentation of all pairs, 2 trials of each pair	8 pseudorandom presentation of all pairs, 2 trials of each pair
Probe Tests		
Day 1	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	
Day 2	8 presentations of the pairs, one of A>E, one of B>D, and 6 of the A>B, B>C, C>D, D>E pairings	

Probe Trial Tests

Probe trial sessions were identical to Phase IV of training except that the cups had no food reward and the animals were tested for transitive inference with B>D and non-transitive inference with A>E. The amount of time the mouse spent digging in each cup

was recorded. A preference index was calculated using a preference index, shown as percent preference, developed by Bunsey and Eichenbaum (1996); for B>D ([B-D]/[B+D]) and A>E ([A-E]/[A+E]) where each letter corresponds to the amount of time digging in that cup. When digging in B over D was significantly greater than chance, this provided support for the use of transitive inference since B and D were both rewarded 50% of the time. As a comparison, digging in A over E could be guided by processes other than transitive inference because choices of A were always rewarded and choices of E were never rewarded.

Statistical Analyses:
Main effects for performance in transitive inference were analyzed using one sample and independent sample t-tests. Data from foreground and background contextual fear conditioning were analyzed with independent sample t-tests. Any subjects with values more than 2.5 standard deviations away from the mean were deemed outliers and excluded from analysis, which totaled one mouse throughout all experiments. All tests were performed at the $p < 0.05$ level using SPSS version 16.0.

Results

Fear Conditioning

An independent samples t-test showed a significant effect of strain on background contextual fear conditioning ($t(16)= 6.13$ $p<0.05$) and on foreground contextual conditioning ($t(14)=3.09$, $p<0.05$). In both cases, the DBA/2 mice froze significantly less than the C57BL/6 mice (Figure Eleven). There was no effect of baseline freezing in either strain.

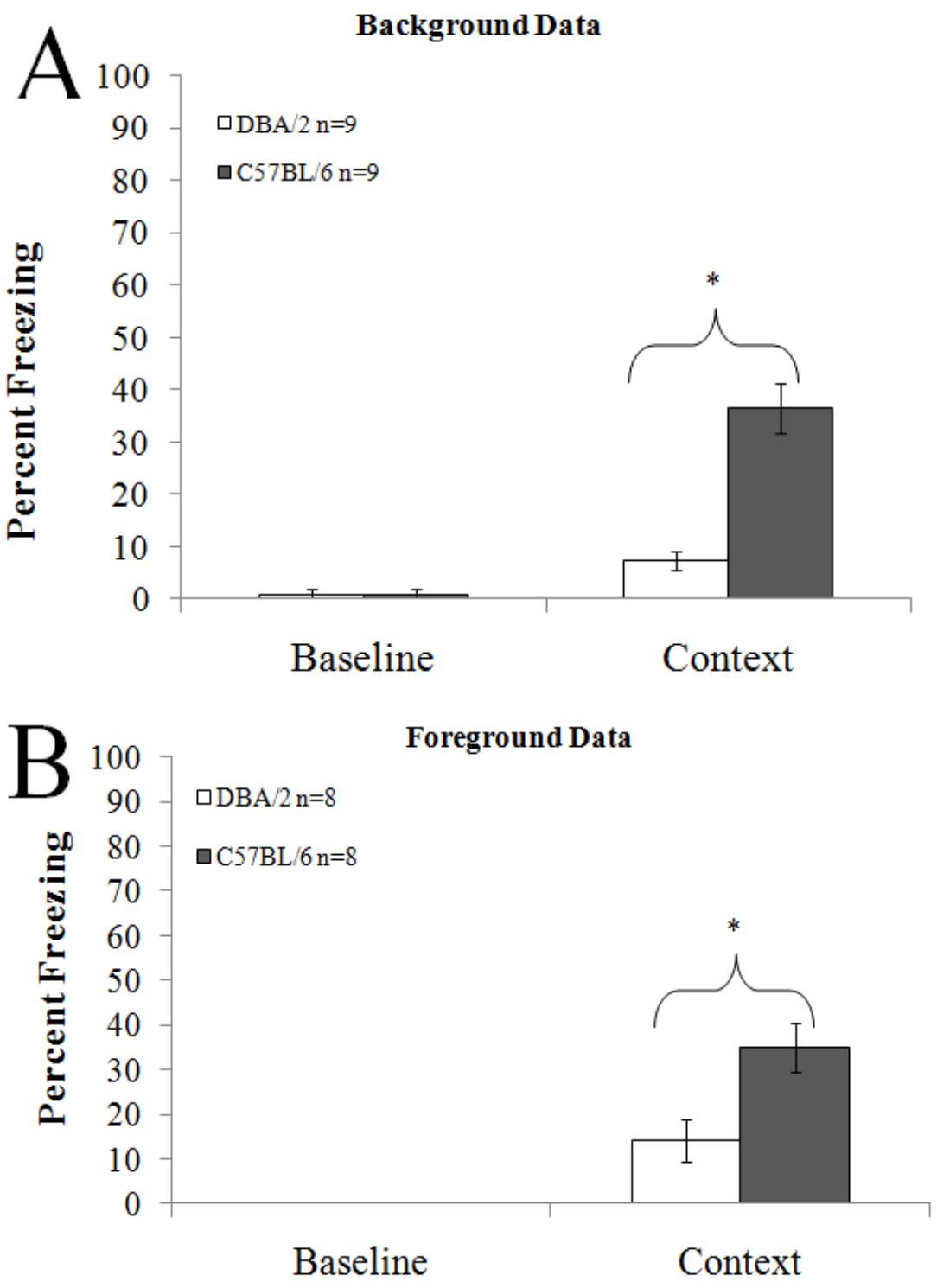

Figure Eleven: Performance in background (A) and foreground (B) contextual fear conditioning. In both cases the DBA/2 strain performed significantly poorer than the C57BL/6 strain. Asterisks represent significance between groups at $p<0.05$. Error bars represent ± the SEM.

Transitive Inference

All mice successfully acquired the learned pairs over an average of 21.27 +/- 0.78 days. The C57BL/6 mice took 22.35+/- 1.42 days and the DBA/2 mice took 20.30 +/- 0.96 days (Figure Twelve A). All mice took longer for the first two stages (8.49 and 5.76 days) than the third and fourth stages (3.24 and 3.76 days). The DBA/2 mice took significantly longer to complete the first phase than third and fourth ($t(38) = 10.04$, $p<0.05$ and $t(38) = 5.39$, $p<0.05$, respectively) and a significantly longer to complete the second phase than the third ($t(38) = 5.67$, $p<0.05$). The C57BL/6 mice took significantly longer to complete the first phase than the second, third, and fourth ($t(32) = 4.13$, $p<0.05$, $t(32) = 9.12$, $p<0.05$; and $t(32) = 8.83$, $p<0.05$, respectively). There was no significant difference between the strains in the time they took to acquire the learned pairs (Figure Twelve B).

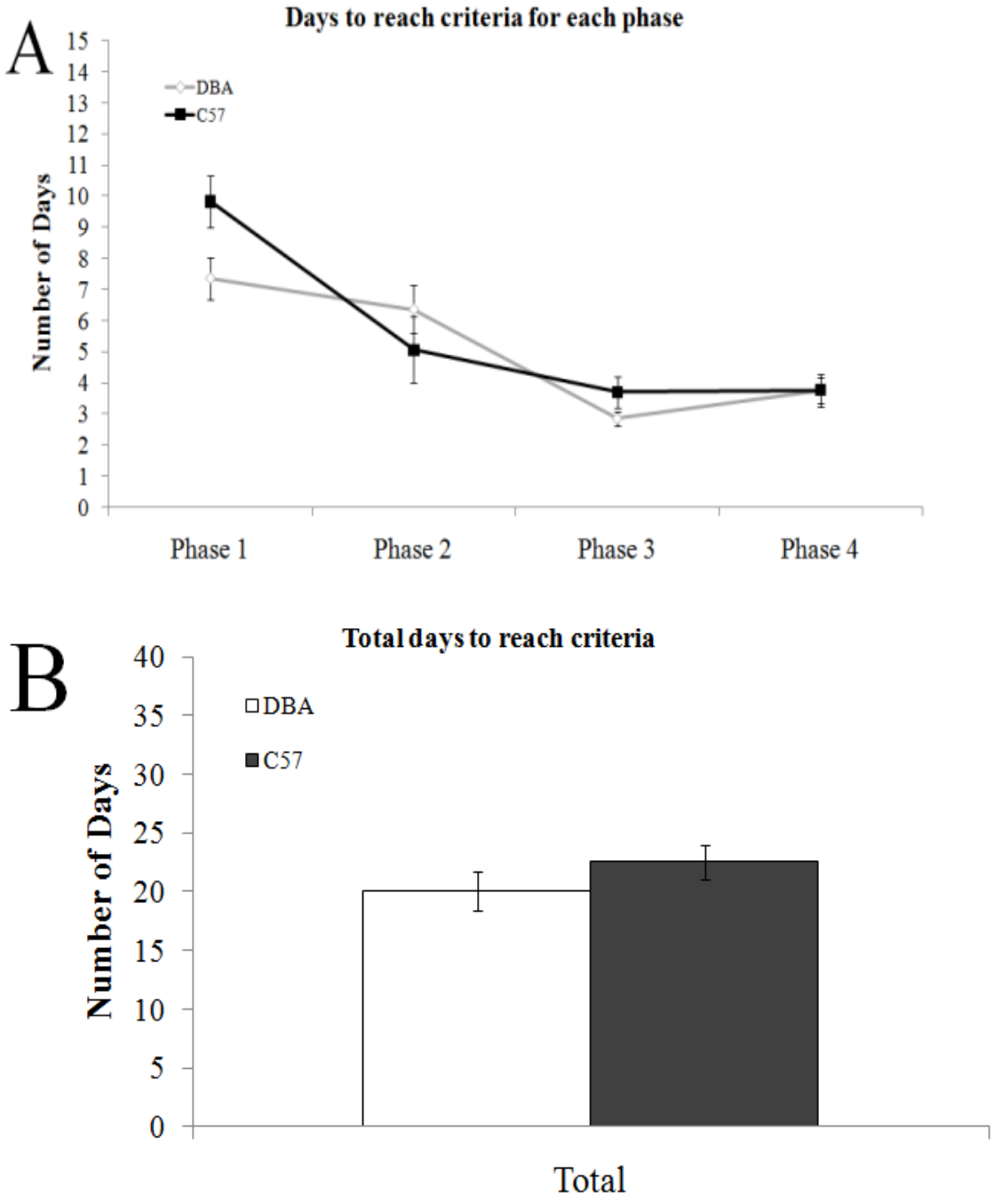

Figure Twelve: A) Days to reach criteria for each phase of training. B) There was no significant difference between strains in total days to reach criteria. Error bars represent ± the standard error of the mean.

Performance on learned pairs: Performance on the learned premise pairs during the probe trial sessions differed across the pairs. The C57BL/6 mice performed significantly

better on the A>B, C>D, and D>E trials than on the B>C **(***t*(32)= 3.82, $p<0.05$; *t*(32)=3.62, $p<0.05$; and *t*(32)=4.96, $p<0.05$, respectively). The DBA/2 mice performed better on the A>B, B>C, and D>E trials than on the C>D trials but there was only a significant difference between B>C and C>D, D>E and C>D, and A>B and D>E (*t*(38)=2.97, $p<0.05$ and *t*(38)=4.48, $p<0.05$, *t*(38)= 2.81, $p<0.05$ respectively). Further analysis found that the DBA/2 mice performed significantly better than C57BL/6 mice on the B>C trials (*t*(35)=4.67, $p<0.05$) and significantly worse on the C>D trials (*t*(35)= 2.19, $p<0.05$) (Figure Thirteen).

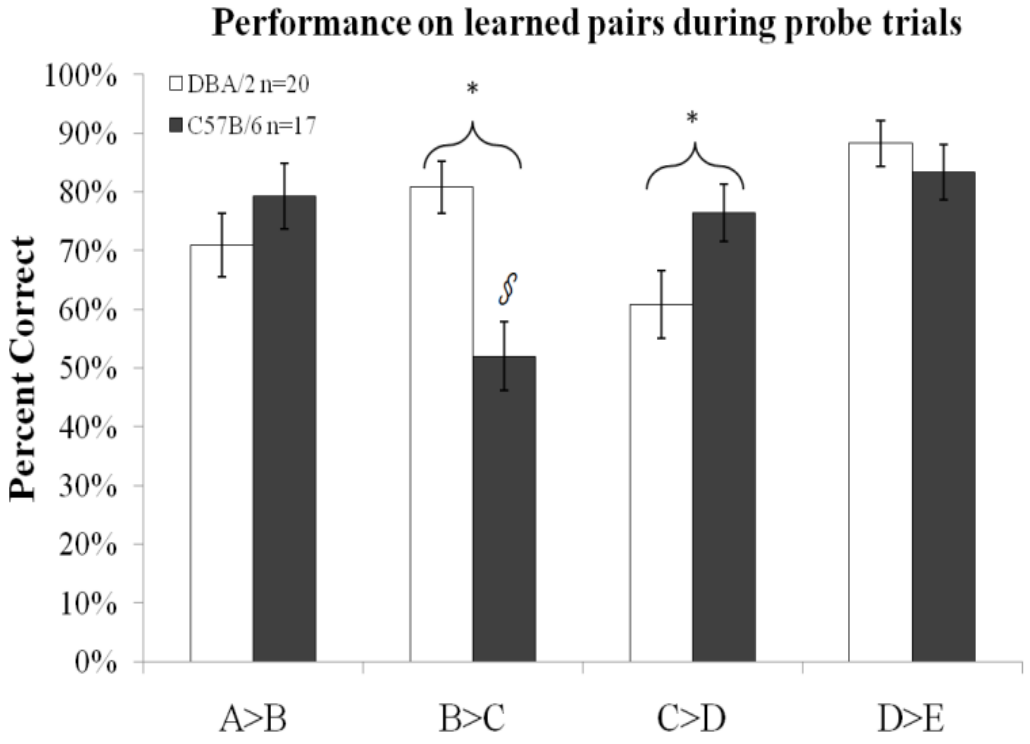

Figure Thirteen: Performance on learned pairs. The DBA and C57 mice differed in performance of the B>C pairing. § represents significantly different from all other pairings for this strain. Asterisks represent a difference at $p<0.05$. Error bars represent ± the standard error of the mean.

Performance on novel transitive inference pairs: Performance on the novel transitive inference pairing B>D and the novel control pairing A>E was above chance for the C57 strain ($t(16)=4.08$, $p<0.05$ and $t(16)=29.45$, $p<0.05$, respectively). The DBA/2 mice performed above chance on the novel control pairing A>E ($t(13)=72.41$, $p<0.05$) but did not perform above chance on the novel transitive inference pairing B>D ($p>0.05$). Furthermore, the DBA/2 mice performed significantly worse than the C57 mice in the B>D trials ($t(35)= 2.36$, $p>0.05$) (Figure Fourteen).

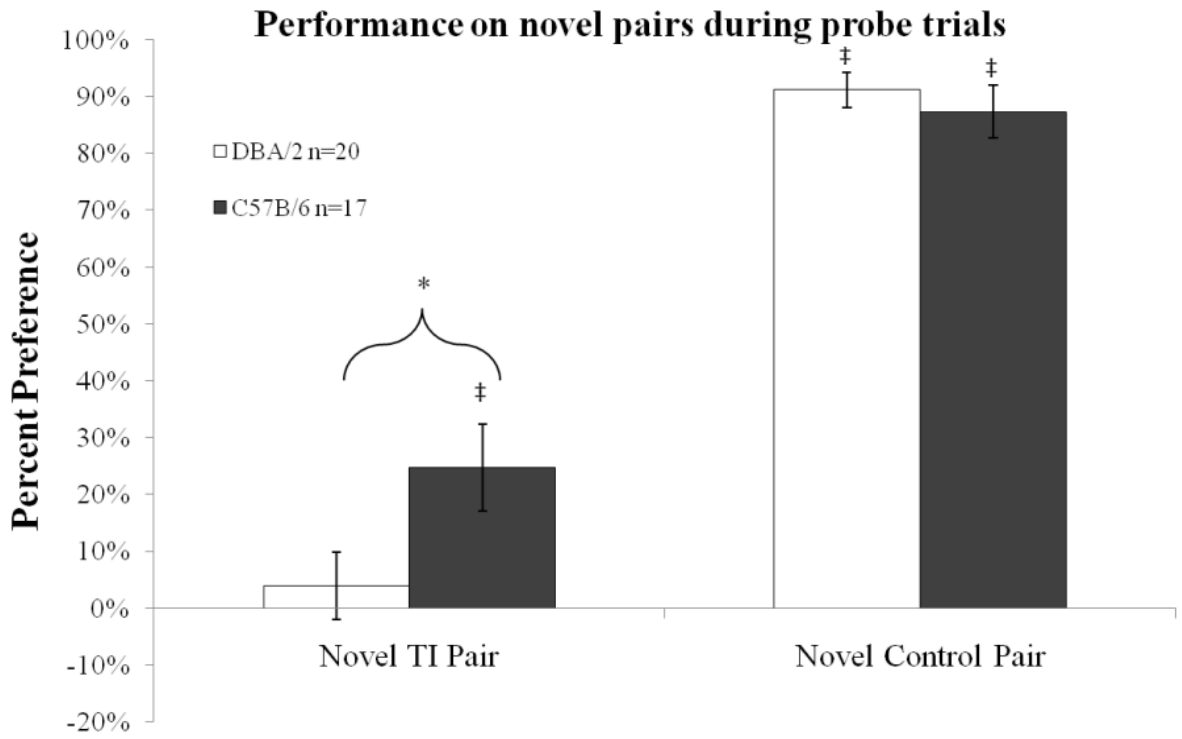

Figure Fourteen: Performance on the novel pairs during probe trials. Performance was significantly different from chance for the C57BL/6 strain in both the novel transitive inference and control pairs but was only above chance for the DBA/2 strain in the novel control pair. Error bars represent ± the standard error of the mean. Asterisks represent significance between groups at $p<0.05$. ǂ represents significantly different from chance.

Discussion

The present study found that the DBA/2 strain of mouse performed worse than the C57BL/6 strain in both foreground and background contextual fear conditioning. The study also found that the DBA/2 mice performed worse than the C57BL/6 mice in transitive inference. Importantly, the DBA/2 mice were unable to perform different from chance in the novel transitive inference pairing (B>D). The fact that the DBA/2 mice performed poorly in all of these hippocampus dependent tasks provides strong evidence that the genetic-based hippocampal abnormalities this strain possesses are the common underlying causes of their learning and memory deficits.

This is the first study to examine the performance of DBA/2 mice in transitive inference. The poor performance of the DBA/2 strain combined with lesion studies in rats and the C57BL/6 strain of mouse support the theory that the hippocampus is critical in the ability to perform transitive inference (Dusek and Eichenbaum, 1997; Devito et al., 2010a). A previous study demonstrated that DBA/2 mice show deficits in learning regardless of whether the context is a foreground or a background stimulus (Stiedl et al., 1999). In this study, however, only one shock was administered during training which makes forming the association more difficult than if two foot shocks are given. Two foot shock presentations were part of the training in many of the other studies that used a training protocol where the context is the background stimulus (Logue et al., 1997; Nie and Abel, 2001; Balogh et al., 2002). The present study examined the performance of the DBA/2 mice in both foreground and background contextual fear conditioning with two presentations of the foot shock during training. A deficit in foreground contextual fear conditioning with two presentations of the foot shock suggests that the learning and

memory deficits of the DBA/2 strain are related to a deficit in forming context-shock associations rather than a lack of attention.

The DBA/2 strain performed better than the C57BL/6 strain in the B>C learned pair. In general for this task, a correct choice in the internal pairs B>C and C>D is considered more difficult than the external pairs A>B and D>E because all of the internal pairs yield reward fifty percent of the time. The fact that the percent correct for the B>C pair is higher in the DBA/2 strain and not both internal pairs was unexpected. It may be that the choices made for the learned pairs during probe trials are not mediated by declarative, relational learning processes, but are instead the result of choosing based on reinforcement values that are learned implicitly through the striatal-dopamine system (Frank et al., 2003; Van Elzakker et al., 2003). Specifically, A receives a strong positive salience which carries over to B. Therefore, choosing A over B and B over C relies on the positive salience attached to A and B during training rather than the hippocampus-dependent associations that are normally formed. In this manner the hierarchy is not learned; however, learning B>C would be easier than learning C>D and percent correct for the B>C pair would be higher than for the C>D pair. However, the B>C improvement is most likely not solely due to the hippocampal abnormalities of the DBA/2 strain for same results are not observed with lesions of the hippocampus (Devito et al., 2010a). An abnormality related to the prefrontal cortex in DBA/2 strain may be involved. In support, lesioning the prefrontal cortex in C57BL/6 leads to performance that closely resembles the DBA/2 strain (DeVito et al., 2010b). Further research is needed to understand why impairing the prefrontal cortex would cause a higher percent correct in the B>C pair.

Patients with schizophrenia have hippocampal abnormalities and show deficits in many cognitive tasks relative to normal controls (For review see (Harrison, 2004)). The DBA/2 strain of mouse also possesses hippocampal abnormalities and show behavioral deficits not unlike patients with schizophrenia making this strain of mouse a useful animal model for schizophrenia (Stevens et al., 1996; Stevens and Wear, 1997; Stevens et al., 1998; Radek et al., 2006). One task that has been extensively studied in both the DBA/2 strain and patients with schizophrenia is pre-pulse inhibition (PPI). PPI is a paradigm used to measure sensory gating - the ability to process important information and filter out extraneous stimuli or noise. PPI and sensory gating in general have been shown to be hippocampus dependent (Stevens and Wear, 1997; Adams et al., 2008; Wolf et al., 2010). Patients with schizophrenia and the DBA/2 strain of mice both show similar deficits in PPI. In PPI, presentation of a weak stimulus inhibits the reaction to a stronger subsequent stimulus. The first stimulus (also known as the prepulse stimulus) elicits an excitatory response that activates inhibitory neural mechanisms which diminish the response to the second, stronger stimulus. The reduction of the response to the second stimulus is a measure of sensory gating and reflects the ability of the nervous system to adapt to a strong sensory stimulus when the preceding weaker stimulus warns the organism of its onset. A high PPI value indicates good prepulse inhibition; conversely, a low PPI value indicates poor prepulse inhibition (Paylor and Crawley, 1997). The PPI value for the DBA/2 strain is low, meaning that this strain responds as much and sometimes more so to the second stimulus than the first. More than 85% of patients with schizophrenia have abnormally low PPI values similar to the DBA/2 strain and problems

with sensory gating (Adler et al., 1982; Clementz et al., 1998; Erwin et al., 1998; Yee et al., 1998; Patterson et al., 2000).

Similarly, patients with schizophrenia show deficits in transitive inference compared to normal populations (Titone et al., 2004) and functional magnetic resonance imaging were able to implicate dysfunction of the hippocampus (Ongur et al., 2006; Wendelken and Bunge, 2010). One would then expect that the DBA/2 strain would also show deficits in transitive inference because of their hippocampal abnormalities and similarities to patients with schizophrenia; and indeed they did not perform different from chance and they performed significantly worse than the C57BL/6 strain. The circuitry involved in transitive inference is only just beginning to be understood. By gaining a better understanding of the circuitry involved in transitive inference we may be better able to understand and model the cognitive impairments exhibited by different populations and consequently develop better therapeutics.

CHAPTER FOUR:

CONCLUSION

The experiments conducted above examined the neural substrates involved in the transitive inference task. Abnormalities of the hippocampus were modeled pharmacologically using temporary inactivation. It was found that inactivation of the dorsal hippocampus, but not ventral hippocampus, produces impairment in transitivity. Next, the DBA/2 strain of mouse, a strain that has altered hippocampal function, was tested in the task. The DBA/2 strain performed poorly compared to the C57BL/6 strain and did not perform different from chance.

From the results of the inactivation study and the DBA/2 strain study it appears clear that an intact dorsal hippocampus is needed to perform the transitive inference task. Impairing dorsal hippocampus function through inactivation with muscimol caused deficits in the performance of these mice. Furthermore, the DBA/2 strain was not able to perform similarly to the C57BL/6 strain meaning that the genetic-based abnormalities that the DBA/2 strain possesses also impair performance. Taken together, the results from the current studies suggest that there is a strong link between the hippocampal abnormalities seen patients with schizophrenia and the poor performance of these patients in cognitive tasks. It is important to further investigate the underlying neural substrates involved in these tasks, such as transitive inference, in order to gain a better understanding of how abnormalities may affect performance in patients with schizophrenia.

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