ABSTRACT

DORSAL HIPPOCAMPUS, VENTRAL HIPPOCAMPUS AND MEDIAL PREFRONTAL CORTEX IN TRACE AND CONTEXTUAL FEAR MEMORY EXPRESSION: IMPORTANCE OF THE LESION TO TEST INTERVAL

by Christopher Beeman

Previous work has shown that lesions of the dorsal hippocampus (DH) at recent but not remote times following training, produce deficits in memory expression. The opposite pattern was observed with lesions of the medial prefrontal cortex (mPFC). In Experiment 1, excitotoxic lesions of the DH, ventral hippocampus (VH) or mPFC were made at 1 or 30 days following trace fear conditioning. Results showed a temporally-graded deficit in the DH and VH and mPFC lesioned animals in trace fear recall, but not simultaneously-learned contextual fear. In Experiment 2, the same lesions were made one day following training and testing was delayed for thirty days. This allowed for recovery of trace fear memory, but not contextual fear. These results suggest that systems-consolidation of trace fear memory occurs during the course of 30 days, is similar in both VH and DH, and is not disrupted in animals without intact HPC or mPFC.
DORSAL HIPPOCAMPUS, VENTRAL HIPPOCAMPUS AND MEDIAL PREFRONTAL CORTEX IN TRACE AND CONTEXTUAL FEAR MEMORY EXPRESSION: IMPORTANCE OF THE LESION TO TEST INTERVAL

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Introduction

Systems memory consolidation refers to a gradual redistribution of long-term memories such that the critical anatomical substrates supporting memory expression change over time (Marr 1971; McGaugh 2000; Squire et al. 2004; Frankland and Bontempi 2005; Wiltgen et al. 2005; Squire and Wixted 2011; Sutherland and Lehmann 2011). While independent brain circuits mediate different forms of memory, the contribution of a specific brain region may also depend upon the age of the memory (Ribot 1882; Squire 1992; Knowlton and Fanselow 1998). The characteristic pattern of temporally-graded retrograde amnesia following insult to the hippocampus (HPC) suggests that at least some forms of explicit memory undergo a transition from HPC-dependence for recent memories to HPC-independence for remote memories (e.g., Scoville and Milner 1957; Zola-Morgan and Squire 1990; Kim and Fanselow 1992; Kim et al. 1995; Anagnostaras et al. 1999; Clark et al. 2002; Takehara et al. 2002, 2003; Ross and Eichenbaum 2006; Quinn et al. 2008; Broadbent et al., 2009; Parsons and Otto 2010; Gaskin et al. 2011).

There is significant interest in identifying the extra-HPC substrates underlying remote memories that have become independent of the HPC (Hoffman and McNaughton 2002; Dash et al. 2004; Frankland et al. 2004; Wiltgen et al. 2004; Frankland and Bontempi 2005, 2006; Teixeira et al. 2006; Tse et al. 2007; Restivo et al. 2009; Gusev and Gubin 2010; Wang and Morris 2010; Vetere et al. 2011). In particular, the medial prefrontal cortex (mPFC) supports long-term expression of memories that initially depended upon the HPC (Takehara et al. 2002, 2003; Takehara-Nishiuchi et al. 2006; Quinn et al. 2008; Takehara-Nishiuchi and McNaughton 2008).

Trace fear and trace eyeblink conditioning procedures have been particularly useful for studying temporally-graded retrograde amnesia because of the robust inverse gradients that are produced following insult to the HPC or mPFC. Lesions of the HPC made 1 day following trace eyeblink conditioning substantially decrease subsequent test performance, whereas HPC lesions made 1 month following conditioning do not (Kim et al. 1995; Takehara et al. 2002, 2003). Conversely, lesions of the mPFC made 1 day following trace eyeblink conditioning have no effect while mPFC lesions made 1 month
following conditioning significantly impair subsequent test performance (Takehara et al. 2003). We have observed a very similar pattern in trace fear conditioning with lesions of HPC or mPFC made 1 day or 200 days following training (Quinn et al. 2008). The deficit following mPFC lesions made at this very remote time-point suggests that the mPFC contribution to remote memory expression may be permanent. It is also important to note that all but one of these trace conditioning studies (Kim et al. 1995) used lesions that were restricted to the dorsal hippocampus (DH). Anatomical and functional distinctions between DH and ventral hippocampus (VH) have been reported (Swanson and Cowan 1977; Henke 1990; Moser et al. 1995; Fanselow and Dong 2010) and a role for VH in trace fear conditioning has recently been established (Rogers et al. 2006; Yoon and Otto 2007; Czerniawski et al. 2009). To date, no studies have assessed whether retrograde amnesia following VH insult is temporally-graded.

The present study assessed whether trace fear memories and simultaneously learned contextual fear similarly become HPC-independent within 30 days of acquisition and whether mPFC involvement in remote trace fear memory expression becomes apparent within that same time frame. In addition, we assessed whether retrograde amnesia for trace fear conditioned and simultaneously learned context fear memories following insult to the VH is temporally-graded (as with DH) or sustained (i.e., flat gradient).

In a second experiment, we investigated the importance of the lesion-to-test interval in the expression of trace and contextual fear memories following insult to the DH, VH, or mPFC. Importantly, previous studies used lesion-to-test intervals of approximately 10 days. Experiment 2 uses a 30 day lesion-to-test interval to determine if a fully intact HPC or mPFC is necessary during the systems consolidation window in order for the remote expression of trace and/or contextual fear memories.

**Results**

Experiment 1 Histology. Figure 1 shows minimum (gray) and maximum (black) lesion extent (a-c) and representative DH, VH, and mPFC lesions (d) and sham controls (e). The DH lesions (M = 73% of total DH, range 54-91%) generally targeted the CA1 and dentate gyrus cell populations, with some spared tissue in lateral CA3. Very little or no
damage to intermediate HPC was observed and there was no damage to VH in DH lesioned animals. The VH lesions (M = 87% of total VH, range = 65-100%) included damage to CA1, CA3, and dentate gyrus of the VH. Limited damage to DH (M = 11%) was observed in VH lesioned animals due to the passage of the injector. Medial PFC targeted the prelimbic (PL; M = 71% of total PL, range = 49-94%) region and generally spared infralimbic (IL; M = 6% of total IL) and anterior cingulate (ACC; M = 12% of total ACC) cortices.

**Experiment 1: Tone Test.** During the first three minutes of the tone test (baseline), freezing was assessed for generalization between the training and novel (tone test) contexts. ANOVA revealed no main effects of surgery or surgery time and no interaction during the baseline period of the tone test (Figure 2a). Across the three test tone presentations, repeated measures ANOVA showed no main effect of tone presentation number; therefore, the averaged tone freezing (across all three tones) was used for all subsequent analyses. The average percentages (±SEM) of time spent freezing during the tone are presented in Figure 2b. ANOVA revealed a significant surgery X surgery time interaction \( F(3, 59) = 4.002, p = 0.01 \). Animals that received surgery one day following trace fear conditioning differed in their levels of tone fear as a result of surgery \( F(3,30) = 4.914, p< .01 \). DH and VH lesioned rats froze significantly less than both sham controls and mPFC lesioned rats \( (p < .05) \). Sham controls and mPFC lesioned rats did not differ from one another (Fig. 2b). Rats that received surgery 30 days following trace fear conditioning did not show a significant main effect of surgery on freezing during the tone. However, planned comparisons \( (p < 0.05) \) revealed that mPFC lesioned rats froze significantly less than sham controls while DH and VH lesion rats did not (Fig. 2b).

**Experiment 1: Context Test.** The percentages of time spent freezing (±SEM) during the 8 minute context test are displayed in Figure 2c. Overall, DH, VH, and mPFC lesion rats froze significantly less than sham controls at both surgery time-points. Analyses revealed main effects of surgery \( F(3, 59) = 12.350, p < 0.001 \) and surgery time \( F(1, 59) = 7.346 p < 0.01 \), but no surgery X surgery time interaction. Planned comparisons \( (p < 0.05) \) revealed that 1-day post-training DH, VH and mPFC lesion rats froze less than sham controls with no differences between lesion conditions. Further, 30-day post-
training VH and mPFC lesioned rats froze less than sham controls, but DH lesioned rats did not.

**Experiment 2. Histology.** Figure 3 shows minimum (gray) and maximum (black) lesion extent (a-c) and representative DH, VH, mPFC lesions (d) and sham controls (e). The DH lesions targeted the CA1 and dentate gyrus cell populations with some damage to CA3 as well. In DH lesioned animals (M = 85% of total DH, range = 68-96%), there was no damage found in either intermediate or VH. VH lesions (M = 85% of total VH, range = 63-100%) targeted all cell populations, including CA1, CA3 and dentate gyrus. There was minimal damage to DH (M = 26% of total DH) in VH lesioned rats due to the passage of the injector. The mPFC lesions targeted the PL (M = 84% of total PL, range = 68-100%). Minimal damage occurred in the IL (M = 12% of total IL) and ACC (M = 10% of total ACC). Thus, the lesions produced in Experiment 2 were very similar to those produced in Experiment 1.

**Experiment 2: Tone Test.** During the first three minutes of the tone test (baseline), freezing was assessed for generalization between the training and novel (tone test) contexts. ANOVA revealed no main effect of surgery during the baseline period of the tone test. However, pair-wise comparisons (p < .05) revealed that DH and VH lesioned rats froze significantly less than sham controls (Figure 4a). Across the three test tone presentations, repeated measures ANOVA showed no main effect of tone presentation number; therefore, the averaged tone freezing (across all three tones) was used for all subsequent analyses. The average percentages (±SEM) of time spent freezing during the tone are presented in Figure 4b. ANOVA revealed no significant effects of surgery on freezing to the tone.

**Experiment 2: Context Test.** The percentages (±SEM) of time spent freezing during the 8 minute context test are presented in Figure 4c. There was a main effect of surgery on freezing to context (F (3, 34) = 4.57, p < .01). Planned comparisons (p < .05) showed that mPFC lesions produced significantly less freezing compared to sham controls, with DH and VH lesioned rats not being significantly different from shams.
Discussion

These data show a time-limited contribution of both DH and VH to the expression of trace fear memories, with recent memories being dependent on a fully intact HPC while remote memories are HPC-independent. To our knowledge, this is the first demonstration of temporally-graded retrograde amnesia following lesions of the VH. Furthermore, we showed that expression of trace tone fear becomes critically dependent on the mPFC within 30 days of training. Simultaneously learned contextual fear showed a dissimilar pattern from tone fear, with recent memory relying on an intact DH, VH, and mPFC while remote memories required VH and mPFC. This indicates at least partially dissociable memory processes for trace tone and simultaneously learned contextual fear memories.

Surprisingly, in Experiment 2 we showed that a lesion-to-test interval of 30 days allows for full recovery of trace fear memory after HPC damage was made 1 day following surgery. Previous studies have used lesion-to-test intervals of approximately 10 days (when reported). This is the first experiment showing recovered expression of fear memory following either DH or VH lesions made shortly following training. This experiment also shows no effect of mPFC lesions on trace fear memory. Simultaneously learned contextual fear shows a similar pattern of recovery following a long lesion-to-test interval for HPC, but remains dependent on the mPFC. Together, these data suggest that the HPC, or alternative compensatory system, may be capable of maintaining fear memories in the absence of an intact mPFC.

Experiment 1 data extends the double dissociation of HPC and mPFC in the expression of recent versus remote memory using trace fear conditioning. This adds to a growing literature demonstrating temporally-graded retrograde amnesia for trace conditioning following hippocampal insult across species in both fear and eyeblink conditioning (Kim et al, 1995; Takehara et al, 2002; Takehara, et al, 2003; Quinn et al., 2008). Although examinations of retrograde amnesia often yield mixed results, trace conditioning is uniquely consistent in showing a temporal gradient (Sutherland & Lehmann, 2011; Frankland & Bontempi, 2005). Flat gradients have been observed frequently following hippocampal damage in spatial navigation tasks (Mumby & Glenn, 2000; Clark, et al., 2005; Martin et al, 2005). Active navigation during testing may
necessitate "online" hippocampal processing; thus, the absence of a temporal gradient following hippocampal damage may reflect a test performance, rather than long-term memory, effect (e.g., Knowlton & Fanselow, 1998). Mixed results have been obtained using contextual fear conditioning. While some studies report very clear temporal gradients (e.g., Kim & Fanselow, 1992), others have yielded either slower, or entirely flat, gradients (Lehmann, et al., 2007; Sutherland et al., 2008). There are numerous parametric differences across studies (e.g., lesion type, lesion size, signalled versus unsignalled fear conditioning) that likely contribute to these differences. It is also possible that trace conditioning requires fundamentally different hippocampal processing that critically depends upon systems consolidation for its long-term expression. As was observed in Experiment 1, there was no remote memory impairment for trace tone freezing following HPC damage, yet the context freezing deficit was maintained in these same animals (at least for VH lesioned rats). The functional differences found here between DH and VH lesioned rats in the context test may help explain the dichotomy in the field with some studies showing temporal gradient (Wiltgen, 2010) and others flat gradient for contextual fear (Sutherland, 2008). It may be possible in the case of contextual, but not trace fear, that the larger VH lesion size, compared to the DH lesions, contributes to greater deficit. Sutherland et al (2008) found that lesion extent positively correlated with the magnitude of the performance deficit in contextual fear learning, which may help explain the difference in results at remote time-points between DH and VH for contextual fear in Experiment 1.

In Experiment 2, neither DH nor VH lesions made 1 day after training produced a deficit in trace tone fear when tested 30 days following surgery. One possibility for this apparent “recovery” over the 30 day lesion-to-test interval is that dual traces are laid down in both the HPC and mPFC at the time of training and the mPFC trace is gradually strengthened over time, even in the absence of a fully intact HPC, until it is capable of supporting full expression of the trace fear memory. This idea is supported by recent evidence that an intact mPFC is necessary for the acquisition of trace fear conditioning (Gilmartin and Helmstetter, 2010). Gilmartin and Helmstetter (2012) inactivated contralateral HPC and mPFC to detect whether interaction between the structures was necessary during training, however even lone unilateral HPC lesions produced deficits,
so results were ambiguous. Darling et al. (2011) showed however that when training using trace eye blink classical conditioning (tEBCC) was triggered based on HPC brain state (theta oscillations, 3-7 Hz) animals in the theta+ learned significantly quicker than theta- animals. Interestingly, in T+ animals, mPFC response was more robust implying some dependence of mPFC function based on HPC state and possible interaction between the two structures at the time of training. Interaction between HPC and mPFC may also be required within the first 24 hours following training. During this first 24 hour period, awake recall and neural replay during sleep may play an important role in consolidating memories into both HPC and mPFC. Peyrache et al (2009) showed mPFC replay of neural patterns reflecting previously acquired information during slow wave sleep which frequently corresponded to HPC ripples. This replay is a reactivation of particular patterns of brain activity seen during training. The correlation between this replay and HPC activity strongly implies interaction. Whether this interaction continues beyond 24 hours is unclear since most sleep studies only involve the first sleep period following training. Popa et al (2010) showed that amygdalocortical theta coherence and not HPC-cortical coherence correlated with improved retention 24 hours after learning, however this study used delay fear conditioning and it would be interesting to test if trace conditioning would reverse these results. Regardless, this study shows that shortly after training cortical structures and subcortical structures are still interacting and these interactions have a role in memory retention. Thus the 24 hour cellular consolidation window and specifically sleep during that time may play an important role in long term memory retention, and interactions between these structures during training shortly thereafter should be explored especially as they relate to remote fear recall.

The mPFC lesioned group in Experiment 2 also does not show a deficit in freezing to tone when testing occurs at a long lesion-to-test interval. This leads to several competing hypotheses. It is possible that in the absence of an intact mPFC recall of trace fear memories never becomes hippocampus-independent. This would imply that an intact mPFC is necessary to allow the HPC to "release" or forget the memory. There is a mechanism that could indicate the presence of just such a process. Long-term potentiation (LTP) is a commonly used model for memory formation in which synapses are strengthened following high frequency stimulation (Morris et al., 2003).
The formation of LTP and previously formed LTP is disrupted in vitro in HPC slices following naturally occurring and experimentally-induced sharp wave ripple oscillations (SWR) (Colgin at al., 2004). Sharp wave ripples are naturally occurring firing patterns in the HPC characterized by very high frequency (140-200 Hz) bursts of activity. Interestingly, these SWR’s are common during slow wave sleep and an increase in SWR’s has been shown following stimuli that produce LTP (Behrens, et al., 2005). These findings, taken together along with the retention of memory expression in mPFC lesioned animals in experiment 2, show a potential mechanism by which HPC SWR (which correlates with mPFC replay of a learned task (Peyrache, 2009)) interacts with the mPFC during sleep shortly after training and allows for consolidation of a memory trace in the mPFC while at the same time "releasing" or inhibiting memory retention in the HPC.

Alternatively, there may be other regions capable of supporting recall of trace fear in the absence of mPFC, indicating that memory consolidation can occur in regions other than mPFC, given enough time following training. Multiple trace theory (Moscovitch et al 2005) would attempt to explain these findings by saying the remaining HPC tissue, while incapable of supporting memory performance initially following insult (i.e., Experiment 1), is able to lay down additional memory "traces" over the longer lesion-to-test interval and eventually support expression of the entire memory. This could explain the results in Experiment 2, but is not consistent with the deficit in remote recall seen in the mPFC lesion group in Experiment 1. Evidence at this time would favor HPC-neocortical interactions being involved shortly following training, helping consolidate the memory in mPFC while releasing the memory from the HPC.

In simultaneously learned contextual fear conditioning we found no deficit in either HPC region and a significant deficit in mPFC lesions with a long lesion-to-test interval. This shows that contextual fear learning may be mPFC dependent for the life of the memory. It may also show that given enough time to allow recovery following insult, contextual fear may become HPC-independent following lesions made shortly following surgery.

In conclusion, these data support systems consolidation for trace fear memories, while simultaneously learned contextual fear conditioning appears to rely on different
mechanisms. Recovery of memory expression in Experiment 2 suggests that systems consolidation can occur in the absence of a fully intact HPC. In the absence of an intact mPFC, either the HPC or another region is capable of trace fear expression given a long lesion-to-test interval.

**Materials and Methods**

**Animals:** One hundred seventy seven experimentally naïve, male, Long-Evans adult rats (approximately 80 days of age; Harlan, Indianapolis, IN) were used in this experiment. They were pair housed on a 12:12 hour light:dark schedule with *ad libitum* access to food and water and were handled for 5 consecutive days prior to training and testing. All experimental procedures were performed during the light cycle and in accordance with the Miami University IACUC.

**Behavioral Apparatus:** Four conditioning chambers (32.4 W x 25.4 H x 21.06 D cm; Med-Associates, Inc.) were used for fear conditioning and context testing (Context A). The front door and ceiling were made of clear Plexiglas, the sidewalls were made of aluminum, and the back wall was made of plastic. The floor consisted of 19 equally spaced stainless steel rods. The grid floor in each chamber was wired to a shock generator and scrambler (MED-Associates, Inc.). A stainless steel pan coated with diluted vanilla flavor (50% in water; Meijer) was placed beneath the grid floor to provide an odor, and white light (125 lux) remained on inside the chamber. The chambers were cleaned with 5% NaOH before each trial. Chamber fans provided background noise (80 db).

Four novel chambers were used for tone testing (Context B). Each chamber contained a white Plexiglas floor and a triangular black Plexiglas insert. A 5% glacial acetic acid solution was used for cleaning between each trial and to provide an odor in the floor pan. Testing was performed under near infrared lighting (0 lux).

The rats were continuously monitored by a progressive scan video camera with a visible light filter (VID-CAM-MONO-2A; Med Associates Inc.) connected to a computer in the experiment room running VideoFreeze software (Med Associates Inc.) designed
for automated assessment of motion, including defensive freezing (Anagnostaras et al., 2010).

**Surgery Experiment 1:** Animals were anesthetized with sodium thiopental (40 mg/kg; i.p.) and mounted into a stereotaxic apparatus. An incision was made, the skin covering the skull was retracted, and the skull was leveled before holes were drilled into the skull above the desired lesion sites. Coordinates for lesion sites can be found on Table 1. DH and mPFC lesion coordinates were held the same from Quinn (2008). While VH lesion coordinates were used from Otto and Yoon (2007) NMDA (20 µg/µl; Sigma, St. Louis, MO) was dissolved in phosphate buffered saline (PBS) for infusion. For DH lesions, 0.4 µl of NMDA was infused, and the injector was left in place for an additional 5 min. For mPFC and VH lesions, 0.2 µl of NMDA was infused and the injector was left in place for an additional 5 min. Sham surgeries were similar except that no injector was lowered and no drug infused.

**Surgery Experiment 2:** Surgeries in experiment two were identical to experiment one except in the following ways. Anesthesia was induced using 5% Isoflurane (Vedco, St Joseph, MO) and held at surgical plane using 2-3% isoflurane at 2 L/min. Lesioned animals were given diazepam (0.2ml, 5mg/ml, i.p.) as a prophylaxis against seizure activity with additional doses given as needed.

**Procedure Experiment 1:** Rats received 10 trials of trace fear conditioning, which consisted of a 16 sec tone (2 kHz, 90dB) followed by a 28 sec “stimulus-free” trace interval and then a 2 sec footshock (0.9mA), with an inter-trial interval (tone-onset to tone-onset) of 256 sec. Half of the animals underwent surgery 24 h after conditioning, while the other half remained undisturbed in their homecages. This latter group of animals underwent surgery 30 days after conditioning. Approximately ten days following surgery (range 9-12, m=10), animals received one tone test session (Context B) and one context test session (Context A) the following day. The tone test consisted of a 180 sec baseline period followed by three presentations of the tone used during conditioning with a 256 sec inter-trial interval. Context testing consisted of placing the rat into the original training context for 8 min with no tones or footshocks. Defensive freezing, defined as the absence of movement except that necessitated by respiration (e.g., Fanselow, 1980), was used as the dependent measure.
Procedure Experiment 2: All procedures in Experiment 2 were identical except the following: All lesions occurred 1 day following trace conditioning, animals were left undisturbed in home cages and were tested for freezing to context 29 days following training and freezing to tone the following day (30 days post-training).

Histology: Following the completion of all behavioral testing, animals were anesthetized with Euthasol (50 mg/kg) and perfused with 0.9% saline followed by 10% formalin. The brains were stored in 10% formalin. Approximately two days prior to slicing, brains were transferred to a 10% formalin/30% sucrose solution. Brains were sliced in 50 μm coronal sections, mounted onto glass slides, and stained using 0.5% thionin solution. Images of whole sections at the rostral-caudal levels shown in Figures 1 and 3 were captured, lesion area was measured using an image processing and analysis program (ImageJ; NIH) and a percentage of total target area was calculated.

Data Analysis:
All statistics were calculated using SPSS version 17.0. Baseline period for tone tests, and tests for main effects of tone, surgery day and context freezing were analyzed using omnibus ANOVA test, with critical value $\alpha = .05$. A priori planned comparisons between groups in experiment 1 tone and context tests were done using Fisher's LSD with $\alpha = .05$. Post hoc comparisons between groups in experiment 2 tone and context tests were done using Bonferroni correction.
References


Gilmartin, M., Kwapis, J., & Helmstetter, F. (2012). Trace and contextual fear conditioning are impaired following unilateral microinjection of muscimol in the ventral hippocampus or amygdala, but not the medial prefrontal cortex. *Neurobiology of Learning and Memory, 97*(4), 452-64. doi:10.1016/j.nlm.2012.03.009


Takehara-Nishiuchi, K., Kawahara, S., and Kirino, Y. 2005. NMDA receptor-dependent processes in the medial prefrontal cortex are important for acquisition and the early stage of consolidation during trace, but not delay eyeblink conditioning. *Learning & Memory* **12**: 606-614.


Table and Figure Captions

**Table 1**: Lesion coordinates for DH, VH, and mPFC lesions used in both studies all measurements are relative to bregma except where noted with * which were measured relative to dura.

**Figure 1**: The extent of bilateral lesions in DH (a), VH (b), and mPFC (c) with representative lesions (d) and sham controls (e). Lesions were bilateral with light gray representing the minimum and dark gray representing the maximum extent of damage at each level with contralateral side left blank for comparison. The mean lesion size was DH (73%), VH (84%), and mPFC (PL=72%; IL = 6%; ACC =12%). Atlas images taken and modified from Paxinos and Watson, 1998.

**Figure 2**: The percent of time spent freezing during baseline period (first 3 minutes) of tone test (a) show no differences in the amount of time spent freezing to tone at either recent or remote time-points (all *p*'s >.05). The amount of time spent freezing to tone expressed as a percentage of time during tone presentations (b) show a significant deficit in memory expression in both DH and VH (*p < .05*) but not mPFC as compared to sham controls when lesions occur at 1 day following training (recent memory). The opposite pattern is observed when lesions are made 30 days following surgery (remote memory) with no deficit compared to sham in DH or VH groups and a significant deficit in mPFC lesioned animals (*p < .05*). Simultaneously learned contextual fear expressed during the context test (c) shows all three lesioned groups have a deficit compared to sham animals when lesions occur 1 day following training (*p*'s <.05). In remote memory groups, only VH and mPFC show a deficit in freezing compared to sham controls(*p < .05*).

**Figure 3**: The extent of lesion (a), VH (b), and mPFC (c) with representative lesions (d) and sham controls (e). Lesions were bilateral with light gray representing the minimum and dark gray representing the maximum extent of damage with contralateral side left blank for comparison. The mean lesion size was DH (80%), VH (85%), and mPFC (PL=84%; IL=12%; 10%). Atlas images taken and modified from Paxinos and Watson, 1998.

**Figure 4**: The mean percentage of freezing during the baseline period (first 3 minutes of tone test) (a) show significantly less freezing in HPC lesioned animals compared to
sham controls ($p<.05$). The mean percentage of time spent freezing during presentation of the tone (b). There was neither main effect nor any between group differences when compared to sham controls (all $p$'s $>.05$). Percentage of time spent freezing to training context during test (c). Only mPFC group showed a significant difference from sham ($p <.05$). There were no other differences amongst groups.
Table 1.

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* relative to dura
Figure 1.

A) Dorsal Hippocampus

B) Ventral Hippocampus

C) Medial Prefrontal Cortex

D) Lesion

E) Sham
**Figure 2.**

a) Tone test baseline

![Baseline Freezing Chart](image1)

b) Tone test

![Freezing to Tone Chart](image2)

c) Context test

![Training Context Freezing Chart](image3)
Figure 3.

a) Dorsal Hippocampus

b) Ventral Hippocampus

c) Medial Prefrontal Cortex

d) Lesion

e) Sham
Figure 4.

a) Tone Test Baseline

b) Tone Test

c) Context Test