DISSOCIATING SPACE AND TRACE IN DORSAL AND VENTRAL HIPPOCAMPUS

By

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ABSTRACT OF THE THESIS

Dissociating space and trace in dorsal and ventral hippocampus

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There are inconsistencies regarding the role of the hippocampus in many forms of spatial and nonspatial learning, including Pavlovian trace fear conditioning (McEchron et al., 1998, Quinn et al., 2002, Rogers et al.,2006; Otto & Yoon, 2007). Emerging evidence suggests the hippocampus can be functionally dissociated along its septotemporal axis into dorsal and ventral hippocampus, which may explain some of these inconsistencies (Moser & Moser, 1998; Pitkanen et al., 2000). Excitotoxic lesions of ventral, but not dorsal, hippocampus impair the acquisition of trace fear conditioning, while lesions of either dorsal or ventral hippocampus made after conditioning impair the expression of trace fear conditioning (Yoon & Otto, 2007). The present study examined the contributions of dorsal and ventral hippocampus to a delayed reinforced alternation task and trace fear conditioning by using inactivation with the $GABA_A$ agonist, muscimol. The findings demonstrate that there is a double dissociation of dorsal and ventral hippocampus: inactivation of dorsal, but not ventral, hippocampus, dramatically impaired performance in the delayed reinforced alternation task, while inactivation of ventral, but not dorsal hippocampus attenuated the acquisition and expression of trace fear conditioning.

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1. Introduction

   Converging evidence indicates that the hippocampus is involved in encoding and

retrieving information in various spatial (O'Keefe & Nadel, 1978; Jung et al., 1994; Moser

& Moser, 1993; Moser & Moser, 1998) and nonspatial (Eichenbaum, Otto, & Cohen;

1992; Eichenbaum, 1996; Hock & Bunsey, 1998; Kennedy & Shapiro, 2004; Otto &

Poon, 2006) memory tasks. While it has been proposed that the different subregions of

the hippocampus work together to support a unitary function of memory (Squire & Zola-

Morgan, 1991), recent evidence suggests there is a likely functional dissociation along

its septotemporal axis (Moser & Moser, 1998, Pitkanen et al. 2000; Bannerman et al.,

1999; Richmond et al., 1999). In order to better characterize the role of the

hippocampus in learning and memory, further study of the potentially dissociable roles of

these subregions is necessary.

   Anatomically, the hippocampus can be divided along its septotemporal axis, with

the septal two-thirds comprising the dorsal subregion and the remaining one-third

comprising the ventral subregion (Moser & Moser, 1998). These subregions differ with

respect to neuronal organization and afferent and efferent connections with other brain

areas. For example, dorsal hippocampus receives primarily visual, auditory and

somatosensory information from entorhinal cortex and other cortical areas (Pitkanen et

al., 2000). Conversely, ventral, but not dorsal, hippocampus has direct reciprocal

connections with the amygdala, particularly with the lateral, basal, accessory basal,

central nuclei, and amygdalohippocampal area, as well as heavy connections with the

hypothalamus (Pitkanen et al., 2000). Taken together, these different anatomical

connections suggest that there may be a functional dissociation between dorsal and

ventral hippocampus.

It is generally accepted that dorsal hippocampus is critical for spatial learning (O'Keefe & Nadel, 1978; Jung et al., 1994; Moser & Moser, 1993; Moser & Moser, 1998; Mao & Robinson, 1998; Pothuizen et al., 2004; Bannerman et al., 1999; Ferbinteanu & McDonald, 2001). This follows from anatomical connections relaying sensory information to dorsal hippocampus. This notion is further supported by the presence of place cells in both dorsal and ventral hippocampus, with a greater proportion of place cells in dorsal hippocampus and with better resolution per cell than in ventral hippocampus (Jung, 1994). These data suggest that while both subregions might participate in spatial learning, the dorsal hippocampus may be more importantly involved.

While considerable research effort has focused on the function of dorsal hippocampus, relatively few studies have explored the function of ventral hippocampus. The few studies which have examined the role of ventral hippocampus suggest that it participates in anxiety-related behaviors (Bannerman et al., 2004, Trivedi & Cooper, 2004). These findings are consistent with the aforementioned connections between ventral hippocampus and the amygdala. Collectively these data indicate that dorsal and ventral hippocampus may subserve functionally dissociable roles, with dorsal hippocampus being preferentially recruited for spatial learning and ventral hippocampus playing a relatively stronger role in anxiety-related behaviors. Given that these hippocampal subregions differ with respect to spatial learning and anxiety, it is likely that they may also differentially participate in other forms of learning, such as Pavlovian fear conditioning.

While it is well known that the amygdala is critical for the acquisition and maintenance of fear conditioning (Phillips & LeDoux, 1992; Maren et al., 1996; Kim & Jung, 2006), there is conflicting data concerning the role of the hippocampus in this form of associative learning. In delay conditioning the CS typically overlaps and coterminates

with the US, whereas in trace conditioning there is a trace interval between the offset of the CS and onset of the US. With respect to trace fear conditioning, there are apparent differences in the extent to which each hippocampal subregion participates in the acquisition and expression of fear memories. Ventral hippocampus appears to contribute to both the acquisition and expression of trace and delay fear conditioning (Yoon & Otto, 2007; Bast et al., 2001; Maren & Holt, 2004; Rogers et al., 2006). While some studies suggest dorsal hippocampus is important for the acquisition of trace fear conditioning (Fendt, Fanselow, & Koch, 2005, Burman et al., 2006; Misane et al., 2005), others suggest dorsal hippocampus is instead critical for the expression of previously learned trace fear memories (Quinn et al., 2002; Yoon & Otto, 2007). Because of the inconsistencies in the current literature, it is important to systematically examine the potentially dissociable roles of both dorsal and ventral hippocampus in trace fear conditioning.

In one systematic study, pre-training lesions of ventral but not dorsal hippocampus resulted in an attenuation of the acquisition and subsequent expression of auditory trace fear conditioning, while post-training lesions of both the ventral and dorsal hippocampus resulted in an attenuated freezing response during testing (Yoon & Otto, 2007). However, lesions are permanent and may result in excessive damage, potentially disrupting neighboring brain systems or pathways. In order to further characterize their respective roles in the acquisition and maintenance of fear conditioning, the current study examined the effect of temporary inactivation, using the GABA$_A$ agonist muscimol, of dorsal or ventral hippocampus on trace fear conditioning. An additional experiment examined the roles of dorsal and ventral hippocampus in a delayed reinforced alternation task. A final experiment assessed locomotor activity in an open field to examine whether muscimol affected basal levels of locomotion. Together, these experiments suggest that there is a double dissociation between dorsal and

ventral hippocampus. Specifically, dorsal hippocampus is necessary for delayed

reinforced alternation but not trace fear conditioning while ventral hippocampus is

necessary for trace fear conditioning but not delayed reinforced alternation.

2. Materials and Methods

All procedures have been approved by Rutgers University's Institutional Animal Care and Use Committee.

2.1. Subjects

The subjects were 115 naïve male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250-300g at the time of surgery. They were housed individually in shoebox cages in a colony room with a 12hr light/dark cycle and lights on at 7 a.m. All behavioral testing occurred during the light cycle. Subjects had access to food and water *ad libitum,* except during the reinforced alternation task when they were maintained on a restricted diet in order to maintain 90% of free-feeding body weight. All animals were handled for 2 minutes daily for 5 days prior to surgical procedures and behavioral training.

2.2. Apparatus

Reinforced alternation training was conducted in a T-maze made of black Plexiglas. It consisted of a central stem (60l X 16w X 30h cm) with a start box (15cm) and two arms (40(l) X 16(w) X 30(h) cm) situated at the distal end of the central stem. The central stem was separated from the start box by a sliding guillotine door. A sliding food tray (5l X 3.5w X 1h cm) with a circular food dish (diameter 2.5cm, .75cm deep) was located on the bottom and at the end of each side arm for delivery of food reinforcers. The T-maze was located in a room lit with a light fixture (65 W).

Auditory trace fear conditioning was conducted in a behavioral chamber (30 X 24 X 27cm) enclosed in a sound-attenuating enclosure (56 X 41 X 42cm). The floor of the chamber was composed of 16 stainless steel rods equally spaced by 1.9cm and connected to a shock generator (model H13-15, Coulbourn Instruments, Allentown, PA) designed to administer footshock US (0.6mA). Two of the opposing walls were composed of transparent Plexiglas and the other two were aluminum. A speaker was

mounted 14cm above the floor outside one of the aluminum walls. A single 24V light bulb was mounted above the speaker. A motion detector (model H24-61, Coulbourn Instruments, Allentown, PA) was mounted on top of the ceiling, and a 3.8cm diameter hole was drilled through the ceiling where the sensor was located. A one-way glass window on the front door of the chamber allowed an experimenter to observe and score freezing using a hand switch attached to the computer. The training chamber was cleaned with cage cleaner (2.54 % didecyl dimethyl ammonium chloride,1.69% dimethyl benzyl ammonium chloride) between sessions.

The testing session for trace fear conditioning took place in a novel chamber located in a different experiment room. The testing chamber had the same measurements and configuration as the training chamber but the entire floor was covered with black Plexiglas and black and white diagonal striped panel was attached to one set of the opposing walls. As in the training chambers, a motion detector (model H24-61, Coulbourn Instruments, Allentown, PA) was mounted on top of the ceiling with a hole (3.8cm diameter) drilled through the ceiling where the sensor was located. A one-way glass window on the front door of the chamber allowed an experimenter to observe and score freezing using a hand switch attached to the computer. The testing chamber was cleaned with alcohol between sessions.

Locomotor activity was assessed one week after trace fear conditioning in an open-field chamber (85 X 85 X 30 cm) made of black Plexiglas. The floor of the chamber was divided into 36 squares (14cm). The chamber was located in a room lit with a light fixture (65 W). A video camera placed above the center of the chamber was used to record each session. An experimenter unaware of the experimental condition of each subject watched the video on a TV screen in a different room and manually recorded locomotor activity.

2.3. Procedure

2.3.1. Surgery

After anesthetization with a solution of ketamine (80mg/kg)-xylazine (12mg/kg), i.p., all subjects underwent aseptic stereotaxic surgery. The subject's head was shaved, mounted in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA), and cleaned with alcohol and Betadine. Subcutaneous injections of Marcaine (0.1ml, 25%) in several locations below the scalp served as a local anesthetic and vasoconstrictor. The scalp was then incised and retracted. Six small burr holes were drilled into the skull for the cannula and four small jeweler's screws. For subjects receiving muscimol or saline infusions into dorsal hippocampus, double guide cannula (22-gauge, 11mm, Plastics1, Roanake, VA) were implanted bilaterally into the dorsal hippocampus (AP: -3.8mm, ML: ± 2.5mm from bregma; DV: -2.2mm from dura). For subjects receiving muscimol or saline infusions into ventral hippocampus, single guide cannula (22-gauge, 11mm, Plastics1, Roankae, VA) were implanted bilaterally into ventral hippocampus (AP: -5.2mm, ML: ±5mm from bregma; DV: -5.5mm from dura). The cannulae were affixed with dental cement and anchored to the skull via four stainless steel screws. The incision was then closed with stainless steel surgical staples and obdurators were placed into the guide cannula. All animals were closely monitored during the 7-day post surgical recovery period. Before behavioral testing subjects were randomly assigned to the inactivation (muscimol) or control (saline) group.

2.3.2. Drug Infusions

Subjects received microinfusions of either physiological saline (0.9%) or muscimol (1µg/µl; Sigma, St Louis, MO), dissolved in 0.1M phosphate-buffered saline (pH 7.4). The infusions were administered via insertion of an infusion cannula into the guide cannula targeted at the dorsal or ventral hippocampus. The infusion cannula protruded 1mm beyond the tip of the guide cannula, and was connected via polyethylene

tubing to a 10µl Hamilton syringe mounted in an infusion pump (Harvard Apparatus).

For animals receiving infusions into dorsal hippocampus, a volume of 0.25µl (0.25

µl/min) was infused bilaterally for a total volume of 0.5µl. For animals receiving infusions

into ventral hippocampus, a volume of 0.5µl (0.25 µl/min) was infused bilaterally for a

total volume of 1 µl. The infusion cannula was left in position for two minutes following

completion of infusion to allow for drug diffusion. Subjects were then transferred to an

experimental room thirty minutes after the infusion was complete to undergo behavioral

testing.

2.3.3. Delayed reinforced alternation

Following a one week post-surgical recovery period, animals were placed on a

food deprivation schedule to maintain 90% body weight. Once 90% of free feeding body

weight was reached, subjects were pre-exposed to the T-maze. During pre-exposure,

subjects were placed in the T-maze for 20min during which they were allowed to freely

explore and eat sucrose pellets (approximately 30) which were scattered throughout the

T-maze and in the sliding food tray cups. Sucrose pellets were placed in the subject's

home cage afterwards to further acclimate the subjects to the reinforcer.

Reinforced alternation training began one day following pre-exposure. Animals

were trained in one reinforced alternation session per day; each daily session consisted

of 12 trials, each separated by a 30s intertrial interval. The subject was placed in the

start box of the T-maze for 30s and then allowed to enter one of the two arms. Sucrose

pellets were placed in both food cups for every trial to help eliminate odor as a cue. On

the first trial, the animal received 3 sucrose pellets for entering either arm. On

succeeding trials, the animal received reinforcement for entering the arm opposite to the

previously entered arm. Entering an arm was defined as all four paws crossing into the

arm. Once a subject entered an arm, it was confined to that arm for 3s. The sliding trays

were pushed in by the experimenter only for every correct trial; incorrect trials ended with the subject receiving no reinforcer.

Since the first trial was a free choice trial, the score for each session was calculated as: (number of correct alternations / total trials -1) X 100%. Subjects were trained on consecutive days until they achieved at least 80% correct (9/11) on three consecutive days. One day after reaching criterion, animals were infused with either saline or muscimol into dorsal or ventral hippocampus 30min prior to training in one reinforced alternation session. On the following day they were run in a final reinforced alternation session, without receiving any infusions. After completion of the reinforced alternation task, all subjects received *ad libitum* food and water.

2.3.4. Auditory trace fear conditioning

Trace fear conditioning was conducted one week after completion of reinforced alternation. Although animals were infused with the same substance (saline or muscimol) as during reinforced alternation, they were randomly assigned to receive either pretraining or pretesting inactivation (see Table 1). For pre-training inactivation, the dorsal or ventral hippocampus was inactivated thirty minutes prior to conditioning with saline or muscimol. These subjects did not receive any microinfusions prior to the testing session 24hr later. For pre-testing inactivation, the rats underwent the trace fear conditioning training procedure without receiving any microinfusions. Twenty-four hours after training they were brought into the infusion room and received microinfusions of saline or muscimol in the same manner described above. Thirty minutes after the completion of infusion, they were transferred to a novel chamber in a separate room for the testing session.

Auditory trace fear conditioning took place in a single session of 10 pairings of a tone (20s, 3.9 KHz, 80dB) and footshock (2s, 0.6mA), with a trace interval of 30s between the offset of the tone and onset of the shock. The first tone was presented after

a 4min acclimation period and trials were separated by a 4min intertrial interval (ITI). The behavioral measures of freezing and immobility were recorded throughout the entire conditioning session by an observer blind to the subjects' condition and an automated motion detector, respectively.

The testing session for trace fear conditioning was conducted in a novel chamber 24hr after conditioning in one session consisting of 6 trials. The procedure was the same as during conditioning except that footshock was not presented. As with conditioning, behavioral measures of freezing and immobility were recorded throughout the entire conditioning session.

2.3.5. Behavioral measures

An automated infrared motion detector (model H24-61, Coulbourn Instruments, Allentown, PA) with Fresnel lens, dual element differential detector (13nM infrared radiation), and 90-degree viewing angle continuously sensed movement or lack of movement during conditioning and testing sessions, and the information was sent to a computer for subsequent analysis. Data were converted to the percent of time spent immobile during the 4min ITI, 20s CS, and 30s trace interval for each trial. In addition, an observer recorded freezing with a manual hand switch throughout the training and testing sessions in order to ascertain a correlation between immobility and freezing.

2.3.6. Locomotor activity

In order to examine whether muscimol infusions affected basal levels of activity, locomotor activity was measured in an open chamber one week after trace conditioning. Locomotor activity was assessed in 3 sessions over 3 consecutive days. At the beginning of each session subjects were placed in a corner of the open chamber and remained in the chamber for 10min. An experimenter blind to the infusion condition of the subjects recorded the amount of ambulation, defined as the crossing of all four legs from one square to another, and rearing, defined as taking the two front legs off the floor.

On the second day of locomotor activity testing, subjects received microinfusions of either saline or muscimol (each subject received the same solution administered before the reinforced alternation task and trace conditioning) 30min before being placed in the open chamber for the 10min session. On the third consecutive day, animals were placed in the open field for one final 10min session.

2.3.7. Histology

Following completion of all behavioral testing, animals were administered a sub-lethal dose of sodium pentobarbital (100mg/kg, i.p.) and perfused transcardially with 0.9% saline followed by buffered 10% formalin. The brain was removed and placed in a 10% formalin-30% sucrose solution for at least three days. The brain was then frozen and sliced into coronal sections with a thickness of 50µm using a cryostat. Every other slice throughout the dorsal or ventral hippocampus was mounted on gelled glass microscope slides and subsequently stained with cresyl violet and coverslipped. An observer blind to the subject's condition verified cannula placement throughout the dorsal or ventral hippocampus. Subjects with inaccurate cannula placement or extensive damage were excluded from data analysis.

2.3.8. Statistical Analysis

The primary analyses for all three experiments were two-way repeated measures analyses of variance (ANOVAs). An alpha level of .05 was used for all statistical analyses. Post hoc comparisons, where necessary, were conducted using SNK's post hoc test.

3. Results

3.1. *Cannula Placement*

Following histological verification, five animals with cannulae targeted at dorsal hippocampus and seven animals with cannulae targeted at ventral hippocampus were excluded from statistical analyses due to improper cannula placement and/or extensive damage. Figure 1. illustrates the cannula placements for all animals in the dorsal or ventral hippocampus.

3.2. *Delayed Reinforced Alternation*

There was no significant difference in reinforced alternation between animals receiving saline into dorsal hippocampus (n=17) or ventral hippocampus (n=11), (F(1,28)=0.062, p=0.8). Therefore they were combined into one saline group (SAL, n=28). The final group sizes for the dorsal hippocampus muscimol group (DH-MUS) and the ventral hippocampus muscimol group (VH-MUS) were 22 and 11, respectively.

Mean (±*SEM*) percentage of correct alternations exhibited by animals receiving infusions into dorsal hippocampus or ventral hippocampus is shown in Fig. 2. Muscimol inactivation of dorsal, but not ventral, hippocampus dramatically impaired performance in delayed reinforced alternation. A two-way ANOVA with test day as the within-subjects factor and infusion condition as the between-subjects factor revealed significant main effects of infusion condition (F(2,59)= 22.9, p<0.0001), test day (F(4,185)= 69.9, p<0.0001), and a significant interaction between test day and infusion condition (F(4,185)= 37.6, p<0.0001). Subsequent post hoc analyses (SNK) revealed that group DH-MUS differed significantly from both the SAL and VH-MUS groups on the day of infusion only (p<0.05).

3.3. The effect of pre-training inactivation of dorsal or ventral hippocampus on auditory trace fear conditioning

One week following the completion of the reinforced delayed alternation task, subjects were assigned to receive either pre-training or pre-testing inactivation prior to trace fear conditioning. A subset of animals with cannulae targeted at ventral hippocampus (muscimol, n=12; saline, n=9) were trained in trace fear conditioning but not the reinforced alternation task. There were no significant differences between the saline or muscimol groups that were or were not trained in reinforced alternation during the ITI, CS, or trace interval for trace fear conditioning or testing and therefore they were combined for analyses. The final group sizes for animals receiving pre-training infusions of muscimol into dorsal hippocampus (DH-MUS), muscimol into ventral hippocampus (VH-MUS), or saline into dorsal or ventral hippocampus (SAL) were 11, 17, and 21, respectively.

3.3.1. Acquisition of trace fear conditioning

The effect of muscimol or saline infusions on the acquisition of trace fear conditioning is illustrated in Fig. 3. The mean (*±SEM*) percentage of immobility expressed by the different infusion groups during the 4min ITIs are shown in Fig. 3a. Inactivation of ventral hippocampus significantly attenuated the level of immobility across trials. A two-way ANOVA revealed a significant main effect for infusion condition ($F_{(2,46)}= 23.04$, $p<0.001$), a significant main effect for trial ($F_{(18,489)}= 63.23$, $p<0.001$), and a significant interaction between infusion condition and trial ($F_{(18,489)}= 4.47$, $p<0.001$). Subsequent post hoc analyses (SNK) revealed that the group VH-MUS had significantly lower levels of immobility than both DH-MUS and SAL during trials 2-10 with the exception of trial 4, ($p<.05$). There were no significant differences found between the DH-MUS and SAL groups or between all three groups during trial 1.

The mean (±*SEM*) percentage of immobility expressed by the different infusion groups during the 20s auditory CS presentations are shown in Fig. 3b. A two-way ANOVA revealed a significant main effect for infusion condition ($F(2,46)= 13.73$, $p<0.001$), a significant main effect for trial ($F(18,489)= 18.67$, $p<0.001$), and a significant interaction between infusion condition and trial ($F(18,489)= 1.89$, $p=0.015$). Subsequent post hoc analyses (SNK) revealed that VH-MUS had significantly lower levels of immobility than DH-MUS and SAL during trials 2, 3,4, and 6, but not during trials 1,5,7,8,9,10, ($p<0.05$). There were no significant differences between the DH-MUS and SAL groups on any trial.

The mean (±*SEM*) percentage of immobility expressed by the different infusion groups during the 30s trace intervals are shown in Fig. 3c. Inactivation of ventral hippocampus significantly attenuated the level of immobility across trials. A two-way ANOVA revealed a significant main effect for infusion condition ($F(2,46)= 22.71$, $p<0.001$), a significant main effect for trial ($F(18,489)= 28.38$, $p<0.001$, and a significant interaction between infusion condition and trial ($F(18,489)= 2.10$, $p=0.005$). Subsequent post hoc analyses (SNK) revealed that VH-MUS had significantly lower levels of immobility than DH-MUS and SAL during trials 2,3,4,5,6,7 and 10, ($p<0.05$). There was a significant difference between DH-MUS and VH-MUS during trials 8 and 9, but neither muscimol group differed from SAL. There was no group difference for trial 1, nor was there a difference between DH-MUS and SAL for any trial.

3.3.2. Expression of trace fear conditioning during testing

Data from only the first three trials of the testing session were used for statistical analysis, as they were least likely to be affected by extinction. The mean (±*SEM*) percentage of immobility exhibited by the different infusion groups during the 4min ITIs are shown in Fig. 4a. Muscimol inactivation of ventral, but not dorsal, hippocampus significantly attenuated the level of immobility across trials. A two-way ANOVA revealed

a significant main effect for infusion condition ($F_{(2,46)}= 13.85$, $p<0.001$), a significant main effect for trial ($F_{(4,143)}= 49.44$, $p<0.001$), and a significant interaction between infusion condition and trial ($F_{(4,143)}= 4.70$, $p=0.001$). Subsequent post hoc analyses (SNK) revealed that VH-MUS had significantly lower levels of immobility than the SAL and DH-MUS groups during trials 2 and 3, but not during trial 1 ($p<0.05$). There were no significant differences between the DH-MUS and SAL groups.

The mean ($\pm SEM$) percentage of immobility exhibited by the different infusion groups during the 20s auditory CS presentations are shown in Fig. 4b. Muscimol inactivation of ventral, but not dorsal, hippocampus significantly attenuated the level of immobility across trials. A two-way ANOVA revealed a significant main effect for infusion condition ($F_{(2,46)}= 14.10$, $p<0.001$), a significant main effect for trial ($F_{(4,143)}= 4.14$, $p=0.018$), but failed to reveal a significant interaction between infusion condition and trial ($F_{(4,143)}= .731$, $p=0.57$). Subsequent post hoc analyses (SNK) revealed that VH-MUS had significantly lower levels of immobility than DH-MUS and SAL during the first 3 trials ($p<0.05$). There were no significant differences between the DH-MUS and SAL groups.

The mean ($\pm SEM$) percentage of immobility exhibited by the different infusion groups during the 30s trace intervals are shown in Fig. 4b. Muscimol inactivation of ventral, but not dorsal, hippocampus significantly attenuated the level of immobility across trials. A two-way ANOVA with infusion condition as the between subjects factor and trial as the within subjects factor revealed a significant main effect for infusion condition ($F_{(2,46)}= 15.51$, $p<0.001$) but failed to reveal a significant effect for trial, ($F_{(4,143)}= 1.51$, $p=0.22$) or a significant interaction between infusion condition and trial ($F_{(4,143)}= 1.10$, $p=0.36$). Subsequent post hoc analyses (SNK) revealed that VH-MUS had significantly lower levels of immobility than DH-MUS and SAL during the first 3 trials, $p<0.05$. There were no significant differences between DH and SAL.

3.4. The effect of pre-testing inactivation of dorsal or ventral hippocampus on auditory trace fear conditioning

In order to measure the expression or maintenance of trace fear conditioning, animals were trained in the trace fear conditioning session without receiving any infusions beforehand. Twenty-four hours later they received infusions of muscimol or saline 30min prior to testing of trace fear conditioning. A subset of animals with cannulae targeted at ventral hippocampus (muscimol, n=10; saline, n=11) was trained in trace fear conditioning but not in the delayed reinforced alternation task. There were no significant differences between the saline or muscimol groups during the ITI, CS, or trace interval for conditioning or testing and therefore they were combined for analyses. In addition there were no differences between animals receiving saline infusions into dorsal or ventral hippocampus, and thus they were combined into one saline group (SAL, n=26). Final group sizes for subjects receiving pre-testing muscimol infusions into dorsal (DH-MUS) or ventral (VH-MUS) hippocampus were 10 and 16, respectively.

3.4.1. Acquisition of trace fear conditioning

In view of the fact that no infusions were conducted prior to the conditioning session, group differences in acquisition were neither expected nor found. The mean (±*SEM*) percentage of immobility exhibited by the different infusion groups during the 4min ITIs are shown in Fig. 5a. A two way ANOVA revealed there was a significant main effect for trial ($F_{(2,49)}$= 81.88, $p<0.0001$), but not a main effect for condition ($F_{(2,519)}$= 2.23, $p=0.118$) or a significant interaction between condition and trial ($F_{(2,519)}$= 1.43, $p=0.111$). Subsequent post hoc analyses revealed a significant difference between trial 1 and all other trials, between trial 2 and all other trials, and between trials 1 and 2, ($p<0.05$).

The mean (±*SEM*) percentage of immobility exhibited by the different infusion groups during the 20s auditory CS presentations are shown in Fig. 5b. A two way ANOVA revealed there was a significant main effect for trial ($F(2,49)$= 25.044, $p<0.0001$), but not a main effect for condition ($F(2,519)$= 0.699, $p=0.50$) or a significant interaction between condition and trial ($F(2,519)$= 1.064, $p=0.39$). Subsequent post hoc analyses (SNK) revealed a significant difference between trial 1 and all other trials, ($p<0.05$).

The mean (±*SEM*) percentage of immobility exhibited by the different infusion groups during the 30s trace intervals are shown in Fig. 5c. A two way ANOVA revealed there was a significant main effect for trial ($F(2,49)$= 45.87, $p<0.0001$), but not a main effect for condition ($F(2,519)$= 2.69, $p=0.07$) or a significant interaction between condition and trial ($F(2,519)$= 1.43, $p=0.10$). Subsequent post hoc analyses (SNK) revealed a significant difference between trial 1 and all other trials, ($p<0.05$).

3.4.2. Expression of trace fear conditioning during testing

Data from only the first three trials of the testing session were used for statistical analysis, as they were least likely to be affected by extinction. The mean *(±SEM)* percentage of immobility exhibited by the different infusion groups during the 4min ITIs are shown in Fig. 6a. Muscimol inactivation of ventral, but not dorsal, hippocampus significantly attenuated the level of immobility across trials. A two-way ANOVA revealed a significant main effect for infusion condition ($F(2,49)$= 65.2, $p<0.001$), a significant main effect for trial ($F(4,155)$= 81.3, $p<0.001$), and a significant interaction between infusion condition and trial ($F(4,155)$= 10.9, $p<0.001$). Subsequent post hoc analyses (SNK) revealed that VH-MUS had significantly lower levels of immobility than SAL and DH-MUS during the first 3 trials ($p<0.05$). There were no significant differences found between the DH-MUS and SAL groups.

The mean ($\pm SEM$) percentage of immobility exhibited by the different infusion groups during the 20s auditory CS presentations are shown in Fig. 6b. Muscimol inactivation of ventral, but not dorsal, hippocampus significantly attenuated the level of immobility across trials of the testing session. A two-way ANOVA revealed a significant main effect for infusion condition ($F(2,49)= 12.2$, $p<0.001$), a significant main effect for trial ($F(4,155)= 6.3$, $p=0.003$), but failed to reveal a significant interaction between infusion condition and trial, $F(4,155)= 0.55$, $p=0.69$). Subsequent post hoc analyses (SNK) revealed that VH-MUS had significantly lower levels of immobility than DH-MUS or SAL during the first 3 trials ($p<.05$). There were no significant differences found between the DH-MUS and SAL groups.

The mean ($\pm SEM$) percentage of immobility exhibited by the different infusion groups during the 30s trace intervals are shown in Fig. 6c. Muscimol inactivation of ventral, but not dorsal, hippocampus significantly attenuated the level of immobility across trials. A two-way ANOVA revealed a significant main effect for infusion condition ($F(2,49)= 103.46$, $p<0.001$), but failed to reveal a significant effect for trial ($F(4,155)= 0.57$, $p=0.57$) or a significant interaction between infusion condition and trial ($F(4,155)= 1.43$, $p=0.23$). Subsequent post hoc analyses (SNK) revealed VH-MUS had significantly lower levels of immobility than DH-MUS and SAL during the first 3 trials ($p<0.05$). There were no significant differences between the DH-MUS and SAL groups.

3.5. Locomotor Activity

Locomotor activity was assessed one week after trace fear conditioning in an open field during 3 consecutive days (i.e. Pre, Infusion, Post). Six saline and seven ventral hippocampus muscimol animals were excluded due to complications with their head stages. There were no statistically significant differences in ambulation or rearing between animals receiving saline infusions into dorsal or ventral hippocampus, or between groups receiving pre-training or pre-testing infusions. Therefore, data from

these animals were combined, resulting in final group sizes of 41, 26, and 21 for animals receiving infusions of saline into dorsal or ventral hippocampus (SAL), muscimol into ventral hippocampus (VH-MUS), and muscimol into dorsal hippocampus (DH-MUS), respectively.

The mean (±*SEM*) number of ambulation counts exhibited by different infusion groups is illustrated in Figure 7a. Muscimol infusions of ventral, but not dorsal, hippocampus resulted in hypoactivity. A two-way ANOVA revealed a main effect for day ($F_{(2,260)}$= 7.67, *p*=0.0006) and a significant interaction between condition and day ($F_{(2,260)}$= 12.06, *p*<0.0001), but failed to reveal a main effect for condition ($F_{(2,84)}$= 1.17, *p*=0.13). Subsequent post hoc analyses (SNK) revealed a significant difference between VH-MUS and both DH-MUS and SAL on the day of infusion only. There was no difference in ambulation between the DH-MUS and SAL groups.

The mean (±*SEM*) number of rearing counts exhibited by different infusion groups is illustrated in Figure 7b. Muscimol infusions of ventral, but not dorsal, hippocampus resulted in a reduced amount of rearing. A two-way ANOVA revealed a main effect for day ($F_{(2,260)}$= 6.28, *p*=0.0023) and a significant interaction between condition and day ($F_{(2,260)}$= 11.11, *p*<0.0001), but failed to reveal a main effect for condition ($F_{(2,84)}$= 1.43, *p*=0.245). Subsequent post hoc analyses revealed a significant difference between VH-MUS and both DH-MUS and SAL on the day of infusion only. There was no difference in rearing between the DH-MUS and SAL groups.

4. *Discussion*

The present study examined the contributions of dorsal and ventral hippocampus to delayed reinforced alternation and trace fear conditioning by using inactivation with the $GABA_A$ agonist muscimol. The reinforced alternation data indicate that dorsal, but not ventral, hippocampus plays a critical role in performance of this task. Conversely, data from pre-training and pre-testing inactivation indicate a critical role for ventral, but not dorsal, hippocampus in both the acquisition and maintenance of trace fear conditioning. These findings are discussed more fully below.

4.1. Dorsal, but not ventral, hippocampus is necessary for delayed reinforced alternation.

Muscimol inactivation of dorsal, but not ventral, hippocampus dramatically impaired performance in the delayed reinforced alternation task. This finding is consistent with numerous studies demonstrating that dorsal, but not ventral, hippocampus is critically involved in spatial learning (Moser & Moser, 1993; Bannerman et al., 1999; Richmond et al., 1999; Pothuizen et al., 2004; Mao & Robinson, 1998). However, it is important to note that rats can use either a place or a response strategy to learn and perform this T-maze task. Numerous studies have suggested that place learning depends on the hippocampus and response learning depends on the striatum (for review, see Gold, 2004). Therefore, while it seems likely that the observed deficits in reinforced alternation are due to the critical role of dorsal hippocampus in spatial learning, they may in fact be indicative of dorsal hippocampus involvement in nonspatial processing.

Regardless of whether the animals used a place or response strategy to learn delayed reinforced alternation, there was still a working memory component involved in the task. In order to correctly alternate on each trial, the rats had to remember which arm was previously visited over the 30s intertrial interval. Consistent with a role of dorsal hippocampus in working memory, muscimol inactivation of dorsal but not ventral

hippocampus impairs delayed non-matching-to-position performance (Mao & Robinson, 1998) and an operant delayed alternation task (Maruki et al., 2001). In addition, lesions of dorsal but not ventral hippocampus disrupted spatial reference and working memory in a radial arm maze task (Pothuizen et al., 2004). Together these studies suggest dorsal hippocampus may contribute more than ventral hippocampus in working memory tasks. Future studies will aim to determine whether or not the deficits in reinforced alternation are due to dorsal hippocampal involvement in spatial learning, working memory, or a combination of both processes.

4.2. Acquisition of trace fear conditioning depends on ventral, but not dorsal, hippocampus

Pre-training inactivation of ventral hippocampus attenuated freezing during acquisition and subsequent expression of trace fear conditioning, while pre-training inactivation of dorsal hippocampus had no effect during acquisition nor during testing 24hr later. These findings are consistent with previous findings from Yoon & Otto (2007) that pre-training excitotoxic lesions of ventral hippocampus impaired the acquisition and subsequent expression of trace fear conditioning while pre-training lesions of dorsal hippocampus had no effect. The fact that both excitotoxic lesions and temporary inactivation produced the same effects using the same paradigm provides consistent and reliable evidence of an important role for ventral, but not dorsal, hippocampus in the acquisition of trace fear conditioning.

These findings are partially consistent with those of Rogers et al. (2006), who found that pre-training lesions of dorsal CA1 had no effect on the acquisition or subsequent expression of trace fear conditioning. However, unlike in the present study, Roger's et al. (2006) found that only pre-training lesions of ventral hippocampus CA1 attenuated freezing during testing, but not during acquisition of trace fear conditioning. There were several differences between the studies that may account for these

discrepancies. The trace interval used in the present study and by Yoon & Otto (2007) was 30s, as opposed to 10s in Rogers et al. (2006). Another important distinction is that the lesions in Rogers et al. (2006) were selective to the CA1 subfield, whereas the lesions in Yoon & Otto (2007) included CA1, CA3, and dentate gyrus; given the likely spread of muscimol (Martin et al., 1991; Edeline et al., 2002), it is likely that ventral hippocampal CA3 was inactivated as well. It is possible that the observed differences in freezing during acquisition are due to more extensive damage or damage to CA3 or dentate gyrus specifically. Nonetheless, all three studies indicate the importance of ventral but not dorsal hippocampus to the acquisition of trace fear conditioning.

These findings are contradictory with a recent study by Burman et al. (2006) in which pre-training lesions of dorsal hippocampus impaired acquisition of trace but not delay fear conditioning. However, Burman et al. (2006) used electrolytic lesions and measured fear-potentiated startle, not freezing. Excitotoxic lesions of dorsal hippocampus also block fear-potentiated startle response in trace conditioning despite having no effect on contextual fear (Fendt, Fanselow & Koch, 2005). The inconsistency between studies measuring fear-potentiated startle and the aforementioned studies measuring freezing suggest that the dorsal hippocampus may serve a dissimilar role in expressing different behavioral responses to fear conditioning.

There are additional studies supporting the notion of dorsal hippocampal involvement in the acquisition of trace fear conditioning that are inconsistent with the data in the present study. For example, administration of the NMDA receptor antagonist DL-2-amino-5-phosponovaleric acid (APV) into the dorsal hippocampus of both rats and mice has been shown to impair the acquisition of trace fear conditioning (Chowdbury, Quinn & Fanselow, 2005; Misane et al., 2005). The fact that APV infusions into dorsal hippocampus block acquisition but neither lesions nor inactivation do suggest that dorsal hippocampus may participate in trace fear conditioning if it is intact during learning, but

that in its absence there is a compensatory mechanism that participates instead of dorsal hippocampus. Future studies will aim at determining to what extent dorsal hippocampus may contribute to the acquisition of trace fear conditioning when it is intact and what may be compensating for dorsal hippocampal involvement in this task when its integrity is compromised.

4.3. Expression of trace fear conditioning depends on ventral, but not dorsal, hippocampus

Pre-testing inactivation of ventral hippocampus dramatically attenuated the expression of trace fear conditioning during subsequent testing, while pre-testing inactivation of dorsal hippocampus had no effect. These results indicate that ventral, but not dorsal, hippocampus is critical for the expression, maintenance, or retrieval of trace fear conditioning.

The results of the current study are consistent with those of Yoon & Otto (2007) regarding the ventral hippocampus, but not the dorsal hippocampus. Together, these studies demonstrate that lesions or inactivation of ventral hippocampus after training, prior to testing, dramatically attenuate freezing in trace fear conditioning. However, unlike in the present study which found no effect of inactivation of dorsal hippocampus on the expression of trace fear conditioning, Yoon & Otto (2007) observed an attenuation in the expression of trace fear conditioning following post-training lesions of dorsal hippocampus. An important difference between the two studies is the training-testing interval. The animals in Yoon & Otto (2007) were lesioned 24 hours after conditioning and then tested 7 days after surgery. By contrast, the animals in the current study received infusions 30min prior to testing, which occurred 24 hours after conditioning. The other difference that may account for the discrepancy in the results is the difference between lesions and temporary inactivation. Lesions cause more extensive and permanent damage, while inactivation is temporary and less deleterious.

Therefore, it may be the case that lesions of dorsal hippocampus impaired expression of trace fear conditioning because it disrupted connections between dorsal and ventral hippocampus, whereas muscimol did not have as detrimental an effect. The present findings are also inconsistent with data from another study in which post-training lesions of dorsal hippocampus impaired the expression learned trace fear conditioning (Quinn et al., 2002). More research needs to be conducted in order to determine if dorsal hippocampus does in fact play a role in the expression of trace fear conditioning, or if the effects seen after lesions of dorsal hippocampus are due to a disruption dorsal hippocampal projections to ventral hippocampus.

4.4. Inactivation of ventral, but not dorsal, hippocampus induced hypoactivity

Lesions of the hippocampus have been shown to result in locomotor hyperactivity (Good & Honey, 1997). This is a key concern as hyperactivity may be mistaken for a lack of freezing. In the present study, animals who had received inactivation of either dorsal or ventral hippocampus were not hyperactive, as measured by ambulation counts in the open field. Instead, while muscimol infusions of dorsal hippocampus had no effect on ambulation or rearing, muscimol infusions of ventral hippocampus reduced both ambulation and rearing. This hypoactivity is in direct competition with the attenuation of freezing observed during trace fear conditioning. Therefore, the observed deficits in the present study are likely due to a learning deficit, not a performance deficit induced by muscimol.

4.5. Summary and implications

The present study demonstrates that there is a double dissociation between dorsal and ventral hippocampus. Specifically, dorsal hippocampus is critical for delayed reinforced alternation but neither the acquisition nor the expression of trace fear conditioning. On the other hand, ventral hippocampus is not necessary for delayed

reinforced alternation but is critical for both the acquisition and expression of trace fear conditioning.

While the general consensus is that the hippocampus is important for trace, but not delay, conditioning, it may be more complex. Pre-training, but not pre-pre-testing muscimol inactivation of ventral hippocampus produces a mild, but statistically significant attenuation in the acquisition of auditory delay conditioning (Maren & Holt, 2004). In the same study, Maren & Holt (2004) observed that unlike inactivation, post-training electrolytic lesions of ventral hippocampus impaired the expression of delay fear conditioning. However, these lesions caused extensive damage to the ventral subiculum as well as more limited damage to medial entorhinal cortex, ventral dentate gyrus, CA1, and CA3. In the current study both pre-training and pre-testing inactivation of ventral hippocampus produced impairments in trace fear conditioning, suggesting that the role of ventral hippocampus is different in delay fear conditioning than it is in trace fear conditioning. While ventral hippocampus appears to be important only for the acquisition and not the expression of delay fear conditioning, it seems to play a particularly important role in the expression, maintenance, or retrieval of trace fear memories. Dorsal hippocampus, on the other hand, does not appear to be critical for acquiring or maintaining trace fear memories.

The double dissociation observed in the present study may be due to the processing of different information in these regions, such as spatial in dorsal and trace conditioning in ventral hippocampus. Another key distinction is that reinforced alternation is an appetitive task, while trace fear conditioning is an aversive task. Therefore, because of its strong connections with amygdala, ventral hippocampus may contribute more to processing in aversive tasks, which is consistent with studies indicating an important role for ventral hippocampus in anxiety related behaviors (Bannerman et al., 2004). Dorsal hippocampus, on the other hand, may contribute more

to non-aversive trace conditioning. A recent study measuring pyramidal neuron activity during trace eye-blink conditioning in the rabbit observed a greater activation of dorsal hippocampus neurons compared to those in ventral hippocampus (Weible et al., 2006). Therefore, dorsal and ventral hippocampus may both contribute to processing the temporal and event-related information related to both tasks in the current study. However, their involvement in each task may differ due to their different anatomical connections. Future studies should focus on whether functional differences between the functions of dorsal and ventral hippocampus are due to differences in spatial learning vs. trace conditioning, trace conditioning vs. delay conditioning, or appetitive vs. aversive learning.

Nonetheless, the current study demonstrates that there is a double dissociation between dorsal and ventral hippocampus. Muscimol inactivation of dorsal hippocampus dramatically impaired performance of delayed reinforced alternation but in the same subjects affected neither the acquisition nor the expression of trace fear conditioning. Conversey, muscimol inactivation of ventral hippocampus had no effect on delayed alternation but in the same subjects dramatically impaired both the acquisition and expression of trace fear conditioning. The questions of how and why these subregions contribute differentially to these tasks remains to be explored.
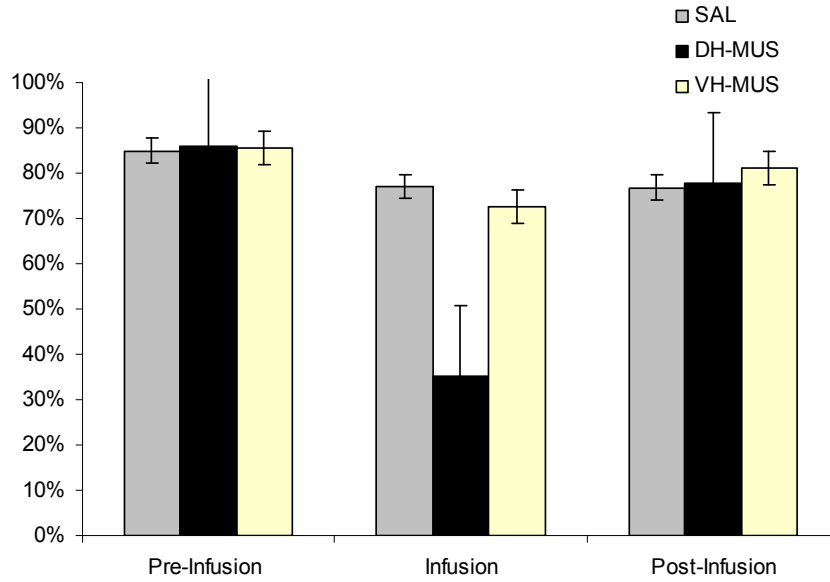
5. Appendix

Trace Fear Conditioning
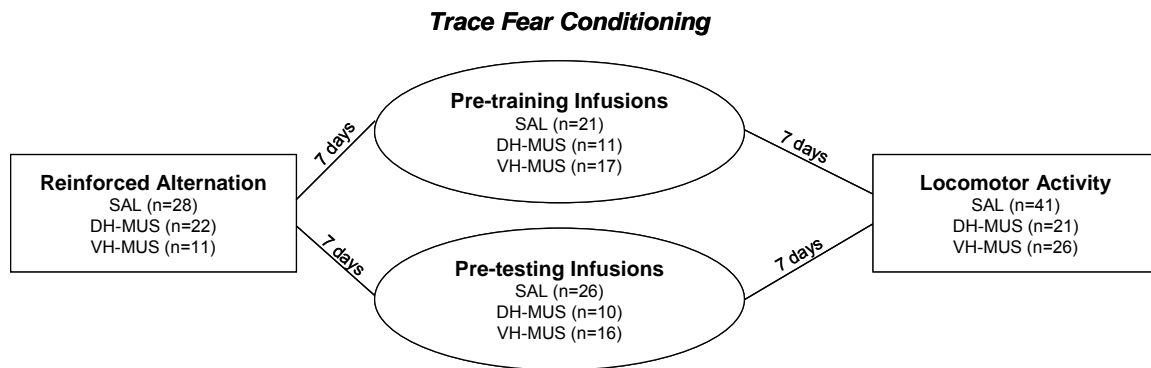
Table 1. Experimental Design and Group Sizes

29
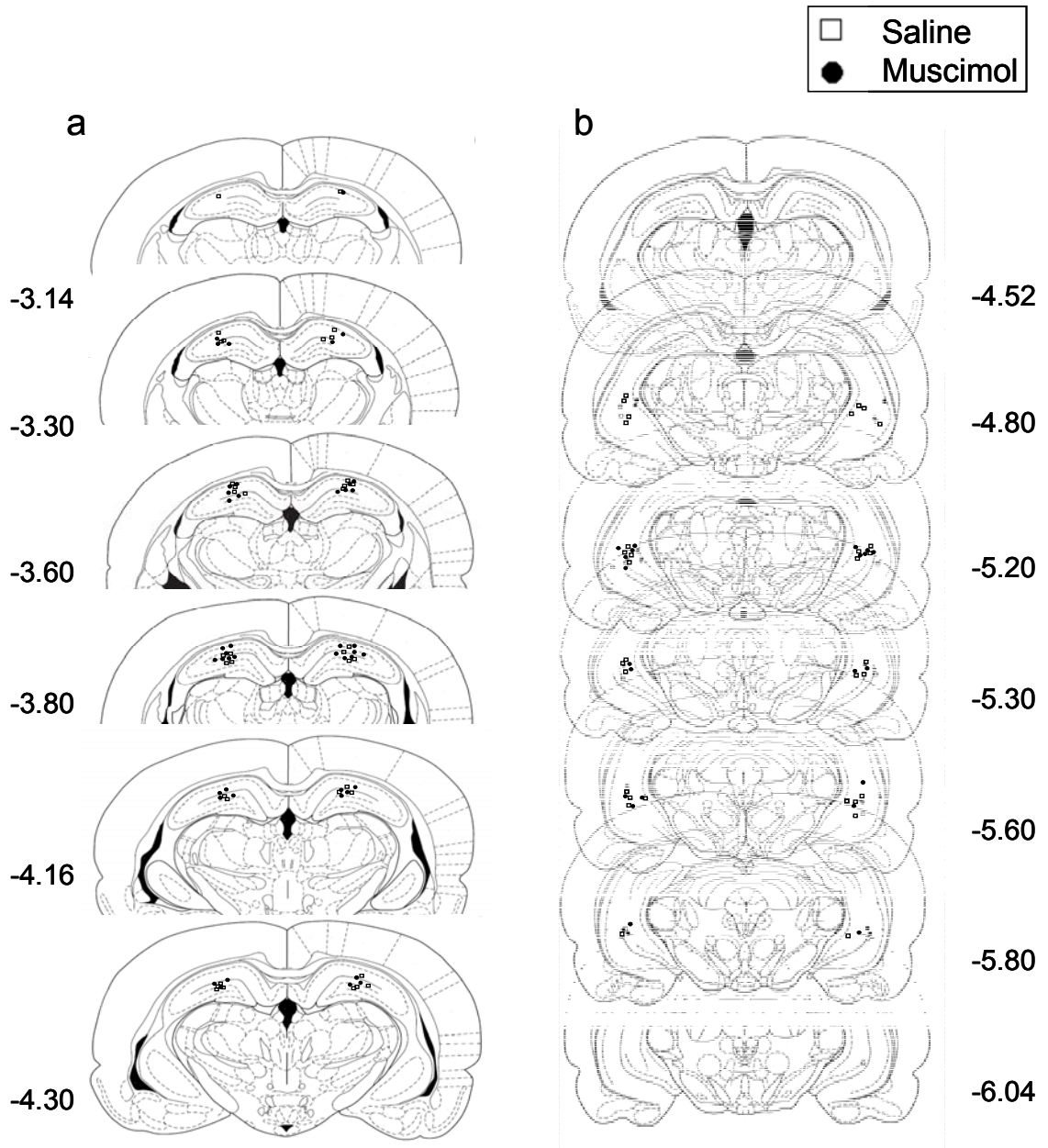
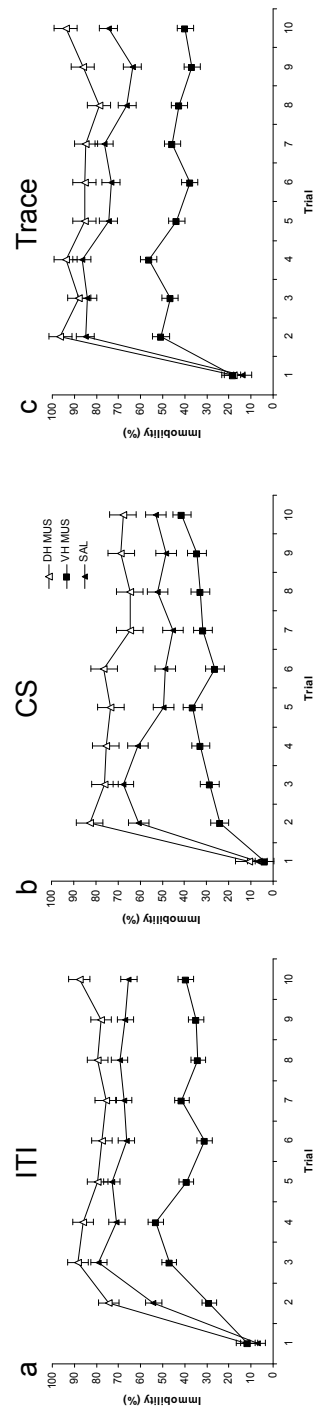**Figure 1**. Schematic representation of cannula placement in coronal sections of a) dorsal or b) ventral hippocampus.

Delayed Reinforced Alternation

Figure 2. Mean (±SEM) percentage of correct alternations for groups receiving infusions of muscimol or saline into dorsal or ventral hippocampus. Muscimol inactivation of dorsal hippocampus dramatically impaired reinforced alternation performance on the day of infusion only. There was no difference between subjects that received muscimol inactivation of ventral hippocampus and control subjects.
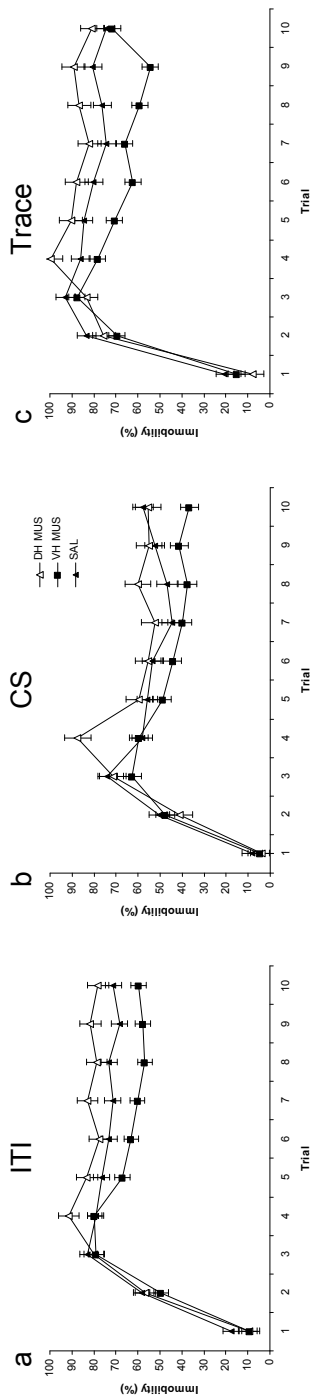
Figure 3. Mean (±*SEM*) percentage immobility during conditioning exhibited by different groups that received bilateral pre-training infusions of muscimol or saline into ventral or dorsal hippocampus. Subjects that received muscimol inactivation of ventral hippocampus exhibited significantly less immobility across trials than subjects that received muscimol inactivation of dorsal hippocampus or saline during the (a) 4min ITIs, (b) 20s tone presentations, and (c) 30s trace intervals. No differences were observed between the dorsal hippocampus muscimol group and controls.

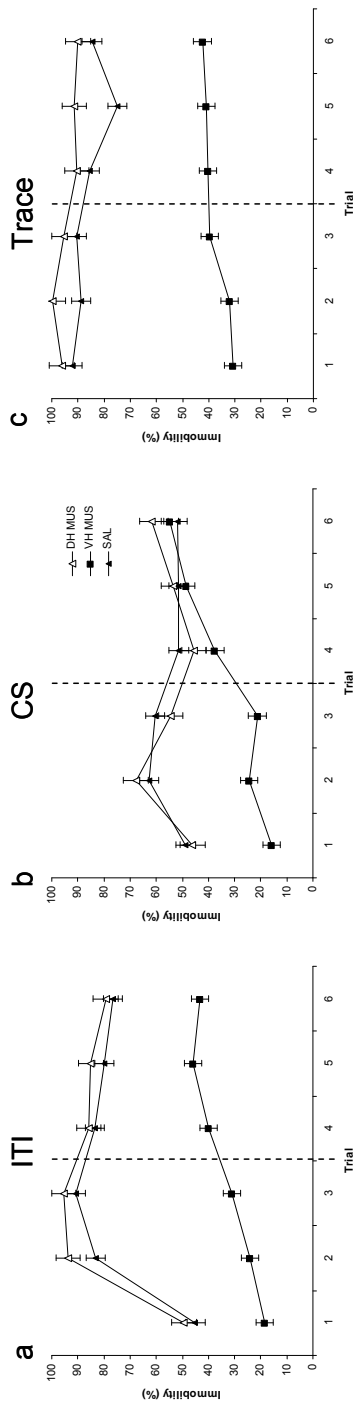# Pre-training Inactivation of Dorsal & Ventral HPC: Testing
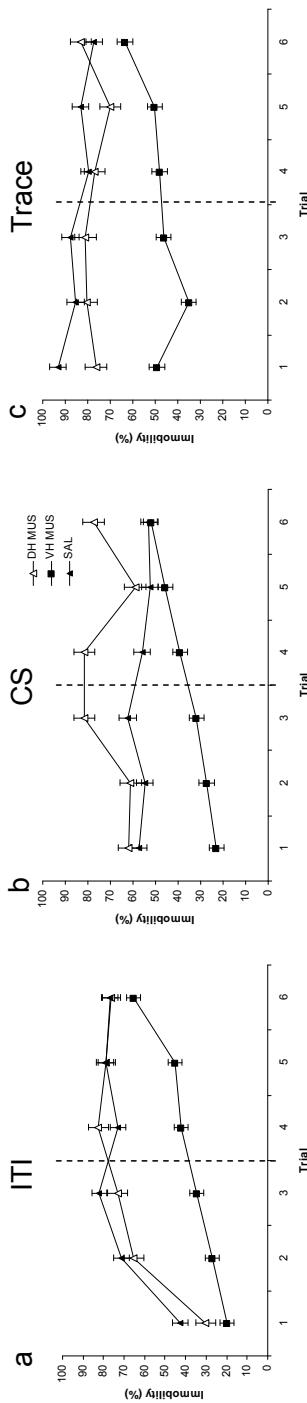
Figure 4. Mean (±*SEM*) percentage immobility during testing exhibited by different groups that received bilateral pre-training infusions of muscimol or saline into ventral or dorsal hippocampus. Subjects that received muscimol inactivation of ventral hippocampus exhibited significantly less immobility across trials than subjects that received muscimol inactivation of dorsal hippocampus or saline during the (a) 4min ITIs, (b) 20s tone presentations, and (c) 30s trace intervals. No differences were observed between the dorsal hippocampus muscimol group and controls. Only the data from the first 3 trials were used for statistical analyses.

# Pre-testing Inactivation of Dorsal & Ventral HPC: Conditioning

Figure 5. Mean *(±SEM)* percentage immobility during conditioning exhibited by different groups that received bilateral pre-testing infusions of muscimol or saline into ventral or dorsal hippocampus. There were no significant differences between groups in the level of immobility exhibited across trials during the (a) 4min ITIs, (b) 20s tone presentations, or (c) 30s trace intervals.

Pre-testing Inactivation of Dorsal & Ventral HPC: Testing

Figure 6. Mean (±*SEM*) percentage immobility during testing exhibited by different groups that received bilateral pre-testing infusions of muscimol or saline into ventral or dorsal hippocampus. Subjects that received muscimol inactivation of ventral hippocampus exhibited significantly less immobility across trials than subjects that received muscimol inactivation of dorsal hippocampus or saline during the (a) 4min ITIs, (b) 20s tone presentations, and (c) 30s trace intervals. No differences were observed between the dorsal hippocampus muscimol group and controls. Only the data from the first 3 trials were used for statistical analyses.
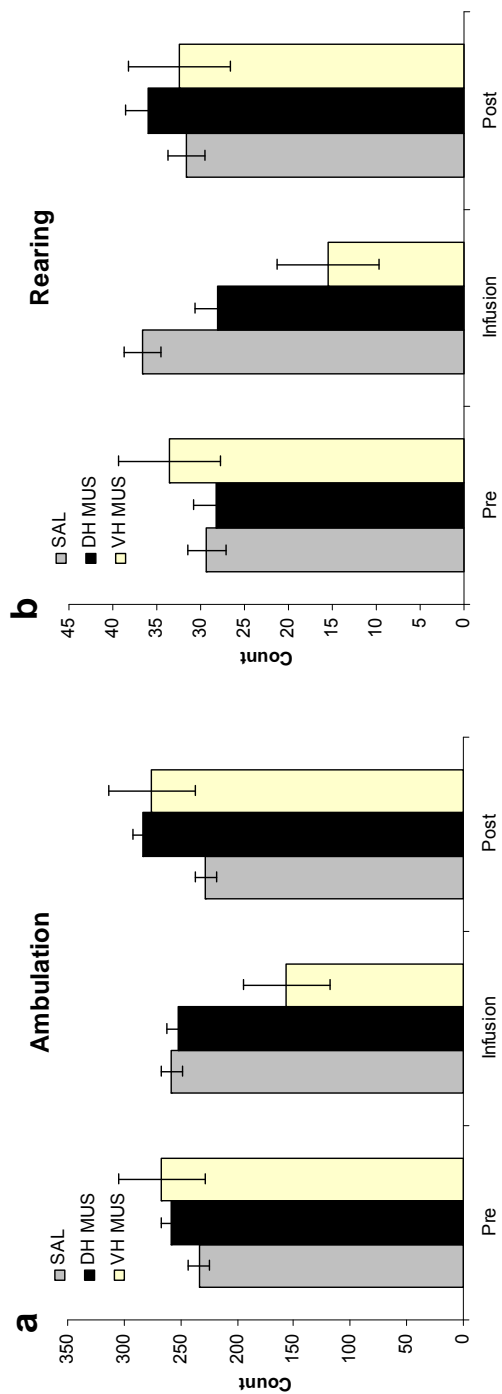
Figure 7. Mean (±*SEM*) number of a) ambulation and b) rearing counts during locomotor activity assessment. Muscimol infusions into ventral, but not dorsal, hippocampus resulted in a reduction of both ambulation of rearing.

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