TIME COURSE OF DORSAL AND VENTRAL HIPPOCAMPAL INVOLVEMENT IN THE EXPRESSION OF TRACE FEAR CONDITIONING

By

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A thesis submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Master of Science

Graduate Program in Psychology

written under the direction of

Timothy Otto, Ph.D

and approved by

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New Brunswick, New Jersey

January, 2013

ABSTRACT OF THE THESIS

Time course of dorsal and ventral hippocampal involvement in the expression of trace fear conditioning

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It is becoming increasingly clear that the time-dependent involvement of the hippocampus in the recall of acquired behaviors is more complicated than once thought. Several early studies demonstrated that hippocampal damage attenuates the expression of recent, but not remotely trained tasks. By contrast, an emerging body of evidence has shown that damage to, or inactivation of, the hippocampus impairs recall across a wide range of training-testing intervals. Collectively, these data suggest that the time course of hippocampal involvement in the storage or recall of previously-acquired memories differs according to hippocampal subregion and the particular learning task under consideration. The present study examined the contributions of dorsal (DH) and ventral (VH) hippocampus to the expression of previously-acquired trace fear conditioning, a form of Pavlovian conditioning in which the presentation of an initially neutral cue or cues and a subsequent aversive stimulus is separated by a no-stimulus (trace) interval. Specifically, either the GABA-$_A$ agonist muscimol or saline was

infused into the DH or VH prior to testing 1, 7, 28, or 42 days after trace fear conditioning. The results revealed a marked dissociation: pre-testing inactivation of DH failed to impair performance at any time-point, while pre-testing inactivation of VH impaired performance at all time-points. Importantly, pre-testing inactivation of VH had no effect on the performance during testing of previously-acquired delay conditioning. Collectively, these data suggest that VH, but not DH, remains a neuroanatomical locus critical to the recall or expression of trace fear conditioning over an extended period of time.

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1. Introduction

It was once widely accepted that damage to the hippocampal system resulted in a lasting anterograde inability to form certain types of memories, as well as a graded retrograde amnesia for some types of information learned prior to the surgical or traumatic event. These data were interpreted to suggest that the neuroanatomical locus for the expression of memories changes over time (reviewed in Squire, Clark, & Knowlton, 2001). More specifically, according to the standard model of systems consolidation, memories for hippocampal-dependent tasks are first encoded primarily in the hippocampus and also by neocortical networks, but initially rely on hippocampal integrity for retrieval (Dudai, 2004). According to this view, early dependence on the hippocampus suggests that not only does hippocampal activity normally overshadow neocortical activity during acquisition of many types of memories (Nadel & Wilner, 1980), but that, importantly, the memory trace is reorganized over a period of days to weeks such that the neocortex eventually maintains an independent representation of the memory and becomes capable of supporting recall without the hippocampus. Finally, the traditional views of systems consolidation suggest that, if systems consolidation for a hippocampal-dependent memory occurs, then post-acquisition hippocampal damage or inactivation should produce temporally-graded retrograde amnesia such that manipulations attenuate expression when administered recently, but not remotely, after training.

The first evidence for a temporally restricted role of the hippocampus in memory retention was observed by Kim and Fanselow (1992), who reported that non-discriminative contextual fear conditioning in rats was blocked by dorsal hippocampal lesions performed 1 day after training, but not 7, 14, or 28 days after training. Several reports followed which demonstrated a hippocampal temporal gradient for non-spatial discriminative contextual fear conditioning (Parsons & Otto, 2008; Parsons & Otto, 2010), social transmission of food preference (Clark et al., 2002; Winocur, 1990), trace eye-blink conditioning (Kim et al., 1995; Takahara et al., 2004), auditory trace fear conditioning (Quinn et al., 2008), object recognition and discrimination (Zola-Morgan & Squire, 1990), and two-choice discrimination (Cho et al., 1993). Recent data, however, suggest that the time-limited involvement of the hippocampal formation in memory consolidation is likely more complicated than once thought. Multiple studies have reported that disruption of the hippocampus can produce retrograde amnesia at both recent and remote time points—i.e. a flat gradient. This effect has been observed in contextual fear conditioning (Lehmann et al., 2007; Sutherland et al., 2008), object recognition (Gaskin et al., 2003), and performance in tasks requiring spatial navigation (Bolhuis, Stewart, & Forrest, 1994; Clark et al., 2005; Martin et al., 2005). These apparently discrepant data indicate clearly that considerably more study is required to fully characterize the role of the hippocampus in systems consolidation.

Although the reasons for the discrepancies both within and across laboratories described above are poorly understood, there are several behavioral and biological factors that may contribute to these inconsistencies. First, most studies exploring the

role of the hippocampus in systems consolidation have examined the effects of damage to either the majority of the hippocampal formation or selective damage to the dorsal hippocampus (DH) alone (see Sutherland et al., 2010 for review). By contrast, relatively few studies have selectively examined the contributions of the ventral hippocampus (VH) to recall in general and systems consolidation in particular (Czerniawski et al., 2009; Martin et al., 2005; Sutherland et al., 2008; Yoon & Otto, 2007). While DH and VH are extensively interconnected, these subdivisions receive notably different afferents and, moreover, the roles associated with the sources of these afferents are consistent with recent data demonstrating the dissociable roles of DH and VH in different forms of learning. Specifically, DH receives substantially denser sensory projections from the entorhinal cortex than VH (Pitkanen et al., 2000), and is critical for spatial learning (Czerniawski et al., 2009; Moser & Moser, 1998). Conversely VH, but not DH, establishes direct and reciprocal connections with the amygdala (Pitkanen et al., 2000), a brain area known to play a prominent role in emotional learning and anxiety states (LeDoux, 1995). Consistent with these neuroanatomical dissociations, we have recently reported a double dissociation between VH and DH in the acquisition and expression of auditory trace fear conditioning and spatial learning, respectively (Czerniawski et al., 2009; Czerniawski et al., 2011; Yoon & Otto, 2007). Collectively, these data suggest that the dorsal and ventral hippocampal subregions may contribute differently to the acquisition and subsequent expression of a variety of mnemonic processes, and that the apparently discrepant data reviewed above may reflect functional differences between DH and VH.

An additional factor that could contribute to these discrepancies is that the time-dependent role of the hippocampus in expression varies across tasks. That is, while the expression of some acquired behaviors may depend on part of the hippocampus for their expression initially, memory for other behaviors may require subregional hippocampal integrity indefinitely. Consistent with this view, there is a general agreement regarding the time-course of hippocampal involvement in the expression of two behavioral tasks. First, for odor or flavor guided tasks, multiple laboratories have reported that lesions of DH (Winocur, 1990) or the majority of the hippocampus (Clark et al., 2002; Tse et al., 2007; Winnocur et al., 2001) result in a temporally graded retrograde amnesia that lasts 1-2 days. On the other hand, it has been repeatedly demonstrated that lesions of DH (Clark et al., 2005; Martin et al., 2005), VH (Martin et al., 2005) or the hippocampus proper (Bolhuis, Stewart, & Forrest, 1994; Clark et al., 2005; Martin et al., 2005) attenuate performance in tasks requiring spatial navigation for at least 100 days after training.

Another factor that may contribute to the apparent discrepancies in the role of DH and VH in memory consolidation is the type of hippocampal manipulation employed. Specifically, lesions and temporary inactivation of the same brain area have in some instances been shown to elicit markedly different effects. For example, we have previously found that post-training excitotoxic lesions of DH performed 24hr after trace fear conditioning dramatically impair subsequent performance during testing (Yoon & Otto, 2007), while temporary inactivation of DH prior to testing has no effect. Similar differences between the effects of lesions and temporary inactivation have been

reported using other paradigms (Holt & Maren, 1999; Resstel et al., 2008). Correspondingly, while we have recently found that pre-testing inactivation of DH results in a temporally-graded retrograde amnesia for non-spatial contextual conditioning (Parsons & Otto, 2010), data from other laboratories has shown that pretesting lesions of DH result in a flat gradient for spatially guided contextual fear conditioning (Lehmann et al., 2007; Sutherland et al., 2008). Although most studies exploring the role of the hippocampus in systems consolidation have employed lesions (c.f. Sutherland et al., 2010), it is important to note that one advantage of temporary inactivation is that it allows for transient disruption of hippocampal activity while preserving the existing neural architecture.

The data reviewed above suggest that the DH and VH may contribute differentially to memory consolidation in a paradigm-dependent manner, and that lesions and pharmacological inactivation may themselves produce differential effects on memory retention and expression. Unfortunately, to date there has been no systematic examination of the potentially dissociable roles of DH and VH in systems consolidation using a single behavioral paradigm and hippocampal manipulation. With respect to trace fear conditioning, one study has evaluated the role of the hippocampus in maintaining expression in this paradigm (Quinn et al., 2008). Importantly, this study only examined the role of DH, and only at two disparate time points. Thus, in the present study, we examined the effect of regionally selective temporary inactivation of DH or VH 1, 7, 28, or 42 days after trace fear conditioning. The results revealed a marked

dissociation: pre-testing inactivation of VH impaired recall at all time-points whereas pre-testing inactivation of DH failed to impair recall at any time-point.

2. General Methods

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Rutgers University (Protocol #96-033).

2.1 Subjects

173 naïve male Sprague Dawley rats (Harlan, Indianapolis IL) weighing between 249-300 grams were used as subjects. Animals were individually housed in plastic cages in an environmentally regulated vivarium maintained on a 12 hour light/dark schedule. All behavioral procedures occurred during the light phase. 5 days prior to surgery, subjects were handled for two minutes a day. Subjects were provided food and water ad libitum.

2.2 Experimental Apparatus

Training sessions occurred in one of two identical conditioning chambers (30 x 24 x 42 cm), each housed in a sound attenuating enclosure (56 x 41 x 42cm). A one-way glass window was installed on the front of the attenuating enclosure through which experimenters could observe subjects' behavior. Two opposing walls of each conditioning chamber were composed of aluminum while the adjacent walls were comprised of transparent Plexiglas. The floors consisted of 16 evenly spaced (1.9cm) steel rods connected to a shock generator (model H13-15, Coulbourn Instruments, Allentown, PA). A wall-mounted speaker was affixed 14cm above the floor of the chamber. Illumination was provided by a single 24V light bulb installed above the

speaker. Between training sessions, the chamber was cleaned with a cage cleaning solution (quatricide).

Testing sessions took place in a novel chamber located in a distal room. While the composition and dimensions of this chamber were the same as the training apparatus, the testing chamber was distinguished by a single black and white diagonally striped wall and a solid black Plexiglas floor placed on top the steel rods. Additionally, the chamber was cleaned with a 10% ethyl alcohol solution between testing sessions.

2.3 Surgical methods

Bilateral cannulae were surgically implanted into the dorsal or ventral hippocampus. Rats were first anesthetized with a mixture of Ketamine (80mg/ml) and Xylazine (12mg/ml). Their heads were then shaved, fixed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA), and cleaned with Betadine and alcohol. Marcaine (0.2ml, 25%) was injected subcutaneously at several sites along the midline and served as a local anesthetic and vasoconstrictor. The scalp was incised along the midline and retracted, and the skull exposed. Two small bilateral burr holes were drilled through the skull at sites overlying DH (3.8mm posterior to bregma and 2.5 mm lateral to midline) or VH (5.2mm posterior to bregma and 5.0mm lateral to midline). Next, four burr holes were drilled around the initial holes and jewelers screws were affixed in them. For subjects receiving infusions into DH, guide cannulae (22-gauge, 11mm, Plastics1, Roanake, VA) were implanted bilaterally such that the cannula tips were situated 2.2mm ventral to dura. For subjects receiving infusions into VH, guide cannulae were implanted

bilaterally with the cannula tips situated 5.5mm ventral to dura. Dental acrylic was then applied around the cannulae and the screws to affix the cannulae in place. Obdurators were then inserted through the guide cannulae and held in place with a cap. The incision was closed with stainless steel staples and obdurators were placed inside guide cannulae. Subjects were returned to their home cages and closely monitored for 7 days before training began. Subjects were handled in the infusion room for 5 minutes a day, for 5 consecutive days prior to behavioral procedures. During this time, the infusion pump was turned on and their guide cannulae were cleaned in order to acclimate subjects to the environment and procedures associated with infusions.

2.4 Infusion Methods

The infusion methods used in the present study were identical to those used previously in our laboratory (Czerniawski et al., 2009; Parsons & Otto, 2008, 2010). Briefly, subjects received infusions 30 minutes prior to training and testing sessions. Each subject was transported to the infusion room in a clear plastic container and their obturators removed. A 30 gauge infusion cannula, connected via polyethylene tubing to a 10 µl Hamilton syringe affixed in an infusion pump (Harvard Apparatus, South Natick, MA), was inserted into each guide cannula. The tip of the infusion cannula protruded 1mm below the tip of the guide cannula. All subjects received infusions of physiological saline (0.9%) prior to training but, prior to testing, subjects received infusions of either saline or muscimol (1µg/µl Sigma, St Louis, MO; counterbalanced across subjects). For

subjects receiving infusions into DH, .25µl was infused into each hemisphere for a total volume of .5µl. For subjects receiving infusions into VH, .5µl was infused into each hemisphere for a total volume of 1.0µl. Infusions occurred at a rate of .25µl/min. Infusion cannulae were left in place for 2 minutes after infusion to allow for diffusion. Following this, the infusion cannulae were removed and replaced by the obturators. Subjects were then returned to the transport container for 28 minutes before behavioral procedures.

2.5 Histological Methods

Following testing, subjects were deeply anesthetized with sodium-pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 100ml of saline (0.9 %), then 100mL of a 10% buffered formalin solution. Brains were extracted and stored in a 30% formalin/sucrose solution for at least 72 hours. The tissue was then frozen and sliced on a cryostat into 50um thick sections, which were then mounted onto glass slides and stained with cresyl violet. Cannula placement was verified under a light microscope.

3. Behavioral Methods

3.1 Experimental Design

All subjects that received auditory trace fear conditioning received infusions of saline 30 minutes prior to training. They were then divided into separate groups that would later receive either muscimol (MUS) or (SAL) infusions prior to testing 1, 7, 28, or

42 days after training. These intervals were chosen based on previous data from our laboratory that demonstrated a time-limited role for the hippocampus in recall of previously-established fear memory (Parsons & Otto, 2008; Parsons & Otto 2010). A final group of subjects was implanted with cannulae into VH and received auditory delay fear conditioning. They were infused with saline 30 minutes prior to training and then, 24 hours later, subjects were infused with either saline or muscimol 30 minutes prior to testing.

3.2 Auditory trace and delay fear conditioning

Conditioning for all subjects occurred 7-10 days after surgery. Trace and delay conditioning paradigms were similar to those used previously in our laboratory (Czerniawski et al., 2009). Briefly, conditioning in both paradigms took place in a single session consisting of 10 pairings of an explicit auditory stimulus (CS) (20 sec, 3.9 kHZ, 80 db) with scrambled footshock (US) (2 sec, 0.6 mA). During trace conditioning, there was a 30 sec interval between the offset of the CS and the onset of the US. By contrast, during delay conditioning, the CS co-terminated with the US. There was a 4 minute acclimation period before the presentation of the first tone, and all subsequent trials were separated by a 4 minute ITI.

3.3 Testing following a 1, 7, 28 or 42 day training-testing interval

Testing for trace fear conditioning occurred 1, 7, 28, or 42 days after training while testing for delay fear conditioning occurred 1 day after training. In both paradigms, the procedural parameters during testing were identical to those during training, except no shock was delivered and there were 3 trials instead of 10.

3.4 Behavioral measures

Conditioned fear during testing sessions was assessed by quantifying subjects' display of the freezing response characterized by a rigid posture and cessation of all movement except that required for respiration (Fanselow, 1980). Freezing was recorded by a trained observer who depressed a hand switch connected to a computer whenever freezing was observed. These data were later transformed into the percent time spent freezing during periods corresponding to the ITI, CS, and trace interval for each trial. Comparisons for the ITI were made between the first trial, before the tone was presented (used as a baseline measure of freezing), and all subsequent trials. Comparisons for the CS and trace periods were collapsed across trials.

3.5 Statistical analysis.

The primary analysis for all three experiments was a two-way analysis of variance (ANOVA). For subjects receiving trace conditioning, infusion condition and

testing time-point were the two principal factors. The alpha level was set at .05 for all statistical analyses. When necessary, the Student-Newman-Keuls test was applied as a Post-hoc analysis.

4. Results

4.1 Histology

Figure 1 illustrates cannula placement for all subjects included in analyses. Cannula placement was verified prior to statistical analysis. For subjects with cannulae in DH, the tip of the infusion cannula was required to be between 3.14mm and 4.3mm posterior to bregma; and between the pryramidal cell layer of CA1 and the molecular layer of the dentate gyrus. For subjects with cannulae in VH, the tip of the infusion cannula was required to be between 4.52mm and 6.04mm posterior to bregma; and between ventral CA1 and dentate gyrus. Thirty-six subjects were rejected because of misplaced cannulae. The final sample size for each of the groups was: (VH-1day: MUS = 8, SAL = 9), (VH-7days: MUS = 6, SAL = 7), (VH-28days: MUS = 7,-SAL = 6), (VH-42days: MUS = 6,SAL = 6), (DH-1day: MUS = 11, SAL = 10), (DH-7days: MUS = 7, SAL = 6), (DH-28days: MUS = 6, SAL = 7), (DH-42days: MUS = 9, SAL = 8), (VH-delay-MUS = 8, SAL = 9).

4.2 Pre-testing inactivation of VH impairs the expression of previously-acquired trace fear conditioning at all training-testing intervals.

The effect of pre-testing infusion of muscimol or saline into VH on the expression of trace fear conditioning at all training-testing intervals is illustrated in Figure 2. A two way ANOVA performed on freezing behavior during the initial ITI (i.e. baseline period; Figure 2a, left) revealed a main effect of infusion condition, ($F(1,47) = 6.211$, $p=0.016$),

but there was no effect of training-testing interval ($F(3,47) = 2.394$, $p=0.080$) nor was there a significant interaction between the factors ($F(3,47)= 1.561$, $p=0.211$). Despite the fact that the interaction between infusion condition and training-testing interval was insignificant, *post hoc* comparisons (SNK) were conducted to further explore potential differences between conditions; these analyses revealed that there was a significant difference between muscimol and saline subjects during the 1 day training-testing interval only. Data for the second and third ITI were averaged; a two way ANOVA on freezing during this time (Figure 2a, right) revealed a main effect of infusion ($F(1,47) = 33.302$, $p < 0.001$), but not for interval duration ($F(3,47) = 0.316$, $p = 0.814$); the interaction between infusion and interval was not significant ($F(3,47) = 1.579$, $p = 0.207$). *Post-hoc* comparisons (SNK) revealed that the saline- and muscimol-treated subjects differed in freezing behavior at all training-testing intervals. For the testing period during which the CS was presented (Figure 2b), a two way ANOVA did not reveal a main effect of infusion ($F(1,47) = 1.472$, $p=0.231$) or testing interval ($F(3,47) = 1.200$, $p=0.320$), nor an interaction between the variables ($F(3,47) = 2.148$, $p=0.107$). For the trace interval (Figure 2c), a two way ANOVA revealed a main effect of infusion condition ($F(1,47) = 89.140$, $p < 0.001$), but not of testing interval ($F(3,47)= 1.534$, $p=0.218$), as well as a significant interaction between infusion and interval ($F(3,47) = 4.488$, $p = 0.008$). *Post-hoc* comparisons (Student–Newman–Keuls) revealed a significant difference between SAL and MUS conditions at all training-testing time points.

4.3 Pre-testing inactivation of VH prior to testing has no effect on expression of delay fear conditioning.

The effect of pre-training infusion of muscimol or saline into VH prior to testing in delay fear conditioning is illustrated in Figure 3. A two way ANOVA was performed comparing freezing for both groups during the first ITI (baseline period) vs the second and third ITI (Figure 3a). The results revealed a main effect of infusion ($F(1,30) = 6.756$, $p = 0.014$) and ITI period ($F(1,30) = 100.313$, $p < 0.001$), as well as an interaction between infusion and ITI ($F(1,30) = 5.812$, $p = 0.022$). *Post hoc* comparisons (SNK) revealed that saline and muscimol treatments differed during the second and third ITI only. Finally, a two-tailed *t*-test compared average freezing for the two groups during the CS presentation (Figure 3b). The results revealed no significant difference between subjects receiving infusions of saline or muscimol ($t(15) = 0.452$, $p = 0.657$) (Figure 3b).

4.4 Pre-testing inactivation of DH had no effect on expression of conditioned fear at any training-testing interval.

The effect of pre-training infusion of muscimol or saline into DH on the expression of trace fear conditioning at all training-testing intervals is illustrated in Figure 4. A two way ANOVA performed on freezing behavior during the initial ITI (Figure 4a, left) did not reveal a main effect of infusion condition ($F(1,56) = 1.653$, $p=0.204$) or training-testing interval ($F(3,56)= 0.715$, $p=0.547$), nor did it reveal an interaction between the factors ($F(3,56)=1.273$, $p=0.292$). Data for the second and third ITI were

averaged; a two way ANOVA performed on freezing during this time (Figure 4a, right) revealed a main effect of interval duration ($F(1,56) = 3.692$, $p = 0.017$), but not for infusion ($F(3,56) = 0.0000984$, $p = 0.992$) or the interaction between infusion and interval ($F(3,56) = 1.241$, $p = 0.303$). For the CS period (Figure 4b), a two-way ANOVA revealed no main effects of infusion ($F(1,56) = 0.0122$, $p = 0.916$) or interval ($F(3,56) = 1.314$, $p = 0.279$), and no interaction was noted ($F(3,56) = 2.081$, $p = 0.113$) (Figure 4b). A final two-way ANOVA examined the freezing during the trace interval (Figure 4c). The results revealed a main effect of training-testing interval ($F(3,56) = 3.525$, $p = 0.021$) but did not reveal a main effect of infusion condition ($F(3,56) = 0.607$, $p = 0.439$) or an interaction of the variables ($F(3,56) = 2.177$, $p = 0.101$).

5. Discussion

5.1 Pre-testing inactivation of VH impaired the expression of trace fear conditioning at all training-testing intervals, but had no effect on the expression of delay fear conditioning.

As described above and illustrated in Figures 2 and 3, pre-testing inactivation of the ventral hippocampus impaired the expression of previously-acquired auditory trace fear conditioning across all training-testing intervals, but did not affect expression of previously-acquired fear in delay conditioning. These results are consistent with those from previous studies in our laboratory (Czerniawski et al., 2009; Yoon & Otto, 2007) and others (e.g. Burman et al., 2006) which strongly suggest that VH is critical to the expression of trace fear conditioning shortly after training, and extend those findings to suggest that integrity of the VH remains necessary for expression for at least 42 days after learning. We measured expression of previously acquired trace fear conditioning as the percent of total time subjects froze during the trace interval and during the second and third ITI. Subjects infused with saline froze robustly during the trace interval and second and third ITI, but not during the presentation of the CS or during the initial ITI. This pattern of freezing behavior of control subjects is consistent with previous data from our laboratory (Czerniawski et al., 2009; Yoon & Otto, 2007) and likely indicates that the trace interval, rather than the CS, best predicts the onset of the US during trace fear conditioning. It is also important to note that the deficit caused by inactivation diminished in a progressive—yet statistically insignificant—manor as the training-testing

interval increased. It is possible that at more remote time points VH may no longer be necessary for recall.

By necessity, the hippocampus' role in trace fear conditioning depends on interactions with the amygdala, which has been implicated in the acquisition and expression of most types of fear conditioning (LeDoux, 1995). Ventral CA1 makes direct reciprocal connections with the basal, accessory basal, and amygdalohippocampal transition area of the amygdala (Canteras & Swanson, 1992; Pitkanen et al., 2000), whereas DH can only interact with the amydala via its connections with VH. Inactivation or damage to VH prior to testing in trace conditioning has been observed to dramatically impair expression of acquired fear (Burman et al., 2006; Czerniawski et al., 2009; Yoon & Otto, 2007). Therefore, from a neuroanatomical perspective, VH, more so than DH, is likely to participate significantly in forming associations between explicit stimuli and aversive events (Bannerman et al., 2004)

Evidence from previous studies suggests that infusions of muscimol in the volumes and concentration used in the present study can exert inhibitory effects up to 2mm from the site of infusion (Edeline et al., 2002; Martin, 1991). So, while it is likely that significant portions of ventral CA1, CA3 and DG were inactivated, there is some possibility that muscimol may have diffused as far as the amygdala, and thus that the behavioral deficits described here could reflect inactivation of the amygdala. However, our data suggest this is likely not the case. Specifically, pre-testing inactivation of VH had no effect on the expression of delay fear conditioning, which has been shown to

depend critically on integrity of the amygdala (Fanselow, 2005; LeDoux, 1995). Although one study has demonstrated that pre-testing lesions of VH can attenuate expression of delay fear conditioning (Burman et al., 2006), those lesions extended beyond the hippocampal formation into portions of the medial temporal lobe including the entorhinal cortex. Thus the data presented here suggest that muscimol infused into VH likely did not diffuse as far as the amygdala, and that the performance deficit observed in trace conditioning was due to inactivation of VH. While it is also possible that muscimol infusions could produce hyperactivity which would conflict with the freezing response, previously published data from our laboratory suggests that the concentration and volume of muscimol infused here has no such effect (Czerniawski et al., 2009). Furthermore, in the present study, we observed a stark contrast between subjects receiving muscimol infusions into VH prior to testing in trace fear conditioning compared to subjects infused prior to testing in delay fear conditioning. The former group of subjects displayed low levels of freezing throughout the testing session whereas the latter group exhibited low levels of freezing during the initial ITI, but froze robustly to the presentation of the CS and during subsequent ITIs. Thus it appears that the freezing deficits observed following infusion of muscimol in VH in the present study were likely due to a deficit in recall or expression rather than hyperactivity or an inability to express the freezing response.

5.2 Pre-testing inactivation of DH has no effect on the expression of trace fear conditioning 1,7,28, or 42 days after conditioning

As described previously and illustrated in Figure 4, pre-testing inactivation of the dorsal hippocampus did not attenuate the expression of previously-acquired trace fear conditioning at any of the training-testing intervals assessed. These results are consistent with previous data from our laboratory demonstrating that neither pre-training nor pre-testing inactivation of DH 1 or 8 days after conditioning affects the acquisition or expression of trace fear conditioning (Czerniawski et al., 2009). In contrast to the present data, previous studies from our laboratory (Yoon & Otto, 2007) and others (Quinn et al., 2008) have demonstrated that post-training lesions of DH attenuated the expression of trace conditioning soon after training. It is possible that the discrepancy between these earlier studies and the present data are due in part to differential effects of lesions and temporary inactivation. Although excitotoxic lesions preserve fibers of passage, cell bodies are extensively damaged (Zapeda et al., 2012), and it is likely that the surviving neurons which surround the damaged area function differently after the lesion (Majchrzak & Di Scala, 2000). Furthermore, several studies have indicated that functional recovery can occur following lesions in general (Casamenti et al., 1988; Gardiner et al., 1987; Wenk & Olton, 1984) and focal lesions of the hippocampus in particular (Zapeda et al., 2012). It has been suggested that this functional recovery may result from a reorganization of the surviving neural population (Burns et al., 2009). Temporary inactivation, by contrast, likely does not elicit structural reorganization because its effects are only transient, and selectively alter receptor

activity. As such, muscimol inactivation has advantages over lesions because it leaves the existing neural architecture intact and thus preserves normal neural activity after treatment. Data from our laboratory (Czerniawski et al., 2009; Yoon & Otto, 2007) suggest that there is likely an important dissociation between the effects of lesions and inactivation with respect to the time-limited involvement of the hippocampus. By contrast, there is data from other laboratories which demonstrates similar effects of DH lesions and inactivation on recall in the Morris Water Maze (compare Broadbent, Squire, & Clark, 2006 with Martin et al., 2005). It is possible that differential effects of lesions and inactivation may occur in some tasks but not others. The mechanisms that drive these dissociations remain to be fully characterized, however, and are beyond the scope of the current investigation.

We have previously reported that pre-training inactivation of DH has no effect on the acquisition of trace fear conditioning (Czerniawski et al., 2009). By contrast, we (Czerniaswki et al., 2011) and others (e.g. Misane et al., 2005) have found that NMDA receptor antagonism within DH prior to training blocks the acquisition of trace fear conditioning. These data collectively suggest that DH may normally participate in both the acquisition and consolidation of trace fear conditioning, but is not necessary for either acquisition or recall. If this is the case, these data may suggest that, in trace fear conditioning, learning-induced plasticity in DH may reflect a process whereby the memory trace is temporarily distributed across the hippocampus, but eventually consolidates in VH.

5.3 Implications of the present data to contrasting theories of consolidation

To our knowledge, this is the first systematic investigation of the dissociable roles of dorsal and ventral hippocampus in the long term maintenance of trace fear conditioning. The results add to an emerging body of evidence suggesting that the time-limited role of the hippocampus in memory consolidation likely varies across hippocampal subregions, and may account for at least a subset of seemingly contradictory results (see Sutherland et al., 2010 for review). The data presented here are consistent with the view that systems consolidation, insofar as it refers to reassignment of the neural architecture necessary for memory storage and/or recall, likely occurs only under specific circumstances. It is becoming increasingly clear that observations of systems consolidation are limited to certain learning tasks. Although the present data suggest that extra-hippocampal systems consolidation does not occur following trace fear conditioning, previous data from our laboratory (Parsons & Otto, 2010) do suggest that extra-hippocampal consolidation occurs following non-spatial contextual discrimination.

There are other factors not addressed by the present study that may also contribute to systems consolidation. For instance, training that occurs over multiple distributed sessions can mitigate the necessity of the hippocampus for recall (Lehmann et al., 2009; Winocur et al., 2005). This idea was first suggested by Nadel and Moscovitch (1997) in their description of the Multiple Trace Theory of hippocampal-dependent systems consolidation. They argued that the maintenance of episodic memories requires

hippocampal activity indefinitely, but that repeated training could support recall independent of the hippocampus by facilitating acquisition by extra-hippocampal circuits which nominally participate in a task, but are overshadowed by hippocampal activity. This effect has been noted following repeated training in contextual fear conditioning (Lehmann et al., 2009) and spatial navigation (Winocur et al., 2005). Importantly, these data support the argument that memory traces can be strengthened and consolidated outside the hippocampus, yet do not support the argument for an inherently time-limited role of the hippocampus in the expression of acquired behaviors. Future investigations may examine whether repeated training in trace fear conditioning can support recall independent of the ventral hippocampus.

It is important to note that neither Multiple Trace Theory nor the data presented here can account for data demonstrating a time-limited role for the hippocampus after single training sessions. It has been suggested that, in tasks where temporally graded retrograde amnesia is detected, the hippocampal trace may undergo recurrent activation during periods of implicit processing; projections from these neurons may stimulate plasticity in neocortical neurons, encouraging the development of a robust neocortical trace (Dudai, 2004). This view may be supported by studies which have indicated that patterned replay can occur for hours (e.g. Karlsson & Frank, 2009) and up to a day (Kudirmoti et al., 1999) after training. If a short period of recurrent activation is necessary to consolidate an extra-hippocampal trace for some tasks, this is consistent with the observation that span of temporally graded amnesia in flavor/odor cued conditioning is 1-2 days (Clark et al., 2002; Tse et al., 2007; Winocur et al., 2001;

Winocur, 1990). These results are not congruent, however, with data from our laboratory which demonstrated time-limited retrograde amnesia up to 28 days in odor guided discriminative contextual conditioning (Parsons & Otto, 2010). Despite these discrepancies, these findings are equally supportive of the notion that, while the hippocampus may participate in the maintenance of some forms of memory, extrahippocampal circuits may also develop sufficient strength to support recall independent of the hippocampus. In contrast to traditional models of systems consolidation, these data do not necessarily support the argument that hippocampal-dependent memory traces relocate outside the hippocampus under all circumstances. Rather, they suggest that, for some tasks, extra-hippocampal circuits may support recall through their own recurrent activation, independent of additional training.

5.4 Summary

Collectively, our data support a key role for the ventral, but not dorsal, hippocampus in supporting recall of previously acquired trace fear conditioning for an extended period of time after learning. These results are consistent with a model of consolidation where, at least for many tasks, the hippocampus remains critical to the storage or recall of some forms of memory in a subregion-specific manner. This does not preclude consolidation from occurring in systems outside the hippocampus. Rather, the extent of extra-hippocampal consolidation likely varies according to specific behaviors and specific training paradigms.

6. Appendix

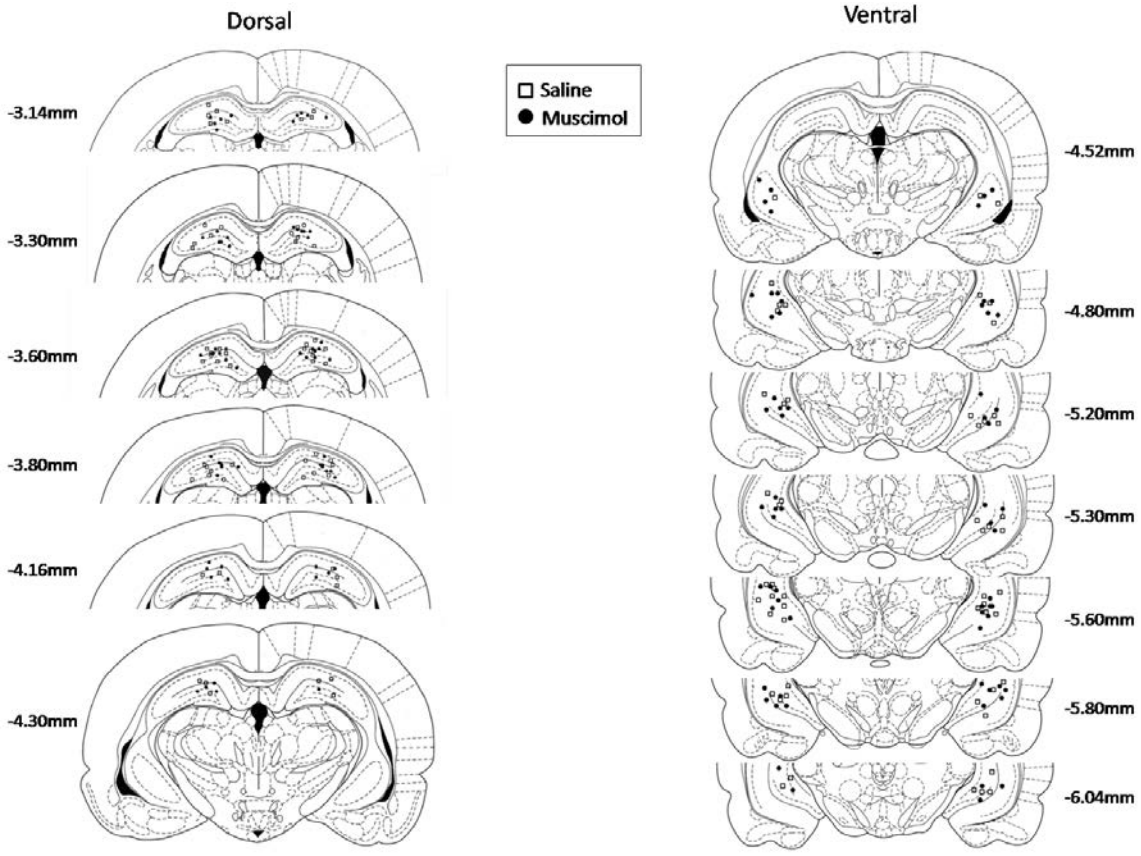

Figure 1. Cannula placement in the hippocampus. Coronal sections of a) dorsal or b) ventral hippocampus; location of cannulae tips are indicated by black squares or white boxes.

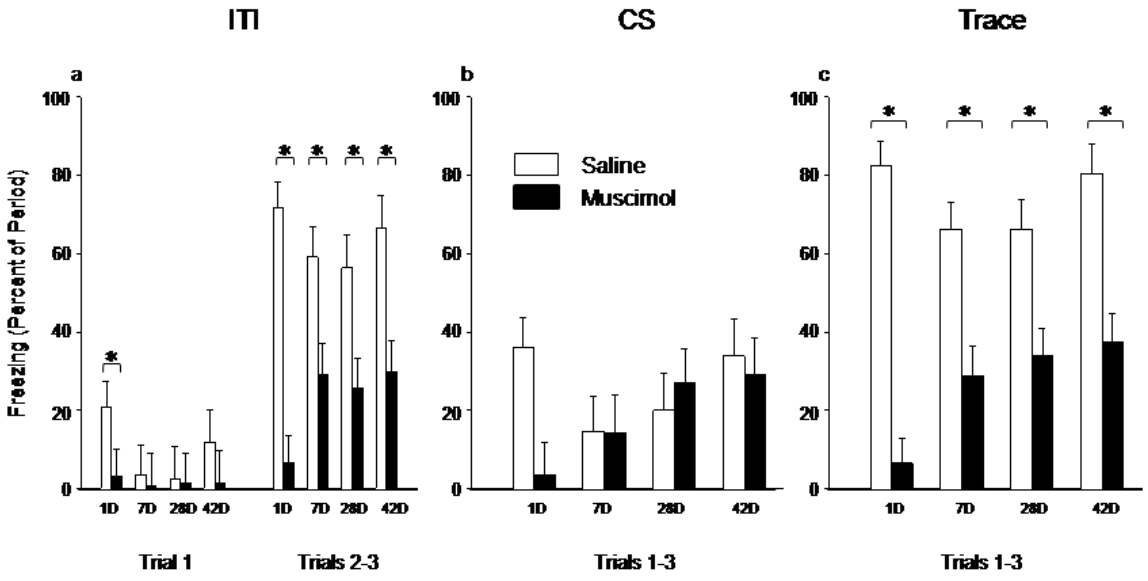

Figure 2. Pre-testing Inactivation of VH: Trace Fear Conditioning. Mean (± SEM) percent freezing during testing for groups that received infusions of muscimol or saline into VH prior to testing in trace fear conditioning. There were significant differences between infusion conditions during the trace interval and the second and third ITI.

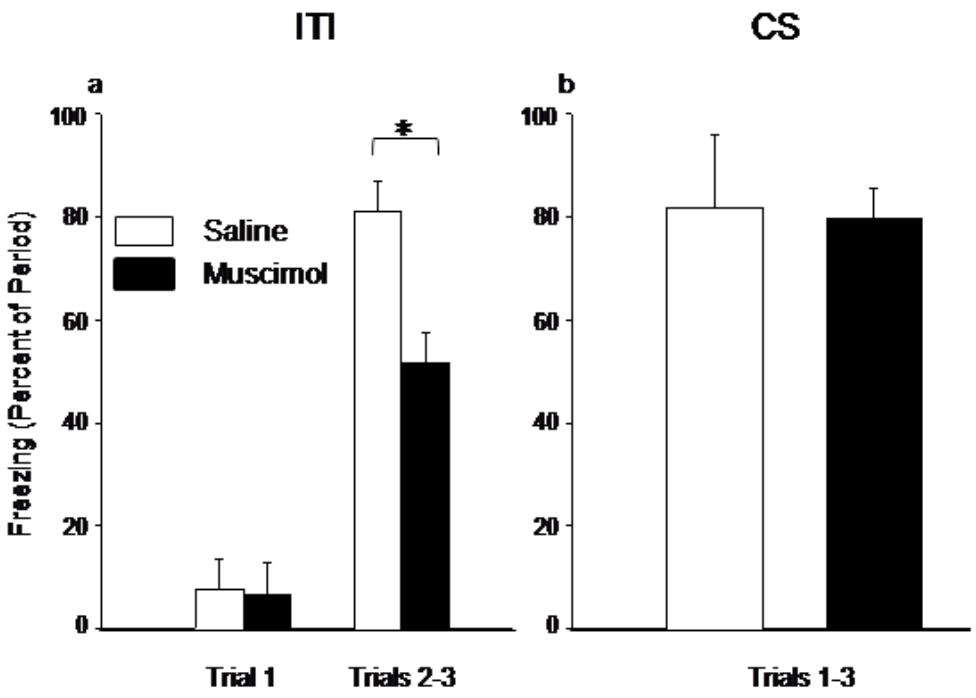

Figure 3. Pre-testing Inactivation of VH: Delay Fear Conditioning. Mean (± SEM) percent freezing during testing for groups that received infusions of muscimol or saline into VH prior to testing 1day after delay fear conditioning. There were significant differences between infusion conditions during the second and third ITI, but not during the CS.

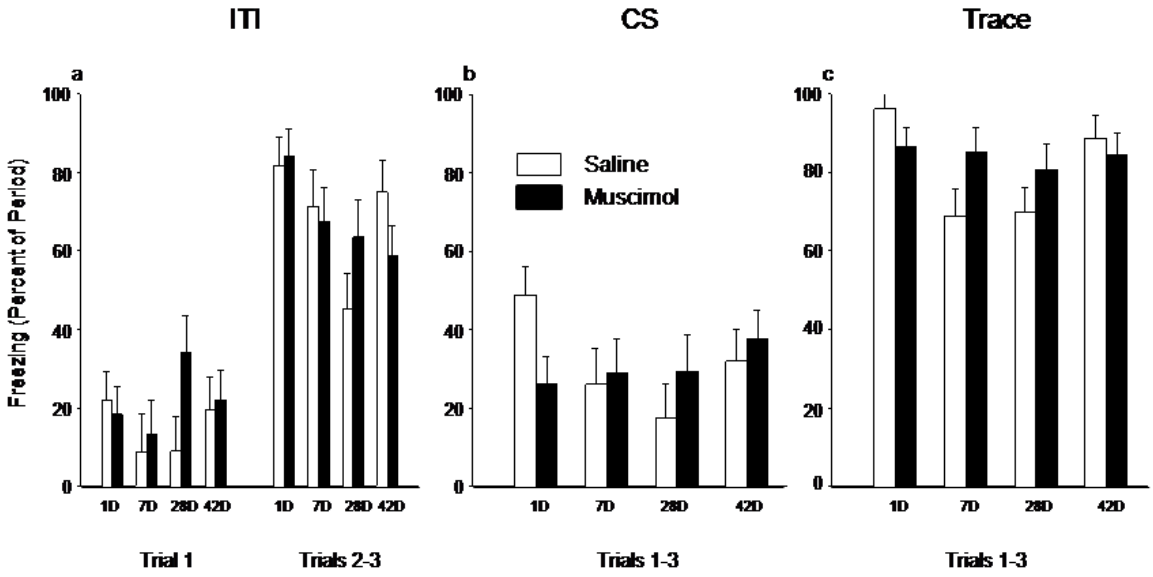

Figure 4. Pre-testing Inactivation of DH: Trace Fear Conditioning. Mean (± SEM) percent freezing during testing for groups that received infusions of muscimol or saline into DH prior to testing in trace fear conditioning. There were no significant differences between infusion conditions during any of the testing periods assessed.

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