# The Role of the Dorsal Hippocampus in the Contextual Control of Appetitive Responding

by

Vincent Campese

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Andrew R. Delamater

_____ _____

Date Chair of Examining Committee

Maureen O'Connor

_____ _____

Date Executive Officer

Anthony S. Sclafani

Frank Grasso

Devin Mueller

Mark E. Bouton

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK

# Abstract

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Advisor: Dr. Andrew R. Delamater

Four experiments were run using rat subjects in order to assess the impact of manipulations to the dorsal hippocampus (DH) on the contextual and temporal control of extinguished appetitive learning (e.g., magazine approach). Subjects were trained to associate discrete stimuli with food in specific locations or at specific times. The subjects then had these associations extinguished by means of omitting the food reinforcers following stimulus presentations. In order to assess contextual and temporal modulation of learning the stimuli were tested within as well as outside of the contexts or times where/when they were extinguished. Control subjects showed reduced responding when stimuli were presented within their extinction contexts (physical and temporal) whereas responding recovered outside of these extinction contexts (i.e., renewal and spontaneous recovery). In order to assess DH function in these different instances of response recovery, neurotoxic lesions of the DH prior to tests or temporary muscimol-induced inactivation of the structure were used. The results of these studies indicate that while DH manipulations fail to affect conditional control of appetitive extinction learning by physical contexts, they do impair control when temporal contexts are used as a conditional cue.

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Classical conditioning consists of repeatedly pairing a conditioned stimulus (CS) such as a tone with an unconditioned stimulus (US) such as a foot shock. This procedure has the effect of rendering the previously neutral CS capable of evoking behaviors formerly elicited by the US (e.g., fear and freezing). When some form of these unconditioned behaviors are observed following a CS presentation, they are referred to as conditioned responses (CRs). Learning of this nature has been understood in terms of associative connections being formed between neural representations of CS and US (e.g., Rescorla, 1991; 1992). Neurobiological studies have demonstrated anatomical substrates that are involved in representing the CS, US, and expectations of the US in a variety of learning preparations (for a review see Fanselow & Poulos, 2005; see also Waelti, Dickinson & Schultz, 2001).

The procedure of presenting the CS without the US after conditioning has already taken place is referred to as extinction. This results in a reduction of CRs to the CS. While behavioral research into extinction has produced many psychological theories on its mechanisms, neuroscientific research has begun to identify areas in the central nervous system that are important for extinction learning. The current review will first address some of the more influential psychological theories of extinction before moving into a review of neuroscientific research into central nervous system mediation of extinction learning.

**Psychological Mechanisms of Extinction**

One of the most influential models of learning, the Rescorla-Wagner model (Rescorla & Wagner 1972), explains learning in terms of a quantity, associative strength, that refers to the strength of a connection between the CS and the US. Furthermore, changes in associative strength are assumed to depend on how surprising the US is on a given conditioning trial. Specifically, when an animal is presented with a CS-US pairing on the first trial of training, the US is not expected, and should result in the development of associative strength between the CS and US. As training progresses, given a CS presentation, the US comes to be expected by the animal more so. After sufficient training, (when presented with the CS) the US is fully expected and no further difference exists between the organism's expectations of the US and the actual US on that conditioning trial. At this point, learning is asymptotic with no further increases in associative strength occurring with additional CS-US pairings. A subsequent extinction phase, however, would at first produce a large negative discrepancy between what the animal expects when the CS is presented (i.e., the US) and what actually occurs (nonreinforcement). According to the model, this negative discrepancy results in a loss of associative strength on the extinction trial. Further loss will occur on subsequent trials until the US is no longer expected and the CR is extinguished (i.e., unlearning is said to occur).

While the Rescorla-Wagner model implies that extinction should result in weakened associative strength between the CS and US, a number of experimental phenomena challenge this view. For example, if sufficient time is interpolated between an extinction treatment and a subsequent test for responding to a stimulus, the CS can spontaneously recover its ability to elicit CRs (Pavlov, 1927; see Rescorla, 2004 for a review). Some

learning must survive extinction to result in this effect; if the association were completely unlearned, then no recovery should occur. Pavlov, (1927) suggested that the CR reduction in extinction is reflective of a loss in CS processing (i.e., attention to the CS), and that spontaneous recovery is due to the return of attention at the start of the test session. Alternatively, Konorski, (1948) suggested that extinction may actually result in new learning, specifically, an inhibitory connection between CS and US representations, and that spontaneous recovery is observed because this link weakens over time (see Robbins 1990).

Providing more evidence that extinction does not produce unlearning, extinction has been shown to be sensitive to context manipulations. Bouton and Bolles, (1979a) repeatedly paired a CS with shock in one context, followed by extinguishing the CS in a second context. Testing the CS in the original training context resulted in more fear CRs compared to when that same CS was tested in its extinction context. This contextually gated recovery effect is commonly referred to as renewal (Bouton & Bolles, 1979a; Bouton & King 1983). The renewal effect suggests that a CS retains some residual excitatory control over responding after an extinction treatment. This result, at a first glance, seems to be inconsistent with the assumptions of the Rescorla-Wagner model. However, the Rescorla-Wagner model can account for renewal by assuming that the context combines with the CS to form a context-CS compound stimulus during the extinction phase, and that this context cue can come to signal nonreinforcement. According to this theory, treating the context and CS as a compound stimulus during extinction protects the excitatory CS-US association from total degradation. This is due to the inhibitory strength accrued during nonreinforcement being divided between the CS and context. Reduced CRs are observed when a test occurs in the extinction context because the context has acquired an inhibitory

association with the US. Thus, when the CS is presented in the extinction context, the inhibitory context is thought to antagonize the excitatory effect of the CS. However, if the CS were to be tested in a neutral context (other than that present during extinction) the excitatory effect of the CS would, in the absence of any antagonistic processes, promote the CR (i.e., renewal).

There is some evidence to suggest that the context may not come to possess the inhibitory properties proposed by this interpretation (Bouton & King, 1983; Bouton & Swartzentruber, 1986; 1989; but also see Polack, Labdora & Miller, in press). However, Larrauri and Schmajuk (2008) ran computer simulations of a learning model that combines associative and attentional mechanisms to assess context-US associations in extinction. In this model, decreases in attention reduce the extent to which associative connections are expressed. While their model, contrary to Bouton's findings (Bouton & King, 1983; Bouton & Swartzentruber, 1986; 1989), resulted in the formation of inhibitory context-US associations, further summation and retardation simulations also failed to detect any inhibitory influence of the context. These authors suggest that the difficulty in detecting this inhibition is due to attention to both the stimulus and context decreasing as a result of extinction. In essence, this model explains the lack of a demonstrable inhibitory effect to the context because subjects pay less attention to it and therefore process it less well by the end of extinction.

However, despite the alleged development of inhibitory context-US learning, renewal can occur even when contexts are equated for training history (Delamater, Campese & Westbrook 2009). In this study, two CSs were trained in two different contexts (e.g., Context 1: A+ & Context 2: B+). Each CS was then extinguished in the other CS's training

context (e.g., Context 1: B-, Context 2: A-) before both were tested in each context. Therefore, at the time of testing, each context should possess an equivalent inhibitory associative connection with the US. Nevertheless, under these conditions more responding was still observed when the CSs were tested in their acquisition compared to their extinction contexts. These results suggest, perhaps more persuasively, that the renewal effect is not due to inhibitory context learning; rather, renewal may reflect some other process.

Another extinction phenomenon that suggests learning survives extinction is Reinstatement (Pavlov 1927; Rescorla & Heth 1975). Following extinction, presenting the US alone results in a reinstatement of CRs to the CS in the subsequent test compared to animals that did not receive the un-signaled USs. At least some learning must survive extinction in order for a US presentation to elicit reinstatement of CS induced responding. Reinstatement has also been shown to be sensitive to context, suggesting that the effect may be due to excitatory context-US associations summating with the residual excitatory CS-US association. For example, presenting the reinstating USs in a context other than the test context fails to produce CR recovery in a subsequent test of the CS (Bouton & Bolles 1979b; Baker et al. 1991). Furthermore, extensive nonreinforced context exposure following the reinstating USs eliminates the reinstatement effect (Bouton & Bolles 1979b; Baker et al. 1991). While the Rescorla-Wagner model assumes that context-US inhibitory learning develops during the extinction treatment, the model also assumes that reinstatement occurs because unsignaled USs result in the context becoming less inhibitory and possibly excitatory.

While these phenomena provide some evidence that extinction does not completely erase prior learning, the results of Delamater (1996) and Rescorla (1996) suggest that most, if not all of the information acquired by a CS survives extinction. In each of these studies, rats were trained to associate two CSs with two different USs. Specific CS-US associations were shown to endure, despite extinction. Specifically, Rescorla trained rats to associate two CSs with two USs (i.e., S1-US1 & S2-US2). Half of the animals received extinction of S1 and S2, followed by all animals receiving training of S1 and S2 with a third US (i.e., US3). All animals then had one of the two USs (e.g., US1) devalued by pairing the USs with injections of Lithium Chloride (LiCl - which causes illness). The test session consisted of presenting S1 and S2 without reinforcement. These tests revealed that both extinguished and non-extinguished animals responded less to S1 (which was initially paired with US1, which had been devalued) compared to S2 (which initially predicted an outcome that had not been devalued – US2). If extinction had resulted in unlearning, extinguished animals should not have shown any differences in responding between the two stimuli. Instead these subjects behaved as if they knew which CS was paired with the devalued US and which was not. Furthermore, Delamater (1996) trained rats to perform two different instrumental responses for two different food rewards (i.e., R1-US1, R2-US2). The animals were then trained to associate two different CSs with the two USs trained in the first phase of the study (i.e., A-US1, B-US2). One of these CSs was then extinguished. During the test session, rats had access to both R1 and R2, though these responses did not produce reinforcement during test. The CSs were tested for their ability to augment baseline instrumental responding. For example, when the nonextinguished CS was presented rats elevated instrumental responding on the manipulanda that shared an

outcome with that particular CS (e.g., A: R1>R2). Importantly, when the extinguished CS was tested, it, too, selectively elevated responding on the manipulanda with which it shared an outcome. These data imply that the associations between the CSs and the sensory attributes of their USs survived extinction. If they hadn't, a selective bias in instrumental response should not have been observed when the CSs were presented during the transfer test. Given the evidence above, it seems unlikely that CRs are reduced by extinction because the procedure weakens previously formed associative connections.

An alternative view of extinction was proposed by Bouton (1993; 1994), who suggested that contextual information can come to modulate extinction learning similarly to how discrete CSs can operate in occasion setting discrimination tasks (Holland, 1998). Specifically, Bouton proposed that as a result of extinction, the CS becomes ambiguous. Contexts can be used to disambiguate extinction from acquisition memories (provided the two training phases take place in different locations), as testing the CS outside of the extinction context has been shown to renew responding (Bouton & Bolles 1979a). Bouton and Bolles, (1979a) trained rats to associate a CS with a foot shock US. The stimulus was then extinguished in a second context and tested for its ability to evoke fear in the original training context. Renewed fear CRs were observed under these conditions compared to when that same CS was tested in its extinction context. The renewal effect provides compelling evidence that CR retrieval after extinction depends on the context present at the time of test. As a result of extinction, Bouton (1993; 1994) suggested that a new inhibitory link develops between the CS and US that competes with the excitatory association developed during the initial training phase. Bouton suggested that the extinction context comes to activate the inhibitory CS-US extinction memory when it is

present, and that when it is absent, the original excitatory CS-US training memory can be expressed (i.e., renewal is observed) because he assumed this was less context-dependent.

The suggestion that extinction results in new inhibitory learning has been included in a number of other theories (Konorski 1948, 1967; Pearce & Hall 1980; Pearce 1994; see also Pavlov 1927). Bouton, however, suggested that the extinction context gates the activity of this inhibitory connection formed during extinction. Bouton makes an analogy for this mechanism to an 'and gate' (see figure 1 below). Thus activation of the extinction context representation promotes the retrieval of the extinction memory by additionally activating the inhibitory CS-US memory through this 'and gate' mechanism. Contrarily, when tested outside of the extinction context, renewal is observed because additional activation of the inhibitory CS-US memory is absent. Additionally, because factors such as time (spontaneous recovery) and internal states (state dependent learning) can modulate conditioned responding similarly as contextual information, Bouton views these phenomena as different forms of the same renewal effect. For instance, according to this idea, spontaneous recovery occurs because the delay between extinction and test removes the organism from the temporal context of extinction, hence removing the inhibition that the temporal extinction context engages.

**Figure 1**

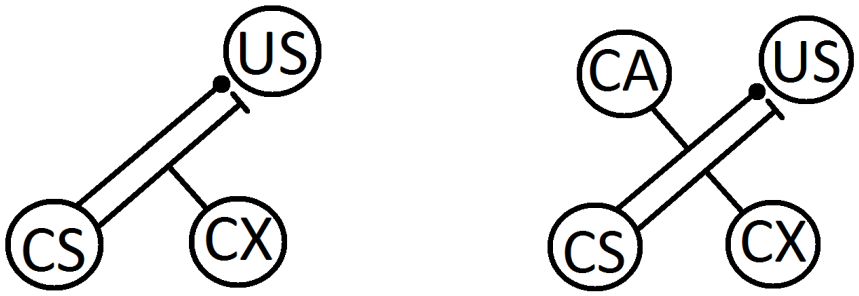



**Figure 1:** On the left, the model proposed by Bouton (1993) to account for contextual control over extinguished responding. The extinction context (denoted as 'CX') provides additional activation to the inhibitory learning acquired during extinction (connections ending with a 'T' denote inhibitory associations). On the right, a modified version of the Bouton (1993) model that incorporates similar context modulation of the excitatory connection acquired during initial training (excitatory connections are denoted by a bullet point). The results of Harris et al. (2000) suggest that upon extinction, both acquisition and extinction memories become specific to the location in which these events took place (the acquisition context is denoted as 'CA').

The ability that context has to modulate memories has received considerable attention from researchers interested in learning processes, in particular with regards to mediating extinguished learning (Bouton & Bolles 1979a; Hall & Minor 1984; Loviond, Preston & Mackintosh 1984; Rescorla, 1984; Bouton 2004). However, in contrast to Bouton's assumption, recent research indicates that the ability of a context to facilitate the retrieval of one memory over another is not limited to extinction contexts. Harris, Jones, Bailey, and Westbrook (2000), provide evidence that Bouton's interpretation is not complete. Harris et al. (2000) demonstrated that nonreinforced CS presentations do not only contextualize extinction training, but that following this experience, the memory for initial training becomes context specific as well. Specifically, they showed that animals respond more to a CS when it is presented in its training environment compared to novel contexts. However, this was true only after that stimulus had undergone extinction in a context other than where it was trained. If the stimulus was not extinguished, conditioned responding

generalized more readily across new contexts. The authors suggested that the most suitable explanation of renewal is found in Bouton's (1993; 1994) ideas on context control, which suggested that the extinction context prevents retrieval of the acquisition memory in an occasion setting fashion as described above. However, Bouton's account is sufficient enough only to provide an explanation of how extinction performance is context-specific, and does not readily explain the results seen in Harris et al. (2000). These authors built on Bouton's suggestion that the extinction context promotes retrieval of the extinction memory, further suggesting in addition that the conditioning context promotes the retrieval of the excitatory CS-US memory. These authors suggest that extinction leaves the CS in a state of ambiguity (as it has been both reinforced and nonreinforced), and that context is used to bias memory retrieval towards what had been learned in the location where the CS is presented following this treatment. These authors propose (not too dissimilarly from Bouton) that the training contexts come to hierarchically control the CS-US association when a CS has undergone extinction outside of its training environment (see figure 1). This control is similar to how facilitative or inhibitory stimuli work to regulate the CR in occasion-setting (see Holland 1985). The presence of the extinction context promotes retrieval of the extinction memory, while the acquisition context does the same for the CS-US memory. Hirsch (1974) proposed that when conflicting information is learned (e.g., ambiguous CS history following extinction) the addition of a "context label' can differentiate between conflicting memories. Context-dependent memory retrieval may be a more accurate explanation of the psychological processes underlying the renewal of extinguished responding.

The evidence reviewed above suggests that Bouton's (1993; 1994) views on extinction and Hirsch's (1974) ideas about contextually facilitated memory retrieval may together provide a more accurate account of the psychological processes that underlie extinction. While the influential Rescorla-Wagner model sees extinction as resulting in inhibitory learning to the context, there is little evidence to support this claim (Bouton & King 1983; Bouton & Swartzentruber 1986, 1989; but see also Larrauri & Schmajuk 2008 and Polack et al. 2011). Furthermore, this account cannot provide an easy explanation of renewal under conditions in which test contexts are equated for training history (Delamater et al. 2009). While this review has focused on the Rescorla-Wagner model and contextual-modulation of memory retrieval, other models that emphasize mechanisms such as attention or inhibitory stimulus-response learning should be acknowledged (see Delamater 2004; Rescorla 2004; Robbins 1990).

Extinction research has not been limited to purely behavioral studies, the neuroscience literature approaches extinction and its related phenomena from a different angle, and has led to additional insights. Studies focused on identifying extinction's underlying neural circuitry have implicated areas in the central nervous system as mediating contextually modulated responding (see Corcoran & Maren 2001; 2004). These data provide support for the idea that context representations modulate responding following extinction. A review of the research pertaining to brain structures found to be crucial for extinction learning is presented in the following section.

**Neural Mechanisms of Extinction**

In addition to developing a theoretical understanding of the principles that govern extinguished behaviors, much effort in recent years has been directed towards identifying and analyzing the neurobiological substrates crucial for the extinction of fear. This research implies the coordinated involvement of three brain regions in regulating responding to extinguished fear stimuli. For example, the ventromedial prefrontal cortex (vmPFC), specifically the prelimbic and infralimbic regions (Milad & Quirk, 2002; Quirk, 2000), the amygdala (Falls, Miserendino & Davis 1992; Hobins, Goosens & Maren, 2003; Ledgerwood, Richardson & Cranney 2003; Walker, Ressler, Lu & Davis 2002) and the hippocampus (Corcoran & Maren 2001; 2004; Holt & Maren 1999; Maren & Holt 2000) have been shown to be involved in regulating responding to extinguished CSs. The next sections will review experimental findings regarding the function of vmPFC, the amygdala and the hippocampus in relation to extinction.

**I. The Amygdala**

Given that the amygdala has been found to be crucial for fear conditioning (Ledoux 2000; for a review see Fanselow & Poulos, 2005) many researchers have investigated the role of this structure in the extinction of fear. A number of empirical findings suggest that the amygdala is a site of plasticity in acquisition as well as extinction of fear learning. For example, localized infusion of the NMDA receptor antagonist AP5 into the basolateral (BLA) and basomedial (BMA) nuclei just prior to extinction training disrupts fear extinction (Falls et al. 1992; Zimmerman & Maren 2010). Rats that received infusions of AP5 into the amygdala showed a dose dependent impairment on a recall test 1 day after extinction

training compared to rats that received either amygdalar vehicle infusions or AP5 into the interpositus nucleus of the cerebellum, a condition which addressed the possibility that treatment with AP5 may be sufficient to induce freezing. In addition, systemic injection of NMDA receptor agonist D-cycloserine (DCS) prior to as well as immediately following extinction training facilitates fear extinction recall the following day (Ledgerwood et al. 2003). Walker et al. (2002), infused DCS into the amygdala prior to extinction training and found that extinction was facilitated by the drug compared to animals that were treated with either vehicle infusions or systemic injection of the DCS blocker, HA-966 prior to DCS injection. While these studies show that DCS infusions facilitate extinction learning and performance, this benefit is limited to the extinction context. Bouton, Vubric and Woods (2008) showed that systemic DCS during extinction training did not prevent fear renewal when animals were tested outside of the extinction context.

While NMDA receptor stimulation can initiate intracellular chemical cascades, which can facilitate fear extinction blocking this process conversely disrupts fear extinction. For example, Lu and colleagues (2001) compared the behavioral effects of infusing the mitogen-activated protein kinase (MAPK) inhibitor PD98059 to vehicle infusions into the BLA or dorsal hippocampus prior to extinction training in fear conditioning. MAPK inhibitors block the initiation of various cellular processes, including synaptic changes associated with learning. Only rats that received PD98059 into the BLA showed a significantly stronger potentiated startle response than control subjects in a test occurring 24hrs after extinction training. This result suggests that these infusions blocked the protein synthesis needed for consolidation of extinction learning in the BLA. Another group of rats that received the MAPK inhibitor infusions into the BLA during extinction was tested the

day following extinction without any drugs and then again the day after that with the MAPK inhibitor. These subjects showed more fear on the drug free test as well as on the subsequent PD98059 test compared to rats treated with vehicle during the infusion sessions, providing an argument against state dependent learning explanations of the extinction impairments observed in animals treated with PD98059 into the BLA during extinction. Because amygdalar infusions of drugs that block either the initiation of cellular restructuring or the protein production necessary for these changes block the behavioral expression of extinction learning, it is very likely that changes in the amygdala are crucial for fear conditioning and extinction. Similarly, drugs that initiate the biochemical events result in better performance, providing further evidence to this end (Ledgerwood et al. 2003; Walker et al. 2002).

Chhatwal, Myers, Ressler and Davis (2005) reported that the levels of messenger RNA (mRNA) as well gephyrin (a GABAa receptor clustering protein) in the amygdala were found to decrease during fear conditioning and increase during extinction. Specifically, animals that received only light-shock pairings prior to having their brains extracted possessed less gephyrin in the BLA than animals presented only with the CS (light), or US (shock) during training. These authors also showed that rats who had undergone extinction expressed higher gephyrin concentrations in the BLA compared to animals who were initially conditioned but then simply exposed to the context. Furthermore, animals who were given more extinction trials (i.e., 120 trials) possessed more gephyrin in the BLA than those who had fewer trials (i.e., 10 trials). These findings suggest that some GABAergic process in the amygdala is responsible for inhibiting the CR in fear extinction (see Harris & Westbrook, 1998). In a related finding, Likhtik, Popa, Apergis-Schoute, Fidacaro and Pare

(2008) reported that intercalated cells (ITC) in the amygdala are required for fear extinction. These authors produced post-extinction lesions of the ITC layer in the amygdala using saporin toxin (which inactivates ribosomes) conjugated to dermorphin, an agonist for μ opioid receptors, which within the amygdala are most abundant in ITC cells. In a subsequent test, only ITC lesioned subjects exhibited significantly higher levels of freezing to the CS during this test, suggesting that ITC cells may be necessary for inhibiting the fear CR. ITC cells possess GABAergic projections to the central nucleus of the amygdala (CeA), that are presumed responsible for inhibiting the CR following extinction, as CeA outputs lead to motor neurons responsible for responding (Fanselow & Poulos, 2005). These findings add to those of Chhatwal et al. (2005) in providing evidence of GABAergic mediation of extinction within the BLA. Proliferation of GABAergic projections may be a fundamental change brought about by extinction learning which when blocked or facilitated as described in the preceding paragraph can have opposing influences on CRs (Falls et al. 1992; Lu et al. 2001; Ledgerwood et al. 2003; Walker et al. 2002).

Electrophyisiological recordings in the amygdala provide further evidence of this structure's involvement in fear conditioning and extinction. For example, BLA cells showed increased firing to a stimulus paired with shock, and furthermore, returned to pretraining firing levels following extinction (Milad, Vidal-Gonzalez & Quirk, 2004; and Repa et al. 2001). Repa and colleagues (2001) reported that a small subset of cells continue to respond to the CS following extinction providing physiological evidence that learning survives extinction. Collins and Pare (2000) showed similar results using a discriminative fear conditioning procedure in which presentations of a CS+ (a stimulus paired with shock) increased activity in the BLA and a CS- (a CS not paired with shock) inhibited activity

relative to baseline firing rates in restrained cats. Following extinction of these cues, activity induced by the CS+ fell back to baseline levels, while CS- induced activity rose back to baseline.

More recently, Herry, Ciocchi, Senn, Demmou, Muller and Luthi (2008) replicated this procedure using mouse subjects and identified two classes of cells in the BLA. Fear neurons showed increased firing during acquisition relative to CS- induced activity. These 'fear neurons' reduced their firing rate during the extinction phase. Extinction neurons in the BLA on the other hand, showed a slight reduction in firing rate to the CS + during acquisition and an elevation in activity to the same CS during the extinction phase. While the location of these extinction cells within the BLA is not known, it is attractive to think that these extinction cells may be directing activity of ITC cells, which as described earlier, are capable of inhibiting motor outputs via the CeA. While these results do not allow for such a conclusion to be made, they do provide further evidence that plasticity within the BLA is crucial in both the acquisition and extinction of learned fear.

Interestingly, Hobins, Goosens and Maren (2003) provide evidence that amygdala responses to CSs are regulated by context. These authors reported that BLA activity mirrors what is observed behaviorally in a renewal procedure. Specifically, just as more CRs are seen when a CS is tested outside of its extinction context, activity of BLA neurons also increases under these conditions compared to when the stimulus is tested in its extinction context. While Herry et al. (2008) showed that BLA activity is stimulus-specific, the current findings provide evidence that the physical context a stimulus is tested in can also gate BLA activity. The authors suggest that the hippocampus may send environmental information via direct connections to the BLA or via indirect connections through the

vmPFC. The possible interactive mechanisms will be discussed later, though from the evidence discussed above, it is clear that a role for the amygdala exists in both fear learning and extinction.

**II. Ventromedial Prefrontal Cortex**

The research described below suggests that prefrontal activity can regulate responsiveness of cells belonging to different nuclei within the amygdala. This section will begin by describing effects of general vmPFC manipulations and then move on to more selective studies looking into vmPFC sub-regions and finally studies that consider vmPFC-amygdala interactions in extinction.

Laurent and Westbrook (2008) demonstrated that both muscimol induced inactivation of, and NMDA blockers infused into the vmPFC impair the retention of extinction learning, but not its development. Specifically, rats were trained to fear a context by having it paired with shock. During extinction rats either had ifenprodil (an NMDA receptor antagonist), muscimol (a GABA receptor agonist which 'turns off' cells it reaches) or the vehicle infused into the vmPFC. During this session rats treated with ifenprodil as well as those treated with muscimol showed within session response reduction comparable to controls. Immediately following this session animals received a second infusion into the vmPFC. Some of the animals extinguished with vehicle were infused with ifenprodil and the others with muscimol. The remaining animals all received vehicle infusions following extinction. The resulting groups were Veh/Veh, Veh/Ifen, Veh/Mus, Ifen/Veh, Mus/Veh. Only subjects in gp Veh/Veh displayed low levels of fear the following day during a drug free test, indicating that ifenprodil blocked the consolidation of extinction learning in the Veh/Ifen

group. Santini, Ren, Pena de Ortiz and Quirk (2004) reported a similar disruption in extinction learning as a result of infusing anicomycin (a protein synthesis inhibitor) into the vmPFC. These disruptive effects on extinction recall imply that the vmPFC may play a role in consolidation, as within-session CR reduction proceeds normally in drug treated animals during extinction. Further support from this conclusion comes from Quirk et al., (2000) showed that rats with lesions of the infralimbic (IL) nucles of the vmPFC exhibited enhanced spontaneous recovery to an extinguished CS that was previously paired with footshock. Based on these results the authors also suggested that the IL may be involved with the consolidation of extinction learning. While the exact function of the IL cannot be discerned from the studies discussed above, it is clear that the IL is necessary for the normal expression of extinction. However, Chang and Maren (2010) have reported that different strains of rats do not all show extinction recall impairments following IL lesions. Specifically, Sprague-Dawley rats were impaired by IL lesions while Long-Evans rats were not.

The subregions of the vmPFC have isolated projections to different amygdala sub nuclei (see figure 2 below), which may be regulated by hippocampally based representations of contexts. This possibility will be discussed in more detail following a discussion of studies into hippocampal contributions to extinction. For now, the description of this figure will be limited to vmPFC-amygdala connections. Projections labeled as pathway 'b' from the IL to the basolateral amygdala (BLA) can inhibit the central nucleus of the amygdala (CeA) via GABAergic ITC cells (Vertes, 2004; McDonald, Mascagni & Guo, 1996; Pinto & Sesack, 2003; see also Chhatwal et al. 2005). Excitatory projections from the BLA to the ITC layer serve to activate inhibitory projections from the ITC layer which inhibit CeA outputs. Alternatively,

via pathway 'a' excitatory projections from the BLA to the CeA can be inhibited by interneurons within the BLA. Conversely, the prelimbic (PL) nucleus of the vmPFC can excite the CeA through efferents to the basomedial amygdala, which sends excitatory projections to the CeA (Vertes, 2004; McDonald et al. 1996). As the CeA is the main motor output of the amygdala, knowledge of the afferents to the CeA just discussed is helpful in understanding the results discussed in the following paragraphs.

**Figure 2**

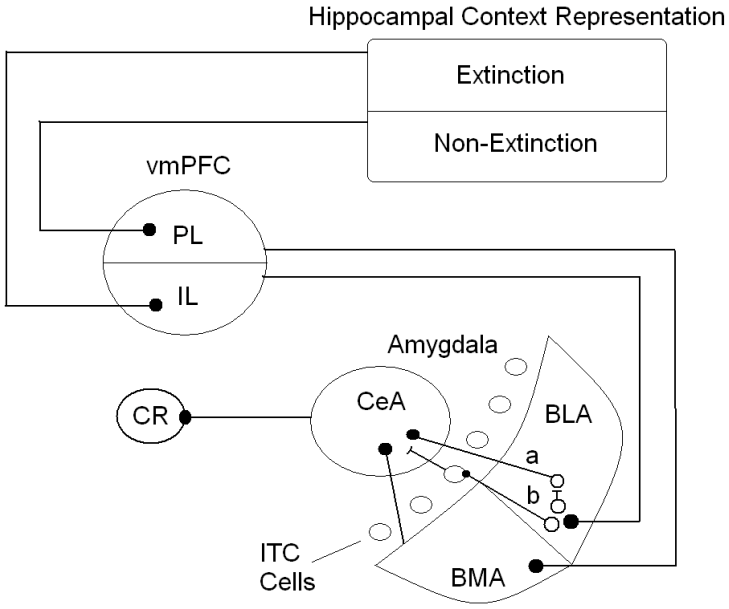



**Figure 2:** Regulation of central amygdala response output (i.e., CR) through hippocampally modulated pre and infralimbic afferents to amygdala subnuclei (based on suggestions from Corcoran & Qurik, 2007). vmPFC = ventomedial prefrontal cortex, PL = prelimbic, IL = infralimbic, BLA = basolateral nucleus, BMA = basomedial nucleus, CeA = central nucleus. Two separate means of inhibition applied to the CeA are denoted as 'a' and 'b'. Path 'a' suppresses CeA outputs by interneuron inhibition of excitatory BLA to CeA connections. Path 'b' inhibits the CeA using excitatory projections to inhibitory ITC (intercalated) cells.

Using electrophysiological recordings, Milad and Quirk (2002) found that whereas IL cells were unresponsive to a CS undergoing extinction, they were responsive the following day to that same CS. This is consistent with the distinction between within- session and the

recall of extinction in so far as how the effects of infusions into the vmPFC exert their influence on retention and not within-session performance. These authors also demonstrated that pairing a fear CS with IL stimulation accelerates extinction to that cue compared to non-stimulated controls. Because IL stimulation reduces fear CRs, but IL activity does not appear until after extinction training, it seems likely that the IL is needed to inhibit CRs to an extinguished stimulus, especially early in a test session. The facilitation IL stimulation has on extinction performance may simply be reflective of the role the IL plays post extinction, as it is not naturally engaged until after a CS has undergone extinction (see Mueller et al. 2010). Conversely, Vidal-Gonzales et al. (2006) showed that PL stimulation paired with a fear CS retarded extinction (this result was marginally significant) and resulted in more freezing on a subsequent test.

Quirk et al. (2003) assessed the possibility that the vmPFC may accomplish the task of mediating CRs by interacting with the amygdala. These authors showed that microstimulation of the IL inhibits downstream CeA activity, suggesting that while the isolated pathway from the IL to the BLA is a key component of the response inhibition seen in extinction, the PL-BMA pathway may serve an opposite function. However, sufficient data is not present to draw that conclusion. In further considering the results of the Laurent and Westbrook (2008) study described above, it seems likely that their infenprodil infusions directly prevented (or indirectly in the inactivation condition) biochemical changes in the IL that allow for post extinction CR inhibition. These findings together with those of Quirk and colleagues provide strong evidence that the vmPFC regulates the activity of amygdala subregions in response to extinguished cues. These subregions can inhibit CeA outputs via ITC cells (Likhtik, et al. 2008).

**III. The Hippocampus**

Investigations into hippocampal function have generated numerous theories as to the structure's importance. On the basis of anatomical connections, early theorists suggested that the hippocampus might be devoted to processing olfactory information (Finger, 2001). Not until Henry Molaison, better known as H.M., had tissue (including that of the hippocampus) removed bilaterally from the medial temporal lobe (in order to treat epileptic seizures) did considerable advancement in the understanding of this structure's function begin to emerge. This procedure left Molaison with an inability to form certain kinds of new memories while leaving other memory formation abilities intact. For example, H.M. showed normal acquisition of motor tasks such as mirror drawing, but no memory for the researchers who had trained him on the task. This deficit with memory led to H.M. being the most studied case in neuroscience (Corkin, 2002). Early animal researchers studying the structure's role in memory noted that animals given surgically induced hippocampal damage exhibited hyperactivity, which led to the hypothesis that the hippocampus may be involved in inhibiting motor responses (Roberts, Dember & Brodwick, 1962; Shull & Holloway 1985). In fact, a considerable amount of research has shown that damage to the hippocampus affects a variety of learned behaviors including aversive trace conditioning (Esclassan, Coutureau, Di Scala, & Marchand, 2009) and conditioned taste aversion (Stone, Grimes & Katz, 2005). While the inhibition and olfactory processing theories have been discarded due to a lack of supporting data, other ideas have come to the forefront of research into general hippocampal functions, the most compelling of which suggests that the hippocampus is involved in spatial learning and navigational abilities (Moser, Kropff, & Moser 2008). Additionally, the structure has been implicated in

renewal phenomena (Corcoran & Maren 2001; 2004). The currently dominant view in the extinction literature is that the structure is able to represent the physical context where extinction occurs. This role is not very unlike the general function thought to currently apply to the hippocampus (i.e., mapping and navigation). Representing the physical context in which an event occurs is an essential component of flexible learning, as the context can then come to associate with other attributes of the learning situation (Corcoran & Quirk 2007; Smith 1910). The first line of research that will be addressed in the following subsections considers data that demonstrate that the hippocampus is critically involved in spatial learning and navigation. Following this, the discussion will move towards data which suggest that hippocampal function is utilized to represent the physical context in which learning episodes takes place. The discussion will then depart onto 1) the modulatory role that context is thought to serve in extinction and 2) assessing the possibility that a network consisting of the amygdala, hippocampaus and vmPFC mediate extinguished CRs using context (Corcoran & Quirk 2007).

**1. Spatial Memory & Navigation**

Recent research on hippocampal function strongly suggests that a primary function of the structure is supporting spatial learning and navigational abilities. For example, specialized hippocampal neurons called place cells, have been shown to keep track of an organism's location within an environment while head direction cells track the animal's orientation (Moser et al. 2008; O'keefe & Nadel 1978). Wilson and McNaughton (1994) recorded from place cells in non-anesthetized rats while they were foraging within a four-arm radial maze. The activity of these cells was also monitored while the rats slept, both

prior to and following the foraging experience. These researchers showed that the same cells which were active during the foraging experience showed correlated activity during subsequent non-REM (non rapid eye movement) sleep, but not during the sleep session prior to the training session. These results suggest that the neural connections coding for the spatial information learned during the foraging session (i.e., locations of food in the maze) were being consolidated during sleep.

Furthermore, researchers have modeled hippocampal-damage-induced memory impairments in laboratory animals. Spatial tasks show heavy dependence on the hippocampus in these studies. For example, in the Morris water maze task a rat is placed into a large vat of opaque liquid, with a safety platform hidden somewhere in the tub an inch or two below the surface of the water. Once the rat locates the platform, the latency with which the animal swims to the platform on subsequent immersions into the pool rapidly decreases. Many researchers have shown that this task is hippocampally dependent (Bannerman et al. 1999; Stone, et al. 2005). Animals that have had their hippocampi lesioned or inactivated do not show a memory for the platform location when tested, suggesting that the structure is critically involved in either the storage or retrieval of these spatially-defined memories.

Another spatial task on which hippocampal animals are found to be impaired is the non-matching to place task (McHugh et al. 2008). Using a T-maze, a rat is placed in the start box and is forced (by the presence of a wall) to make a turn into one of the two arms of the 'T' where the animal receives a small amount of food reinforcement. The rat is placed back in the start box for a delay (e.g., 120 sec) and is then given a choice between the two arms of the maze – with the correct choice being to visit the arm that was not previously visited

(i.e., to alternate the response). For making the correct choice, the rat receives a large food reward, and no reward for making the incorrect choice. Animals that have had their hippocampi lesioned or inactivated show severely attenuated accuracy on this task (i.e., they perform at chance while control animals typically score 80-100% correct).

Collectively, these results suggest that the hippocampus is crucially involved in navigating through environments and relating events of significance to the locations in which they occur. In each of the spatial tasks discussed above the impairments caused by hippocampal damage suggest that some spatially-defined memory is ablated or inaccessible. The evidence for spatial processing in the hippocampus may permit the extension of the structure's function to include that of representing physical contexts. Building from this framework, the next section will review research which suggests that this is the function of the hippocampus when a classically conditioned stimulus undergoes extinction (i.e., representing the context).

**2. Context Representations**

Much attention has been given to understanding the role of the hippocampus in learning and memory in a variety of animals, such as monkeys (Squire & Alvarez 1995), rats (Kim & Fanselow 1992), mice (Cho, Beracochea, & Jaffard 1993), rabbits (Kim, Clarke & Thompson 1995) and humans (Scolville & Milner 1957). In a major review on hippocampal research O'keefe and Nadel (1978) proposed that the structure is involved in context-dependent memory and the psychological representation of physical space. These suggestions were initially based upon the reported deficits seen in hippocampal rats in maze studies (e.g., Olton 1972). The findings and ideas presented by O'keefe and Nadel (1978) presented a

new perspective from which the role the hippocampus in learning and memory could be studied.

Rudy and Sutherland (1989) proposed that the hippocampus 'binds' the elemental components of an organism's experiences. These researchers reported that hippocampally lesioned rats show deficits on negative patterning tasks. For example, rats were rewarded for pressing a lever in the presence of a tone as well as in the presence of a light, but not when the two were presented in a compound stimulus. Control rats learned the negative patterning discrimination perfectly well, while hippocampal rats showed an inability to inhibit responding to the compound. However, other reports have brought the validity of these findings into question (Gallagher & Holland, 1992; Jarrard & Davidson, 1991; Davidson, McKernan & Jarrad, 1993). Davidson et al. (1993) compared rats with different lesion sources (i.e., kainic acid and ibotenic acid) and found that both groups learned a negative patterning task comparably to controls. Kainic acid lesions did however, produce elevated locomotor behavior that Davidson et al. (1993) suggested may have masked rats having acquired the discrimination in Rudy and Sutherland (1989). While Davidson et al. (1993) assessed negative patterning by averaging responding to the compound over an interval, Rudy and Sutherland (1989) recorded responding immediately following the compound presentation. This difference in response requirements suggests that the short probe used by Rudy and Sutherland (1989) was insufficient to assess negative patterning.

More recently, however, Driscoll, Howard, Prusky, Rudy, and Sutherland (2005) reported impairments on a nonlinear spatial discrimination task using visual stimuli. In this task rats were trained to associate a visual stimulus (displayed on a monitor) with the location of a safety platform in the water maze task described earlier. Pairs of these stimuli

were then given discrimination training, one presented with the platform underneath, and the other without. A plexiglass barrier divided the water maze so that the monitors could be placed in fixed positions on either the left or right side of the barrier. The location of the platform was under one of the two monitors, which was signaled by the available stimuli. Three sets of stimuli were used. Two of these sets were trained in linear elemental discriminations (e.g., A+ vs B-, C+ vs D-, and E+ vs F-), while the last set was trained using a nonlinear transverse discrimination (e.g., A+ vs B-, B+ vs C-, and C+ vs A-). Rats given post-training hippocampal lesions showed performance impairments on both the transverse and elemental tasks compared to sham rats. Furthermore, retraining the discriminations in the absence of the hippocampus revealed normal reacquisition of the elemental but not transverse discriminations. The authors interpret these results as implying a few points about hippocampal function. First, that elemental discriminations can be solved by two different systems, one being dependent upon the hippocampus and the other independent, with the hippocampal system dominating in an intact organism. Secondly, that the hippocampus is crucial for forming configural representations that incorporate spatial stimuli (but not discrete stimuli as seen in Davidson et al. 1993). In other words, during the non-linear task, the hippocampus is involved in forming 'A - B' and 'B - C' representations that, despite the presence of a common B element, remain sufficiently separable from one another so that one representation can guide swimming towards 'B' and the other towards 'A'. The inability to bind these elements into distinct representations results in impaired performance by hippocampally lesioned animals on the task, as the non-hippocampal system is not capable of the same feat. The two points brought up by Driscoll et al. (2005)

find support in the work by Fanselow and colleagues who have produced a large body of data looking at the role of the hippocampus in contextual fear learning.

Fanselow (1990) reported that rats given a single context shock pairing displayed subsequent context fear only if the shock occurred 1 minute or longer after placement in the chamber. Fanselow interpreted these results as suggesting that following placement into the chamber, a 'gestalt' representation of the context begins to be developed that can later come to associate with the footshock US and produce fear of the chamber. This interpretation explains why rats shocked immediately upon placement in the chamber fail to exhibit fear to the context in later tests – these subjects have not processed the context sufficiently to produce an aggregate representation of the environment. Upon being shocked, the limited local features of the context being processed at that time cannot support fear to the context as a whole. Further support for this view comes from studies in which protein synthesis inhibitors are injected into the hippocampus following context-shock training and are shown to disrupt context fear (Anagnostaras, Gale & Fanselow, 2001).

Kim and Fanselow (1992) extended these findings to show that electrolytic lesions of the dorsal hippocampus result in a temporally graded retrograde amnesia for contextual fear memories (i.e., more recent events were more likely to be forgotten). Specifically, rats had a training context paired with footshock at various time points prior to (e.g., 1day vs. 1 month) receiving hippocampal ablation. Post surgery context fear was positively correlated with the amount of time between training and ablation. Lesions made immediately following training produced the greatest context fear deficit. Maren, Aharonov and Fanselow (1997) further showed that rats are capable of acquiring normal context fear

when trained following hippocampal lesions. Interestingly, these findings supports Sutherland and colleague's suggestions that 1) a non-hippocampal system can support associations of contextual elements with a US and more importantly for the topic at hand, 2) that the hippocampus stores conjunctive representations of the contextual elements present at training. Although Fanselow and Sutherland use different language to describe the phenomenon, both are suggesting that the hippocampus represents the training context or spatial attributes of an environment in some way. Based on the work from their lab, Fanselow and colleagues suggested that the hippocampus is involved in forming unified 'gestalt' representations of the contextual CS, and that the structure temporarily maintains this representation until it can be transferred to a more permanent store (e.g., neocortical association areas). Regardless of the validity of these claims regarding temporary stores and extrahippocampal consolidation, it is evident from the work of Fanselow and colleagues that the hippocampus is involved in representing physical space in some way.

McLaren, Kaye and Mackintosh (1989) presented a model to account for perceptual learning effects that can apply to the process presumed to be occurring in the hippocampus (i.e., the representation of context). Borrowing from stimulus sampling theory (Estes 1950), McLaren et al. (1989) suggested that when even a simple stimulus (e.g., a tone) is repeatedly pre-exposed, a subset of its elements are sampled on each presentation. Over the course of pre-exposure, as more and more elements are sampled a 'unitized' or central tendency representation of the stimulus can develop. This central tendency representation can facilitate the subsequent discrimination of that stimulus from other pre-exposed stimuli (i.e., the perceptual learning effect; Gibson & Walk, 1956). The facilitation of

discriminative learning as a result of pre-exposure is what McLaren et al. (1989) sought to explain with this theory, though Bennett, Tremain, and Mackintosh (1996) later suggested that this idea could be applied to understanding the immediate shock deficit (Fanselow 1990) discussed above as well. In this framework, a small portion of contextual elements is assumed to be randomly sampled when a rat is put into a chamber and immediately shocked. However, when the rat is again placed into the chamber for a test of fear, the elements of the context that are sampled this time will be mostly previously un-sampled elements, and hence untrained ones, resulting in little to no exhibition of conditioned fear. By pre-exposing rats to the chamber, not only can more individual elements get sampled, they can also become associatively linked with one another. Thus, when the pre-exposed rat is again placed into the chamber for a test of context fear, the nonconditioned elements of the context that may be sampled are capable of activating the other elements of the context that were sampled and learned about during training through interelement associations. The result of this is that more fear should be displayed in pre-exposed animals compared to those given shock immediately upon placement into the chamber.

To investigate this idea, Bennett et al. (1996) used four groups of rats in a one-trial flavor aversion learning paradigm. Two groups of rats were conditioned (i.e., had illness paired) with 4mL of a complex solution while the other two groups were trained with only 1mL (referred to as Gps 1 & 4). Two further groups received similar treatment in addition to having a single pre-exposure session with 3mL of the training solution (referred to as Gps 3/4 and 3/1). The use of these parameters served to make this procedure and the context fear procedure in Fanselow (1990) analogous to one another. For instance, the 1 vs 4mL comparison mirrored the different groups that were put into the context and shocked

immediately (i.e., 1mL) compared to those allowed to explore the chamber (i.e., 4mL). The use of a complex flavor solution (i.e., a mix of monosodium glutamate, quinine and sucrose) took the place of the complex contextual stimulus. Furthermore, the pre-exposure of flavors was analogous to the context pre-exposures shown to eliminate the immediate shock deficit (Fanselow 1990). Bennett et al. (1996) found that without pre-exposure, consuming 4mL prior to illness inducing lithium chloride injections produced a stronger aversion to the training solution than consuming 1mL. In addition, pre-exposure to the training solution in groups 3/1 and 3/4 resulted a stronger aversion than that showed by the non-pre-exposed 1mL group. Moreover, this effect was not seen if a simpler flavor solution was conditioned (e.g., sucrose or hydrochloric acid). These results provide support for a unitized representation interpretation of the results of Fanselow (1990). Though, Fanselow (1990) and Bennett et al. (1996) propose somewhat different interpretations of the immediate shock deficit, a common attribute between the two is the formation of some central tendency representation. While Fanselow (1990) refers to a 'gestalt' representation and Mclaren et al. (1989) argue for interelement associations creating a "unitized" representation, a central theme is the binding or connection of multiple sensory elements into some aggregate representation of the stimulus - which in the case of a contextual stimulus, has been shown to depend upon the hippocampus (Fanselow 1990). It is unclear, however, whether the hippocampus is required for the effect obtained by Bennet et al. (1996) in flavor-aversion learning (see Ward-Robinson, Coutureau, Good, Honey, Killcross & Oswald, 2001; Stone et al. 2005).

**3. Contextual Modulation of Memory**

There is evidence that contextual-modulation of memory retrieval is a hippocampally dependent phenomenon (Holt & Maren 1999; Maren & Holt 2000; Corcoran & Maren 2001, 2004; Ji & Maren 2005). One instance of contextual modulation, renewal refers to the re-emergence of an extinguished CR brought about by presenting the CS outside of the physical context in which it was extinguished (Bouton 2004). Investigation of the neural circuits of extinction learning has produced evidence that the hippocampus is involved in the contextual control over extinguished aversive learning or 'renewal' (Holt & Maren 1999; Maren & Holt 2000; Corcoran & Maren 2001, 2004; Ji & Maren 2005). This section will discuss both the role of context as a modulator and data that implicate the hippocampus as being involved in contextually modulated memory retrieval.

Some early studies showed that while full hippocampal damage eliminated reinstatement of fear, fear extinction itself as well as its context dependence were unaffected (Wilson et al. 1995; Frohardt et al. 2000). More recently, Corcoran and Maren, (2001; 2004) reported that temporarily inactivating the dorsal hippocampus (DH) prior to testing can influence the renewal of extinguished aversive learning. These researchers compared test performance of rats that received muscimol infusions into the DH to control animals that received infusions of the vehicle. Muscimol is a potent GABAa receptor agonist that can hyperpolarize neurons for a number of hours, preventing them from firing action potentials and communicating with other cells (see Edeline, Hars, Hennevin & Cotillon 2002).

Specifically, Corcoran and Maren (2004) trained rats to fear a CS in one context (A). The CS was then extinguished in either the same context (A) or a different one (B). Rats were

then tested for retention in either context A, B or a third context (C). Combining these treatment sequences results in 3 conditions in which renewal of responding would be expected, AAB, ABA and ABC (the letters refer to the sequence of context exposures experienced throughout the experiment). For example, a subject in an AAA condition was in context A throughout all three phases of the study (i.e., acquisition, extinction and test). This subject would not show renewal when tested because the test occurs in the extinction context (the other control condition is ABB). Renewal in AAB, ABA and ABC conditions is due to the test occurring outside of the extinction context. According to Bouton (1993), this circumstance contributes additional activation to the inhibitory CS-US extinction memory via the modulatory context link. Corcoran and Maren (2004) treated half of the rats tested in each context with muscimol and the other half with the saline vehicle. Their tests revealed that DH inactivation disrupts both AAB and ABC renewal relative to vehicle treated control rats, but leaves ABA renewal intact. While muscimol induced DH inactivation did not eliminate ABA renewal, Ji and Maren (2005) reported that electrolytic DH lesions did. The authors attribute this discrepancy to differences in the extent to which muscimol and electrolytic treatment of brain tissue disrupt neurochemical activity. Additionally the timing of the neural manipulations differed with lesions occurring 1-week prior to test and muscimol infusions 20-25 min prior. The authors assert that the results of Holt and Maren (1999), Maren and Holt (2000), Corcoran and Maren (2001: 2004) and Ji and Maren (2005) strongly suggest a role for the hippocampus in context-dependent memory retrieval and that interactions with the amygdala may be involved in this process. More recently, however, the emerging consensus in the literature is that the hippocampus, vmPFC and amygdala interact to control extinguished CRs (Corcoran & Quirk 2007; Milad,

Wright, Orr, Pitman, Quirk & Rauch 2007; Sotres-Bayon, Cain & Ledoux 2006; Herry et al., 2008, Bouton, Westbrook, Corcoran & Maren 2006). The following section will describe the data relevant to this suggestion.

**IV. vmPFC & Hippocampus Work Together to Regulate Amygdala Activity**

Corcoran and Quirk (2007) and others (Milad, et al. 2007; Sotres-Bayon, et al. 2006; Herry et al., 2008; Bouton, et al. 2006; Ji & Maren 2007) have suggested that projections from the hippocampus to the vmPFC (see Swanon 1981; Conde Maire-Lepoivre, Audinat, & Crepel 1995; Tierney, Degenetais, Thierry, Glowinski, & Gioanni 2004; Ishikawa & Nakamura 2003) may regulate amygdala activity. According to this idea, hippocampal-vmPFC interactions serve to process the present context, which can then modulate the retrieval of the CR via the amygdala (see figure 2). As described above, the amygdala is capable of both the promotion of a CR via connections between its basomedial nucleus (BMA) and its central nucleus (CeA) as well as the inhibition of a CR through connections between the basolateral nucleus (BLA) and the CeA (Likhtik et al. 2008). Figure 3 shows the circuit comprised of the vmPFC, amygdala and hippocampus proposed to exert contextual control over extinguished responding. As two possible mechanisms by which the BLA can exert inhibitory control over the CeA exist, both are present in this model (specified as 'a' or 'b' and described earlier).

According to this model, when a CS is tested in its extinction context the hippocampus excites the infralimbic nucleus of the vmPFC. The IL then suppresses activity in the CeA through either one of the two inhibitory mechanisms in the BLA (i.e., a or b), which inhibits CR output. However, when a CS is tested outside of the extinction context, the hippocampus

excites the prelimbic nucleus of the vmPFC. The PL promotes conditioned responding by exciting the BMA, which directly excites the CeA. The hippocampally based context representations are referred to as 'extinction' or 'non-extinction'. While the results of Harris et al. (2000) suggest that extinction results in the contextualization of both excitatory and inhibitory CS-US memories, renewal has been shown to occur in contexts without training history (e.g., AAB and ABC renewal) in addition to that of acquisition (e.g., ABA renewal). Therefore any context representations that do not promote retrieval of the inhibitory CS-US memory are referred as non-extinction representations in this framework.

Evidence of these structures interacting in extinction has accumulated in recent years. For example, Milad et al. (2007), showed that in humans, that the recall of fear extinction activates the vmPFC and hippocampus in synchrony using fMRI imaging, suggesting that these structures are both engaged by extinguished CSs (see also Lang, Kroll, Lipinski, Wessa, Ridder, Christmann, Schad & Flor 2009). Furthermore, Kalisch, Korenfeld, Stephan, Weiskopf, Seymour and Dolan (2006) showed that this synchronous activity is seen only when testing occurs in the extinction context. Perhaps most compelling are the results of electrophysiology studies. For example, Farinelli, Deschaux, Hugues, Thevenet and Garcia (2006) showed that low frequency stimulation of neurons in the DH evokes potentials in the rat vmPFC (i.e., IL). The potentials evoked were similar in rats that had tone-shock pairings, or just tone presentations. However, extinction training was shown to amplify this activity only in rats that received tone-shock pairings. This provides support that the hippocampus communicates with the vmPFC, and that extinction engages this pathway. Furthermore, Siapas, Lubenov and Wilson (2005) demonstrated that the activity of a large group of neurons in the medial prefrontal cortex of awake, freely behaving rats are phase

locked to the theta rhythms generated in the hippocampus, with hippocampal activity preceding that of prefrontal by 50 ms. This further suggests that the hippocampus transmits signals to the vmPFC, as the same patterns of activity are seen first in the hippocampus and then 50 ms later in the vmPFC.

In considering the emerging picture from neurophysiological as well as behavioral and ablation/inactivation studies it seems that the process Bouton (1993; 1994) proposed to explain how contexts retrieve 'different meanings' of an extinguished CS possesses an anatomical correlate in how hippocampal-vmPFC processing may regulate the ultimate output of the amygdala. The possibility that the amygdala receives regulation by hippocampal-vmPFC context processing may provide a mechanism for the reports of how amygdala activity tracks training and extinction, as well as its sensitivity to the test context (see Hobin, et al. 2003). In fact, Quirk et al. (2003) and Vidal-Gonzales et al. (2006) have provided evidence along these lines as mentioned above. Further support comes from Maren and Hobin (2007) who showed that the context-dependent activity shown to occur in the amygdala (see Hobin et al. 2003) is dependent upon the hippocampus. These authors showed that by inactivating the DH with muscimol the activity in the amygdala was no longer context-sensitive. Collectively these studies provide strong support that hippocampal-vmPFC interaction can regulate activity in the amygdala, which has been shown to be a crucial component in the expression of fear CRs. Corcoran and Quirk (2007) have suggested that further investigations in the viability of this circuits' control over extinguished CRs should attempt to record vmPFC activity in response to extinguished CSs tested inside and outside of the extinction context. This should provide useful information

on the extent to which the hippocampal-vmPFC connections provide contextually gated information.

While much progress in understanding the neural circuits of extinction has been made, much of the work has focused on fear conditioning. Whether or not one would expect that these same circuits mediate these behaviors in the domain of appetitive learning has not been sufficiently investigated. The potential parallels between the circuits that mediate contextual control over extinguished responding in appetitive and aversive learning will be the focus of the following section.

## V. Parallels Between Investigations in Appetitive & Aversive Learning

Recently, researchers have shown that there is some convergence between the neural substrates found to be important for aversive and appetitive learning phenomena (Fanselow & Poulos, 2005; Holland & Gallagher, 2004; Rhodes & Killcross 2004; Quirk et al. 2000). For example, pretraining lesions of the infralimbic nucleus (IL) of the vmPFC have been shown to enhance spontaneous recovery and reinstatement of extinguished responding in aversive learning (Quirk et al., 2000). Rhodes and Killcross (2004) ran an appetitive version of the Quirk et al., (2000) study and obtained the same result of increased spontaneous recovery in IL lesioned animals. Though, Rhodes and Killcross interpret their data differently in that they suggest that the IL may be involved in contextual modulation of extinguished CRs, as spontaneous recovery has been interpreted as an instance of renewal, with time acting as contextual stimuli (see Bouton, Westbrook, Corcoran, & Maren 2006).

Additionally, the BLA, has been shown to be important in aversive (e.g., Chhatwal, Myers, Ressler, & Davis, 2005; Fanselow & Poulos, 2005) as well as appetitive learning (e.g., Holland & Gallagher, 2004). There have also been attempts to assess the extent of crossover between the neural processes underlying renewal in appetitive and aversive learning. Delamater, et al. (2009) investigated the role of the GABAa receptor in renewal of appetitive learning while Harris and Westbrook (1998) did so in aversive learning. Both studies utilized systemic injections of the GABAa receptor inverse agonist FG 7142, which reduces GABAergic transmission in the central nervous system, prior to testing rats with extinguished CSs. In Delamater et al. (2009), two CSs were paired with food in two different contexts (e.g., Context 1: A+ & Context 2: B+). Each CS was then extinguished in the other CS's training context (e.g., Context 1: B-, Context 2: A-) before both were tested in each context. Prior to tests, animals were given systemic injections of FG7142 (2.5, 5.0 or 10mg/kg) or its vehicle. FG 7142 treated rats showed a dose-dependent reduction of appetitive response renewal. Looking at fear conditioning, Harris and Westbrook (1998) trained rats to fear a stimulus by pairing it with shock. Rats were then given extinction in either the same or a different context. Regardless of whether or not extinction occurred in the same context as training, when tested, FG7142 treated animals showed comparable levels of fear within and outside of the extinction context compared to control animals who showed reduced fear in the extinction context, but not elsewhere. As the pharmacological manipulation was found to have opposing effects in the two learning domains, the authors suggested that opposing motivational systems may be an important part of the extinction circuit. While these studies have provided some insight into possible parallels between the two motivational domains, analysis of hippocampal involvement in the renewal of

extinguished appetitive learning is lacking. However, attempts have been made to study the hippocampus in other appetitive learning phenomena. Consideration of these findings may provide some expectations for studying the role of the hippocampus in appetitive renewal.

Honey and colleagues have considered hippocampal functions in a variety of appetitive studies. For example, Good and Honey (1991) showed that despite hippocampal animals being able to perform well on a context based acquisition task (i.e., Context 1 paired with food vs. Context 2 not paired with food), they were impaired when CSs had selective context-dependent meanings. For example, two CSs (X & Y) were trained in two different contexts (A & B) as predictors of food. In the next phase, stimuli were trained in both contexts, with a particular CS being reinforced in one context but not in the other (i.e., A: X+, Y- vs. B: Y+, X -). Control animals quickly showed a reduction in CRs when the CSs were presented in their nonreinforced contexts. Hippocampally lesioned rats, however, were slower in learning this task. The authors attribute this deficit to an inability in lesioned rats to use contextual cues to retrieve associative information. However, Coutureau, Killcross, Good, Marshall, Ward-Robinson and Honey (2002) used a similar design and found that animals given excitotoxic lesions of the hippocampus readily acquired the discrimination compared to control rats. Because Good and Honey (1991) used electrolytic lesions, it is possible that this difference in the lesion technique could explain the difference in results between these two studies.

Honey and Good (1993) extended their investigation of contextually-dependent phenomena to latent inhibition. Latent inhibition refers to the depressive effects on learning when one pre-exposes the CS prior to conditioning (Lubow 1973). The only procedural difference between extinction and latent inhibition is the order in which

reinforced and nonreinforced CS presentations occur in reference to each other. However, both of these phenomena show control by context and may be similar in some ways (see Westbrook, Jones, Bailey, and Harris 2000). Honey and Good (1993) pre-exposed animals to two CSs in two different contexts and then subsequently paired the CSs with food in one of the pre-exposure contexts. Control animals showed faster acquisition for the CS that was not pre-exposed in the training context. Hippocampally lesioned animals, however, showed retarded acquisition to both stimuli. Hippocampal lesions impaired acquisition outside of the pre-exposure context because, according to the authors, the contextual specificity of latent inhibition reflects the operation of a contextual retrieval process, which the authors assert is hippocampally dependent. This interpretation is similar to that of Westbrook et al. (2000), who in studying the contextual specificity of latent inhibition drew similarities between the psychological processes underlying extinction and latent inhibition. Westbrook et al. (2000) suggested that upon conditioning the pre-exposed stimulus, it becomes an ambiguous CS (similar to the case of extinction, see Harris et al. 2000). Furthermore, Westbrook et al. (2000) suggested that when a CS is ambiguous, context is used to selectively retrieve the training memory established in that context.

While the hippocampus has been shown to represent context in aversive learning (Fanselow 1990), the same function in appetitive learning seems to not depend on the hippocampus (Good & Honey 1991). However, the structure has been shown to be crucial for the contextual modulation of responding in both appetitive (Good & Honey 1991; Honey & Good, 1993) and aversive tasks (Corcoran & Maren 2001; 2004). Because of this possible similarity it is reasonable to expect that the ability of contextual information to modulate extinguished responding may be hippocampally dependent in appetitive learning

as it has been shown to be in aversive learning (Holt & Maren 1999; Maren & Holt 2000; Corcoran & Maren 2001; Corcoran & Maren 2004; Ji & Maren 2005).

Evidence that hippocampal lesions impair the rate at which rats acquire occasion-setting discriminations in appetitive conditioning was provided by Holland and colleagues (Holland et al. 1999). Specifically, these authors showed that while hippocampal lesions impair the speed with which rats acquire a feature positive occasion-setting discrimination (e.g., A-, AX+), they did not affect the asymptotic level of discriminative responding. The impact of hippocampal lesions on a feature negative task (e.g., A+, AX-) was more severe, with both rate and asymptote reduced by this surgical treatment. However, the results on ambiguous occasion-setting tasks (which include both feature positive and negative discriminations trained concurrently) are mixed. While Han, Gallagher and Holland (1998) reported that ambiguous occasion-setting was enhanced by hippocampal lesions, Gallagher and Holland (1992) reported that this treatment was without effect. It should be noted, however, that while the Gallagher and Holland (1992) study included an operant component in the discrimination, the Han et al (1998) study did not. Interestingly, Holland et al. (1999) point out that while serial occasion-setting discriminations are solved using a true occasion-setting strategy (i.e., the feature's modulatory properties are specific to its target CS) simultaneous occasion-setting discriminations are not. After training on a simultaneous task, the feature is capable of augmenting responding to other CSs in addition to its target, suggesting that the feature comes to directly associate with the US. However, in extinction treatments, because the context is thought to play the role of the feature in the modulatory account of extinction, it is not entirely clear whether simultaneous or serial

conditions are produced. Of importance, however, is the finding by Holland et al., (1999) that hippocampal lesions impair performance on the occasion-setting task.

**Introduction to Present Research**

The following experiments aim to examine the function of the dorsal hippocampus (DH) in the contextual control over an appetitive conditioned magazine approach responses in rats. Experiment 1 will observe the impact of inactivating the DH prior to testing for ABA renewal of magazine approach. Experiment 2 will do the same, however, in an ABC renewal design. Experiments 3 will evaluate the effects of post training excitotoxic lesions of the DH on ABA and ABC renewal. Another extinction phenomenon, spontaneous recovery, will be the focus of Experiment 4. This effect has been theorized to be a form of renewal where time is thought to function as a contextual cue (Bouton 1991; 1993; 1994; Bouton et al. 2006). In order to investigate the possibility that renewal and spontaneous recovery have common underlying mechanisms, the effects of DH inactivation on spontaneous recovery in the magazine approach task will be examined. If the renewal and spontaneous recovery of extinguished responding share an underlying neural mechanism, similar results may be observed when the DH is manipulated in both of these phenomena. Overall the results from the present set of studies should provide important information on how extinction learning is regulated both psychologically and neurologically.

## Experiment 1: The Effects of DH Inactivation on ABA Renewal of Magazine Approach

Studies of fear conditioning have found that while ABA renewal of fear is not impaired by inactivation of the DH (Corcoran & Maren 2004), it is impaired by electrolytic lesions of the same structure (Ji & Maren 2005). There has been no work examining the importance of the DH in appetitive renewal designs. Experiment 1 began exploring the importance of the DH in ABA renewal of appetitive learning by inactivating this structure prior to testing for renewal of magazine approach CRs (investigation of the impact of lesions on this behavior was explored in Experiment 3 below). The design used in Experiment 1 (see table 1 below) examines renewal as a within subjects variable and the effects of DH inactivation as a between groups variable. The design uses two contexts and two CSs in order to equate as much as possible, the history of the contexts at the time of test.

An important issue in understanding the psychological mechanisms of renewal is distinguishing between renewal effects caused by Context-CS summation or contextually modulated processes. Consider a basic renewal design where a CS is conditioned in one context, extinguished in another, and tested either in its training or extinction context. Research has shown that these conditions result in the establishment of an excitatory Context-US association for the acquisition context (see Harris et al., 2000). During extinction an inhibitory Context-US association is thought to develop for the extinction context (Rescorla & Wagner, 1972; but see Bouton & King 1983; Bouton & Swartzentruber 1986; 1989). Therefore, differences in responding to the CS between these two contexts may reflect a simple summation process where the Context-US association during the test phase sums with any residual excitatory CS-US association that remains after extinction. When tests are conducted in the acquisition context, this results in two excitatory

associations summing to produce net excitation and ultimately conditioned responding. This is contrasted with when tests occur in the extinction context. This situation results in the residual excitatory CS-US association summing with the inhibitory Context-US association. These opposing associative relations would cancel each other out and result in very low levels of CRs, thus promoting extinction performance. While summation effects between the CS and context have not been extensively researched, there is empirical evidence that such summation occurs (Grau & Rescorla, 1984; but also see Miller, Grahame & Hallam, 1990).

This summation process differs from the account Bouton (1993; 1994) provides of the psychological mechanisms of extinction. In Bouton's account, extinction performance becomes context dependent because extinction learning (i.e., an inhibitory CS-US association) requires the extinction context in order to be retrieved. The inhibitory CS-US association developed during extinction is assumed to be gated by the extinction context itself. When an extinguished CS is tested in its extinction context, the presence of the extinction context results in the retrieval of the inhibitory CS-US association learned during extinction and this reduces CRs. When the CS is tested in the acquisition context, the extinction memory is not retrieved but the excitatory association learned during acquisition is and this produces CRs. This conditional mechanism is more likely to be the cause of any renewal seen using a design like that used for Experiment 1 below.

In the design for Experiment 1 below, S1 is trained in Context 1 and S2 is trained in Context 2. During extinction this was reversed, so that S1 was extinguished in Context 2, and S2 in Context 1. Each CS was then tested in each context over two test sessions, each occurring after infusions of muscimol or its saline vehicle (see below for details). Because

both test contexts have served as acquisition and extinction contexts for different CSs any excitatory and inhibitory context-US associations should be equated. Therefore, renewal under the test conditions in Experiment 1 would more likely reflect an 'occasion-setting' like or modulatory process similar to what Bouton (1993; 1994) has suggested develops during extinction and is responsible for the renewal effect. However, this issue has not been consistently addressed in studies examining the neural substrates of fear renewal (but see Maren & Hobin 2007). This is an important distinction as the two implied psychological process differ.

| | | Infusions | | Infusions |
|---|---|---|---|---|
| **Acquisition** | **Extinction** | **Test 1** | **Rest** | **Test 2** |
| CX 1: S1+ | CX 1: S2- | CX 1: **(VEH)** S1-, S2- ? | 5 Days | CX 2: **(VEH)** S1-, S2- ? |
| CX 2: S2+ | CX 2: S1- | CX 1: **(MUS)** S1-, S2- ? | | CX 2: **(MUS)** S1-, S2- ? |

**Table 1:** Table 1 presents the design used in Experiment 1. CX 1 & 2 were distinct chambers in which the animals were trained. S1 and S2 were the 15 sec stimuli (tone or light). Plus signs indicate reinforcement with food pellets, while minus signs indicate nonreinforcement. Groups specified with MUS and VEH received either muscimol or its saline vehicle into the dorsal hippocampus 15 min prior to tests.

## Methods

### Subjects

Subjects were 12 experimentally naïve Long-Evans male rats run in two replications. There were six subjects in each replication. The subjects were bred and housed in standard clear-plastic tubs 10.5" x 19" x 8" with woodchip bedding within a colony room on a 14:10 light:dark schedule. The subjects were reduced to 85% of their free feeding body weight following a 1-week recovery period from cannula implantation (see surgery section below for details). Subjects weighed between 330g and 420g at the start of the study and were maintained within a +/-5g range of their target weights with supplemental feedings of

home cage chow given after experimental sessions on a given day. Subjects had free access to water while in their home tubs. Six subjects were assigned to each of the two groups for testing.

Surgeries

Subjects were anaesthetized using a 1% body weight injection of sodium pentobarbital at a 50 mg/ml concentration resulting in a dose of 50 mg/kg. Subjects were treated with a 1.35% body weight injection of atropine sulfate concentrated at 0.4 mg/mL 10min following injection of the sodium pentobarbital in order to aid respiratory function. The scalp was then shaved and the rat placed in a Stoelting stereotaxic apparatus for implantation of bilateral guide cannulae (Plastics One: 22 gauge, 10 mm long beyond pedestal with a 5 mm separation). Lubricant was placed on the cornea to maintain moisture and the head swabbed with a 10 % ethanol solution. An incision was made over the midline of the skull and the fascia pushed aside to reveal bregma and lambda. Once bregma and lambda were confirmed to lie on the same horizontal plane, the unit was calibrated for placement over the dorsal hippocampus by touching both tips of the cannula unit onto bregma and recording the coordinates for each tip. Two holes were drilled over the dorsal hippocampus and an additional 4 holes were drilled around the unit for placement of jeweler's screws. The cannulae were placed at the following coordinates relative to bregma: 3.8 AP, 2.5 ML, 2.5 DV. The unit was lowered and then fixed into place using dental cement. Once the cement was dry the edges of the incision to the scalp were sutured and antibacterial ointment was applied to the wound. The guide cannulae were plugged with obturators (Plastice One: 24 gauge, projected 0.5 mm beyond tips of guide)

and covered with a dust cap on top of the unit. Subjects were placed in a recovery area until locomotor activity was observed and then returned to their home tub for 1-week recovery with food freely available. Following surgery, obturators were changed every other day until the completion of the study.

## Infusions

Infusions into the dorsal hippocampus were done one hemisphere at a time. A 25 μL Hamilton syringe attached to PE50 tubing was connected to a 28 g injector (Plastics One), which extended 1mm beyond the tip of the guide cannula. The syringe was automated by a KD scientific pump (Model no: 780310) dispensing 1 μL of solution into each hippocampi at a rate of 0.32 μL/min. Subjects were restrained during this process and remained connected to the infusion line for an additional 2 min following each infusion. Muscimol was concentrated at 1 mg/mL in physiological saline. Control subjects received the same treatment except that only the saline vehicle was infused. Order of infusion was counterbalanced with regard to cerebral hemisphere.

## Histology

Subjects were anaesthetized using the sodium pentobarbital and transcardially exsanguinated with 0.9% saline and perfused using a 10% formalin solution. When extracted, brains were cryoprotected in a 20% sucrose + 10% formalin solution and stored in 10% formalin until sectioning between 3-4 days later. Sections were cut 40μm thick and placed in distilled water for mounting onto 2% bovine gelatin coated standard microscope slides. The samples were stained with cresyl echt violet for nissl bodies using the standard

protocol. Slides were held in xylene and then covered slipped using permount and let to dry. Sections were scored under an Optimus light microscope using Paxinos and Watson's (2005) atlas of the rat brain as a reference.

## Apparatus

Rats were trained in two contexts. Context One consisted of eight identical standard BRS Foringer conditioning chambers (30.5 cm x 24.0 cm x 25.0 cm) which were housed in individual light and sound attenuating shells. The two end walls of each chamber were made out of aluminum, while the side walls and ceiling were made from transparent Plexiglas. The floors consisted of 0.60 cm stainless steel rods spaced 2.0 cm apart. A recessed food magazine measuring 30 x 36 x 20 mm (length x width x depth) was located on one end wall of the chamber 12.0 mm above the grid floor. Positioned approximately 3 mm deep on the side walls of the food trough and 3 mm above the base of the magazine were an infrared emitter and detector which enabled the automatic recording of head entries inside the magazine. Head entries into the magazine were registered by the breaking of the infrared beam in the food trough.

Context Two consisted of another set of eight conditioning chambers in sound and light attenuating shells located in another room. The chambers that comprised Context Two were a different physical set of chambers in addition to being tailored to promote as distinct of a sensory experience as possible. Context Two was identical to Context One with the exception that stainless steel metal sheets were inserted into the chamber to alter its shape. One sheet completely covered the grid floor, and a second sheet was positioned diagonally (from the bottom of the wall opposite the food magazine towards the front

portion of the ceiling) to give the chamber a trapezoidal shape. With the metal inserts in place, the dimensions of the chamber became 29.2 cm x 30.5 cm x 9.5 cm x 24.1 cm (base x diagonal side x ceiling x vertical side).

All of the conditioning boxes were equipped with a 7.6 cm diameter 8 Ohm speaker (Radio Shack) mounted outside the magazine side of the chamber. This speaker delivered a computer generated 1500 Hz tone (T) amplified by a Radio Shack amplifier. The volume of the tone CS was 4 dB above the 78 dB background noise produced by fans mounted on the light and sound attenuated outer shells of the chambers for ventilation. The boxes also contained a 6 W light bulb (L) positioned at the base of the shell just behind the chamber. When activated these stimuli (T and L) remained on for fifteen seconds. Both stimuli terminated with the delivery of two 45 mg food pellets (Noyes, Formula A/I) delivered to the food magazine.

The T-maze used after the renewal study had metal bar floors (0.60 cm stainless steel rods spaced 2.0 cm apart) and opaque plastic walls with a clear ceiling. The walls of the maze were 6" high and the interior was 3½" wide. The main arm of the maze was 50" long. The upper length of the maze (i.e., the top of the T) was 67½" long, with food wells at each end. The start box at the base of the maze and each of the goal boxes at the top corners of the maze could be closed off by manually lowering an opaque plastic guillotine door. The maze was on a T-shaped wooden table raised approximately 4 ft from the floor. Room lighting was provided by overhead fluorescent lights.

Magazine Training

After the subjects reached their target body weights magazine training commenced. Subjects were placed in each context and had food pellets delivered to the food trough on a variable time 60 sec schedule for 20 min. Subjects were trained in one context on day one and then the other context on day 2 (with context identity counterbalanced).

## Acquisition

Following magazine training subjects were trained to associate two conditioned stimuli with food pellet reinforcers in two distinct contexts (see table 1 above for design). Subjects received two sessions each day for eight days, with one session occurring in each context and one stimulus being trained in each session. These sessions were separated by 30 min and lasted for 28 min. The order of these sessions, the identity of the contexts and the stimuli undergoing training in each location (T, L) were all counterbalanced. There were 8 trials in each session. Each trial consisted of a 15 s stimulus presentation that was immediately followed by the delivery of two food pellets. The average intertrial interval was 3 min with a range from 1-5 min. An additional two minutes were inserted at the end of the session following the last US delivery of each session.

## Extinction

Extinction was run in a similar manner to acquisition except that the stimuli were presented in the alternative context and they were no longer followed by food. Specifically, if T was paired with food in Context 1 and L was paired with food in Context 2, then T was extinguished in Context 2 and L was extinguished in Context 1.

The extinction phase lasted for 3-days.

## Tests

Subjects underwent two test sessions, with one occurring in each context. Test 2 occurred 5-days after test 1 in order to promote some general level of response recovery. During each of these tests, each stimulus was presented without food 4 times with the average intertrial interval and range the same as in the training and extinction phases. The order of stimulus presentations on test 1 was T, L, L, T, L, T, T, L and the order was reversed for test 2. Magazine entries were monitored on each trial starting 15 sec prior to CS onset until 20 sec following CS offset. Animals were infused 15 min prior to these tests with either muscimol or its saline vehicle (see above for details). Group assignments were made by matching for average responding to both stimuli on the last day of each preceding training phase (e.g., day 8 of acquisition and day 3 of extinction). Six subjects were assigned to each infusion condition as a between subjects variable. Between tests, subjects were weighed and maintained at their target weights daily. Test 2 was conducted five days later but in the alternative context.

## Delayed Non-Matching to Place Task

In order to evaluate whether the neural manipulation of DH inactivation was effective the subjects were run on a delayed nonmatching to place task on a t-maze. Performance on this task is impaired by DH inactivation (McHugh, Niewoehner, Rawlins and Bannerman, 2008). Following the second renewal test, animals were given four sessions of exposure to the T-maze over two days, with two sessions on each day. During these sessions both arms of the maze held two food pellets each and were freely accessible. Eight of the subjects from

the renewal study above participated in this follow up. The other subjects were euthanized and perfused as they had become ill either between the second renewal test and the commencement of maze training or over the course of maze testing. Rats were given a total of 10 min to explore the maze and retrieve the food rewards on day 1 and 5 min on day 2. If a rat picked up the food pellets before the allotted time had passed the animal was removed and returned home. If an animal failed to retrieve the food pellets they were removed and given another attempt 15 min later. The two daily sessions were separated by 2 hrs.

During training rats were transported to the maze room in their home tub with water freely available between trials. A single training trial consisted of two runs, one 'sample' run and one 'choice' run. On the sample run, one of the two arms was blocked at the junction while the available arm held a single food pellet. The side which was blocked off varied irregularly across trials. At the start of the trial, the rat was placed in the start box at the bottom of the 'T' and released. Once the rat retrieved the sample food pellet they were removed from that corner of the maze and returned to the start box for the choice run. Released immediately, the animal then was given a choice between the two arms. The correct choice in this task was the arm not visited during the forced sample run. This choice was reinforced with two food pellets. Choosing the incorrect arm resulted in no food, subjects simply remained in the incorrect corner for 10 sec and were then brought back to the waiting area until the next trial. The intertrial interval was approximately 4 min and each rat received 10 trials daily. Testing began once the subjects reached the criterion of performing at 80% accuracy over two consecutive days.

Testing consisted of imposing varying delays between the sample and choice runs 15 min following infusions of either muscimol or its saline vehicle. Tests were done first using a 30 sec interval, then using 60 sec and finally 120 sec. Each subject received 2 sessions at each of these delays, one session followed saline infusions and the other followed muscimol infusions. The order of infusions for each delay was counterbalanced, however, the delays themselves were not. Sessions 1 & 2 used the 30 sec delay, while sessions 3 & 4 used the 60 sec delay and Session 5 & 6 used the 120 sec delay.

Results

Data from the acquisition and extinction phases of the magazine approach task are presented in figure 3 below for each day of training. Preliminary analysis revealed that responding to the auditory stimulus was greater than to the visual stimulus during training $F$ (1,10) = 6.68, $p$<0.05, but this did not interact with either the group or training factors. Therefore, the data have been collapsed across this factor. Additionally, no significant effect of replication was found, so data were collapsed across this factor as well. In order to provide a measure of the conditioned effect of the stimuli, the data were expressed as CS-Pre CS difference scores. The training data were analyzed using a repeated measures ANOVA with an eight level within subjects factor of training day. Because preliminary analyses showed that group assignment did not interact with the factor of training day, the data were collapsed across this factor. The ANOVA yielded a significant main effect of training day $F$ (7, 70) = 7.93, $p$ < 0.05, indicating that as training progressed subjects came to respond more to the stimuli. The extinction data were analyzed using the same approach as for the acquisition data. A significant main effect for training day confirmed that

magazine approach was attenuated over the three days of extinction, $F$ (2, 20) = 19.94, $p$ < 0.01.

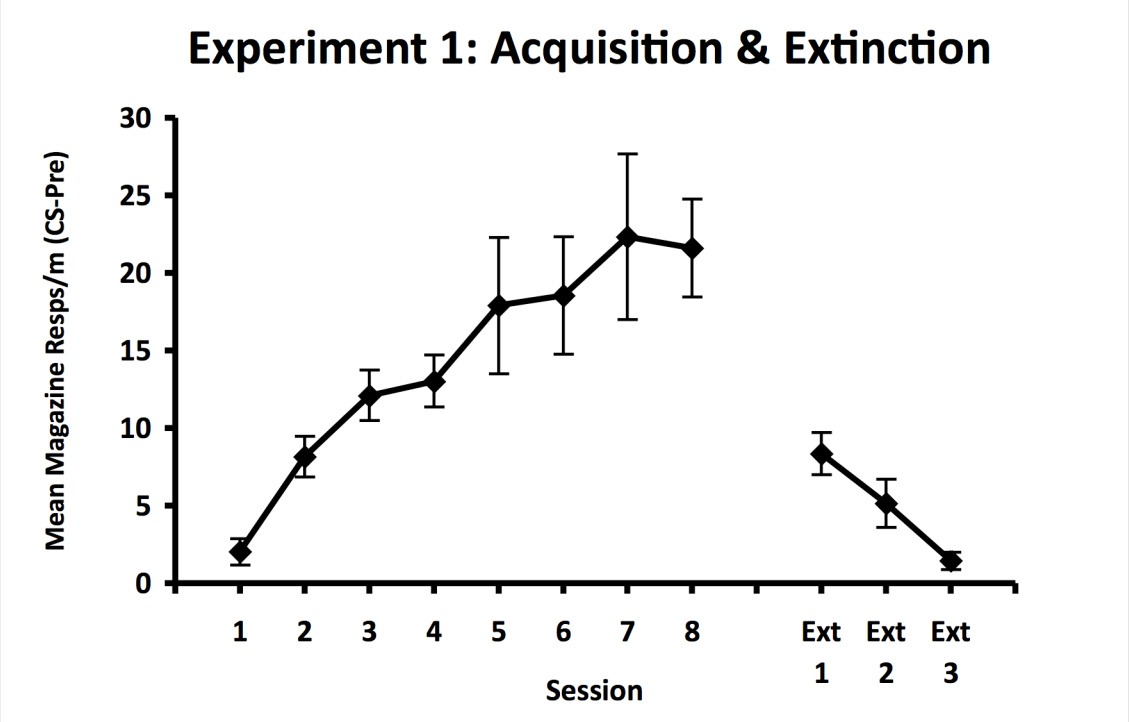



**Figure 3:** Figure 3 depicts mean magazine responses during acquisition and extinction in the form of difference scores (CS-Pre CS).

Data from the test phase are presented in figure 4 below. Because no interactions with test session were found, the data were collapsed across this factor. Responding during the 15 sec stimuli as well as the 20 sec post stimulus periods were converted into difference scores by subtracting from these values, responding during the 15 sec baseline (pre stimulus) period. Responding during the 20 sec period following CS offset was included in the analysis because food had normally been delivered at this point. Therefore, an increase in magazine responding following a CS compared to the baseline period would reflect

anticipation of reinforcement. Because each CS was tested in each context over the two test sessions, responding is expressed as a function of test location. In other words, stimuli that were tested where they had undergone extinction (i.e., CX1: S2, CX2: S1) were averaged together and are referred to as ABB compared to ABA stimuli, which were tested in their acquisition contexts (i.e., CX1: S1, CX2: S2). An analysis on Pre CS responding revealed no significant effects. The average Pre CS rates for subjects infused with vehicle were 2.3, and 1.4 responses per minute for the Pre ABB and Pre ABA intervals respectively. For the subjects infused with muscimol, the average Pre CS rates were 5.0 and 2.7 for the Pre ABB and Pre ABA intervals respectively. The data in figure 4 suggest that subjects infused with the saline vehicle prior to the test sessions showed more responding when a CS was tested in its acquisition context than its extinction context. This Renewal effect was evident during the stimuli as well as during the 20sec post-stimulus period when the food US had normally been delivered. The animals infused with muscimol did not differ from the control group. These impressions were confirmed by a 2x2x2 split plot ANOVA using recording interval (CS, Post CS) and stimulus condition (ABB, ABA) as within subject factors and infusion (Vehicle, Muscimol) as a between subject factor. This analysis revealed a significant main effect of stimulus, $F$ (1, 10) = 6.136, $p$ = 0.033, indicating that subjects responded more when a stimulus was presented in its acquisition context than in its extinction context. Furthermore, a significant effect of recording interval $F$ (1, 10) = 7.50, $p < 0.05$ indicates that subjects responded more during the stimulus than during the 20 sec post stimulus period. Importantly the renewal effect did not interact with the infusion factor, $F$ (1, 10) = 0.039, $p > 0.05$, confirming that both groups equally exhibited the effect. Furthermore, the stimulus effect did not interact with interval, $F$ (1, 10) = 2.17, $p > 0.05$.

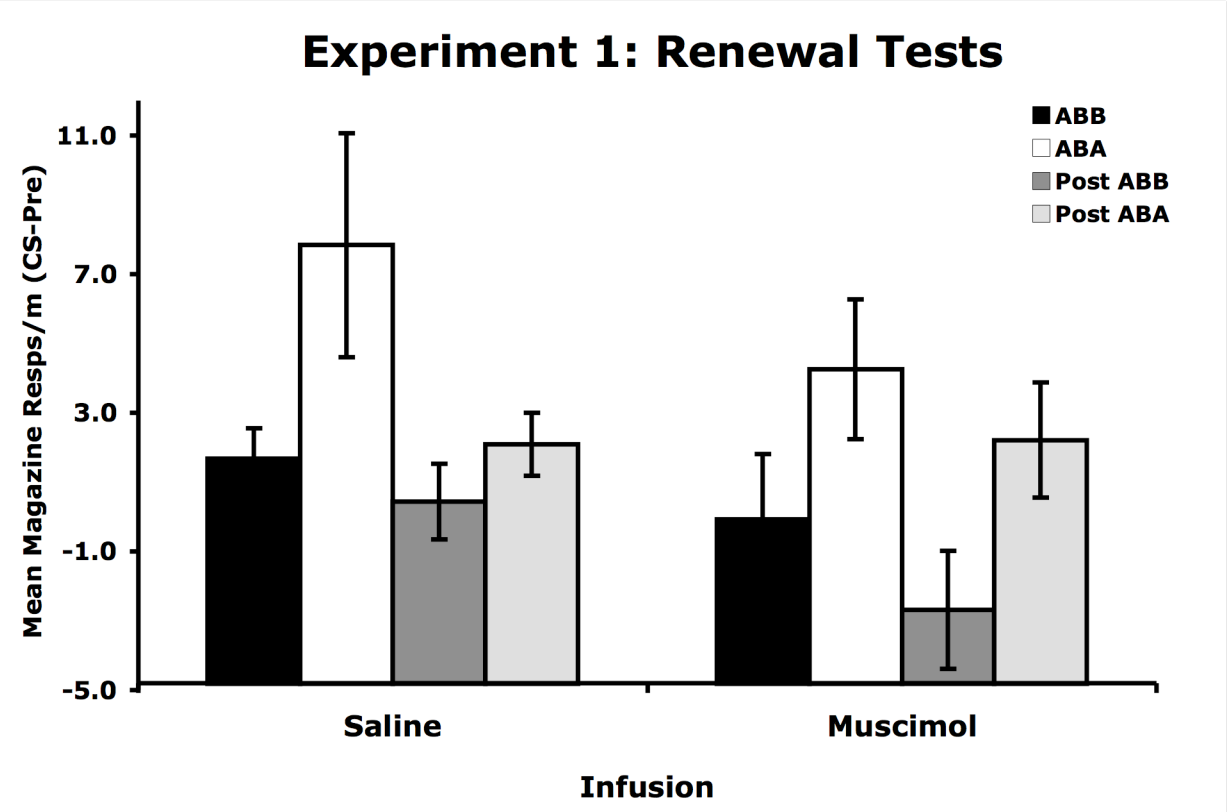



**Figure 4:** Figure 4 displays data from the renewal test phase in Experiment 1 presented in terms of ABB vs. ABA responding (i.e., responding either in the extinction or acquisition contexts). Data are presented in difference score form (CS-Pre CS) for mean magazine responses during the 15sec stimuli as well as during a 20sec post stimulus period during which food had been delivered during initial training.

Data from the T-maze task are presented in figure 5 below in terms of mean percent correct following infusions of muscimol or its saline vehicle irrespective of delay condition. Because preliminary analyses found no interactions between infusion and delay length this factor was ignored in the analysis. A 1-way ANOVA indicated that performance was significantly worse following infusions of muscimol than when testing occurred after saline infusions, $F\,(1, 7) = 7.63$, $p < 0.05$. A supplementary 2-way ANOVA found that the effect of Infusion did not interact with group assignment for the renewal task, $F\,(1, 6) = 0.357$, $p >$

0.05, indicating that animals in both infusion conditions displayed the delayed nonmatching to place impairment.

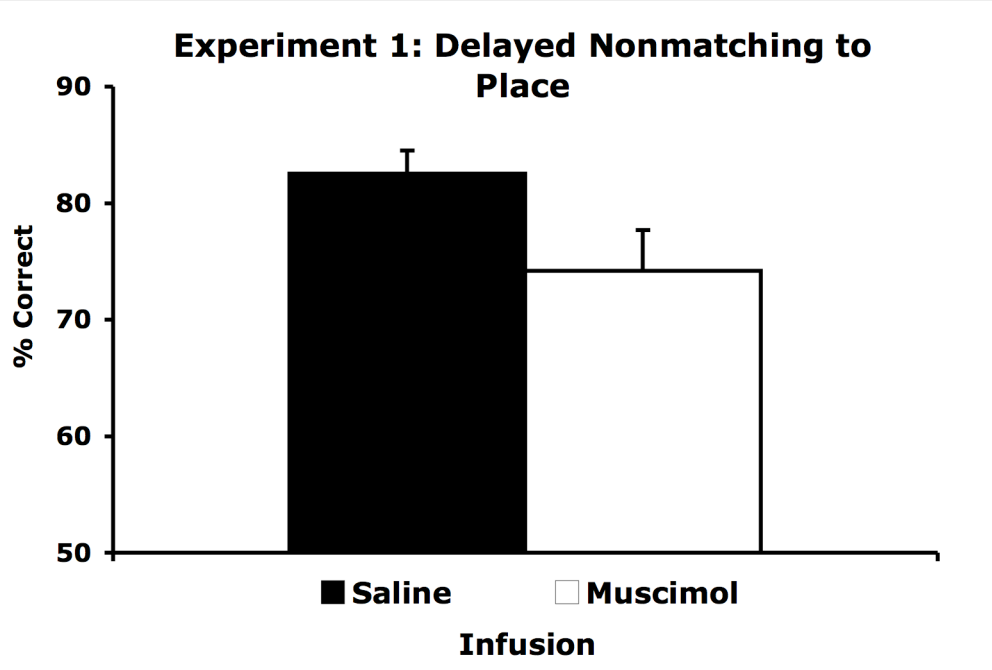



**Figure 5:** Data from the delayed non-matching to place task in Experiment 1 are presented in figure 5. The figure plots mean percent correct on trials following vehicle or muscimol infusions.

The results of the histological analysis are presented in figure 6 below for all animals included in this study. While four of the subjects did not contribute data to the delayed non-matching to place task, they are included in this figure because they did contribute data to the renewal test results. The figure shows the sites of infusion for all animals included in this study. Injection sites are indicated for subjects infused with saline during the renewal tests by white boxes and for subjects treated with muscimol during these tests with black boxes. These sites were identified at the base of the cannula tracks and their locations were determined by using a stereotaxic atlas as a reference (see methods section). The infusion

sites were mostly found to be along the dorsal portions of the hippocampus, with some sites located slightly more ventral towards the anterior part of the hippocampus, however, well within the dorsal most layer of the structure. No subjects were excluded from the analysis due to inaccurate placement of the cannulae.

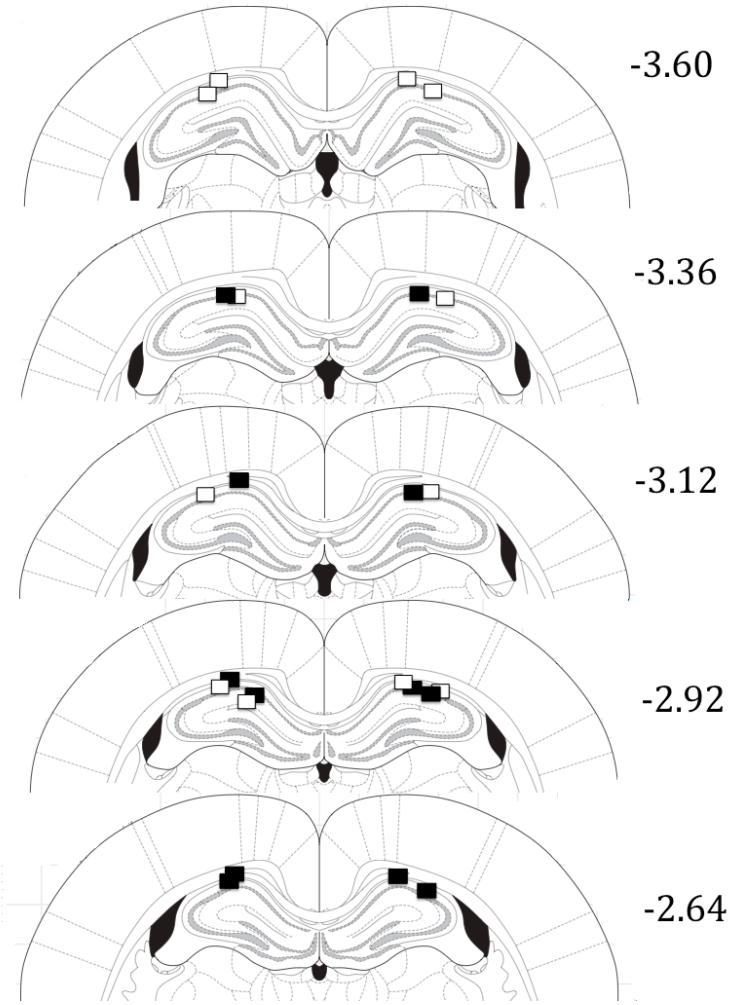



**Figure 6:** Figure 6 depicts the intracerebral infusion sites in each hemisphere of the brain for subjects included in Experiment 1 (each subject has two sites documented, right and left hemisphere). Group designations reflect assignment during the renewal tests with vehicle animal's sites represented by white squares and muscimol animals in black. The coordinates from which the atlas figures derive are found to the right of each atlas figure and are relative to bregma.

## Discussion

Subjects in both the vehicle and muscimol groups showed greater magazine responding when extinguished stimuli were tested in their acquisition as opposed to extinction contexts. In other words, the results from this study showed no effect of DH inactivation on ABA renewal. In the introduction for Experiment 1, it was pointed out that the design used in this study resulted in the test contexts having received equal reinforcement histories. Therefore, this pattern of data cannot readily be explained by the simple process of summation between CS-US and context-US associations and instead likely reflects some conditional learning process similar to that suggested by Bouton (1993; 1994). Furthermore, the fact that muscimol infusions did not disrupt renewal cannot be the result of a failure to accomplish the neural manipulation of dorsal hippocampal inactivation because using the same infusion procedures resulted in impaired performance on the subsequent non-matching to place task in the T-maze, which other research has shown to be dependent on DH (McHugh, Niewoehner, Rawlins & Bannerman, 2008). Finally, the histological analysis confirmed that infusion targets were accurate for all subjects included in this study as seen in figure 4. While no subjects were eliminated from the analysis due to misplaced injection sites, four subjects who participated in the magazine approach task did not survive to contribute to the delayed non-matching to place task. These animals became ill and were perfused so as to preserve their histological data. Overall, the results of Experiment 1 do not contradict those in the fear conditioning literature as Corcoran and Maren (2004) also reported that ABA renewal of fear was unimpaired by muscimol induced inactivation of the DH. Experiment 2 extends the analysis to ABC renewal of appetitive learning.

**Experiment 2: The Effects of DH Inactivation on ABC Renewal of Magazine Approach**

Experiment 2 investigated whether or not ABC renewal of magazine approach requires the dorsal hippocampus. While in ABA renewal the critical comparison is between responding to the CS in either the acquisition or extinction context. In ABC renewal the CS is tested either in the extinction context or in a third context. Because of this procedural difference ABA and ABC renewal may rely on different underlying mechanisms. In both ABA and ABC renewal, the removal of the subject from the extinction context would make it difficult to retrieve the extinction memory. However, in ABA renewal the CS is tested in the acquisition context and research has shown that this promotes retrieval of the acquisition memory more than when testing occurs in a third context (Harris et al. 2000). Thus, ABA and ABC renewal seem to depend upon overlapping yet distinct mechanisms.

Some researchers have shown that while ABA renewal of aversive learning (i.e., fear conditioning) is insensitive to muscimol-induced DH inactivation, ABC renewal is disrupted following this neural manipulation (Corcoran & Maren 2004). To address the possibility that this may also be true in appetitive conditioning, subjects in Experiment 2 received infusions of muscimol or its saline vehicle prior to tests for ABC renewal of magazine approach (see table 2 below for design). The design is similar to that used in Experiment 1 above with regards to equating the test contexts for conditioning history. In the first phase two stimuli (S1 & S2) were trained in the same context (context 1), during the same sessions. During the following phase, each CS was extinguished in a different context. For example, S1 was extinguished in context 2, while S2 was extinguished in context 3. Each stimulus then underwent testing in each of the two contexts used during the extinction phase. For example, test 1 was conducted in Context 2 and test 2 in Context 3, during each

test both CSs were tested under extinction conditions. Importantly, neither test context had any history of reinforcement. In fact, both contexts 2 and 3 served as extinction contexts and should, therefore, have both developed equivalent inhibitory Context-US associations. Any difference seen in responding to CSs during these test sessions is more likely to reflect a conditional learning process than the simple summation process discussed above. Corcoran and Maren (2001; 2004) as well as Maren and Hobin (2007) have reported that ABC renewal of fear is disrupted by muscimol infusions into the DH prior to tests. While the design used by Corcoran and Maren (2001; 2004) did not equate test contexts, the Maren and Hobin (2007) study did by using the same experimental design as that used here.

| | | Infusions | | Infusions |
|---|---|---|---|---|
| **Acquisition** | **Extinction** | **Test 1** | **Rest** | **Test 2** |
| CX 1: S1+, S2+ | CX 2: S1- | CX 2: **(VEH)** S1-, S2- ? | 5 Days | CX 3: **(VEH)** S1-, S2- ? |
| | CX 3: S2- | CX 2: **(MUS)** S1-, S2- ? | | CX 3: **(MUS)** S1-, S2- ? |

**Table 2:** Table 2 presents the design used in Experiment 2. CX 1, 2 & 3 were distinct chambers in which the animals were trained. S1 and S2 were the 15 sec stimuli (tone or light). Plus signs indicate reinforcement with food, while minus signs indicate nonreinforcement. Groups specified with MUS and VEH, received either muscimol or its saline vehicle into the dorsal hippocampus 15 min prior to tests.

## Methods

### Subjects

Subjects were 28 experimentally naïve Long-Evans male rats weighing between 366 g and 534 g at the start of the study. Subjects were bred, housed and maintained as previously described. Of these 28 subjects, 14 had muscimol infused into the dorsal hippocampus prior to tests while 14 received the saline vehicle. Group assignments were made in the same manner as described above. This study was run in two replications with 14 subjects in each.

## Surgeries

Surgeries were done as described above.

## Infusions

Infusion parameters were identical, though the technique used was modified slightly in the current experiment. Infusions were done simultaneously in both hemispheres using bilateral injector units from Plastics One (the units were the same dimensions as those described above). Each side of the injector was attached via PE50 to a 5 μL Hamilton syringe, the two syringes were operated manually. Infusions occurred over an approximately 1 min time window with the rat restrained for a further 2 min following the infusion.

## Histology

Histological procedures were as described above.

## Apparatus

The training chambers used were the same as those described above except that an additional set of inserts were used to produce a third context. This set of inserts consisted of a full Plexiglas floor with 5 cm diameter holes drilled approximately 5 cm apart from one another. Additionally, a solid Plexiglas wall 15'' in length was inserted so that the chamber was reduced to half of its total size resulting in a triangle shaped floor space for the portion of the chamber in which the rat was placed. Two of the chamber walls intersected each

other at a 90-degree angle while the plexiglass insert contacted both of these walls at acute angles. The insert was held in place by a machine bolt fastened with a nut through the plexiglass floor. The same T-maze described for Experiment 1 was used at the end of ABC renewal testing. The same conditioned stimuli, pellet reinforcer and response measure from Experiment 1 were used in this study.

## Procedure

### Magazine Training

Subjects underwent 2 sessions of magazine training, with one session each day for two days. Both sessions were conducted in a single context (Context 1) using the parameters described above. For all subjects, Context 1 was the bare training chamber (i.e., no inserts were in place), while the identities of Contexts 2 and 3 were counterbalanced using the two sets of inserts described above.

### Acquisition

Following magazine training subjects were trained to associate the two stimuli (T & L) with food in a single context. Stimuli were trained in the same session over 8 days. During each 56 min session, each stimulus was presented eight times in a random order and terminated with the delivery of two food pellets to the magazine. The intertrial interval and range were the same as in Experiment 1 with an additional 4 min included after the final US presentation.

## Extinction

During the extinction phase, each stimulus was extinguished in a separate context (Contexts 2 & 3), the identities of which were counterbalanced. Each day, subjects underwent two extinction sessions, one in each extinction context with one stimulus undergoing extinction in each session. During these sessions the appropriate stimulus was presented 8 times without reinforcement. The average intertrial interval and range during extinction were the same as during acquisition but the session duration was half as long as in acquisition. These daily sessions were separated by 30 min and run in a random order.

## Tests

Group assignments for the test phase were done as described for Experiment 1. Subjects underwent two tests, with one occurring in each of the two extinction contexts. The order of stimulus presentations on test 1 was T, L, T, L, L, T, L, T, L, T, T, L, T, L, T, L and this was reversed for test 2. Magazine approach was recorded as in Experiment 1, resulting in the Pre CS, CS and Post CS intervals. These tests were separated by 5 days. During each of these tests, each stimulus was presented without food 8 times with the other session parameters, including intertrial interval identical to the training phase. Animals were infused 15 min prior to these tests with either muscimol or its saline vehicle (see above for details). Between tests, subjects were weighed daily and maintained at their target weights.

## Delayed Non-Matching to Place Task

Maze exposure, training and criteria were as described for Experiment 1 above. Test sessions were conducted 15 min following infusions of either saline or muscimol. During

the test phase delays were inserted between the sample and choice runs. Tests were done using a 60 sec and a 120 sec delay interval. Each subject received 2 sessions at each of these delays, one session followed saline infusions and the other followed muscimol infusions. Both the order of infusions for each delay and the delays were counterbalanced. There were 10 trials per session during tests.

## Results

Out of the 28 subjects that started the study only 19 were included in the magazine approach task (12 subjects were infused with vehicle while 7 received muscimol). Of those excluded, two subjects' cannulae became loose and fell off during training, two rats were excluded due to improper placement of the cannulae and five were excluded due to problems during the infusions preceding the renewal test phase (e.g., animals either became very sluggish or incapacitated as a result of infusions). Out of the 19 rats given renewal testing, 3 subjects did not contribute to the delayed non-matching to place task because these rats became ill prior to or during the course of maze training. Of these three subjects, one had received muscimol during the renewal test phase while the other two served in the control group.

Magazine entry data from the acquisition and extinction phases are presented below in figure 7. Preliminary analysis, once again, revealed more responding to the auditory stimulus compared to the visual cue, $F$ (1, 17) = 35.77, $p$<0.01, but because there were no interactions with group assignment the data were collapsed across this factor. Additionally, there was no significant effect of replication during training and extinction, therefore the data were collapsed across this factor. In order to produce a measure of the conditioned

effect of the stimuli, the data were converted, as before, into difference scores (CS - Pre CS). Responding to the stimuli increased over training sessions. During extinction responding was quickly attenuated and remained low throughout extinction. It should be noted that in this phase the stimuli were presented in novel contexts, and this could account for why responding was immediately suppressed on the first day of extinction. A significant main effect of training day confirmed the impression that the differences in responding between the Pre CS and CS periods increased over training, $F$ (7, 126) = 29.2, $p < 0.01$. By the first day of the extinction phase, responding during the stimuli was attenuated such that no significant effects were observed in the analysis of those data. Responding remained very low through out the extinction phase.

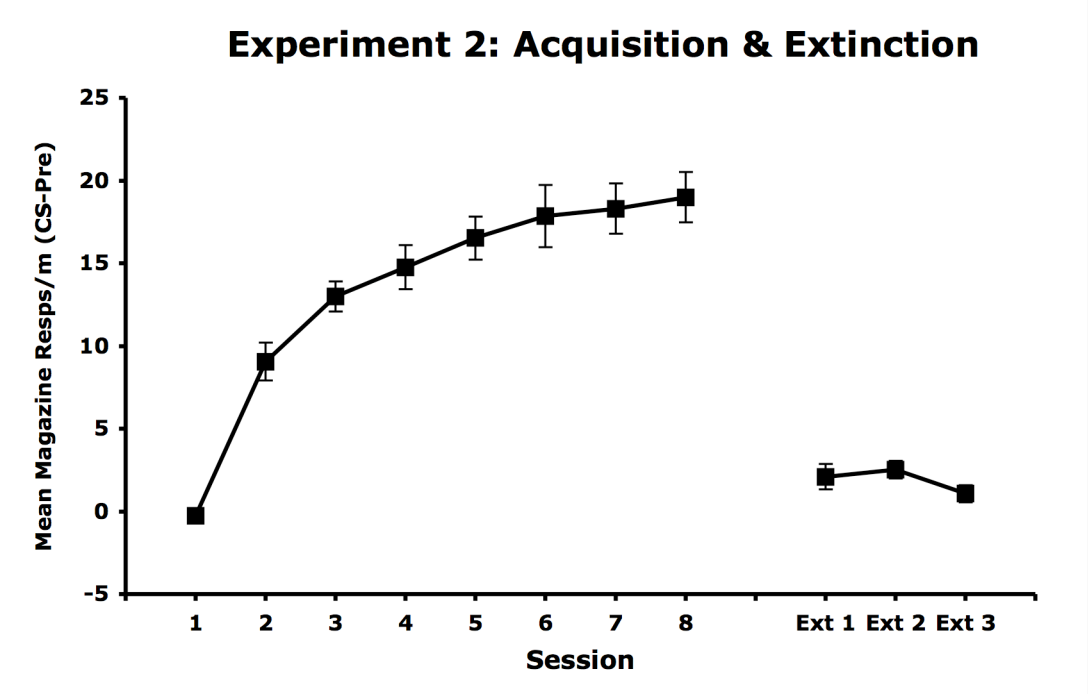



**Figure 7:** Figure 7 presents mean magazine responses during the acquisition and extinction phases in difference score form (CS-Pre CS) for Experiment 2.

Data from the test phase are presented in the form of differences scores (CS - Pre CS) in figure 8 below. Data are expressed as responding to a CS undergoing testing either in its extinction context (ABB) or outside of the extinction context (ABC). For example, when S1 was tested in Context 2, this was coded as an ABB condition, but when S1 was tested in Context 3 this was coded as an ABC condition. An analysis of Pre CS responding revealed no significant effects. For subjects infused with the saline vehicle, the average Pre CS response rates were 1.4 for Pre ABB and 1.3 for Pre ABC. For subjects infused with muscimol, those values are 1.9 and 1.6 responses per minute. Subjects infused with saline prior to being tested showed more CRs when a CS was tested outside of compared to within its extinction context. This effect was seen during the presentation of the stimuli as well as during the 20 sec period immediately following CS offset. Subjects who had their DH inactivated with muscimol prior to testing showed the same pattern of results as the control group. Preliminary analyses did not find a significant effect of test (e.g., test session 1 vs test session 2) or a stimulus x test session interaction, therefore, the data have been collapsed across this factor. Additionally, there were no significant effects of replication, and so the data were collapsed across this factor as well. A split plot ANOVA (Renewal (ABB, ABC) x Interval (CS, Post CS) x Infusion (Veh, Mus) revealed a significant main effect of Renewal, $F(1, 18) = 4.51$, $p<0.05$, which did not interact with any of the other factors. Finally, there was no significant main effect of Infusion despite the trend of overall reduced performance in the animals infused with muscimol.

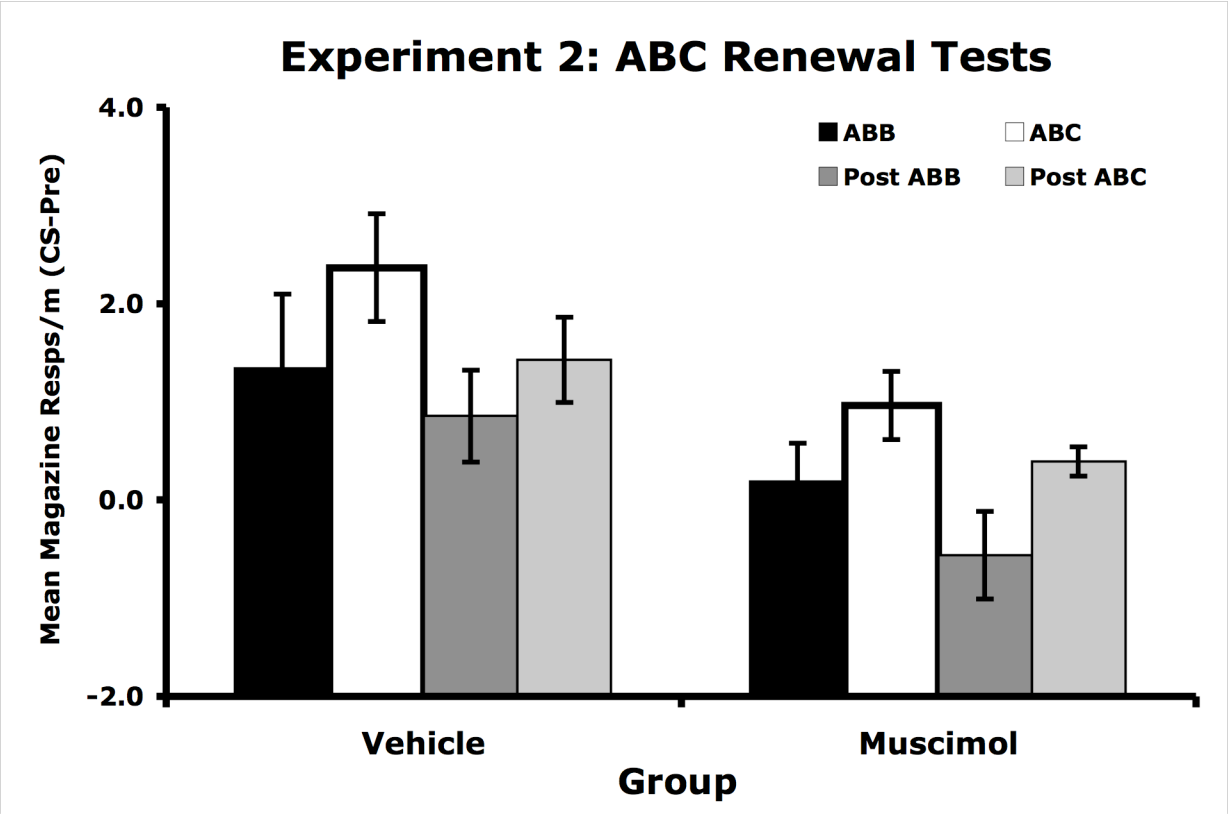



**Figure 8:** Figure 8 displays data from both renewal tests collapsed across stimulus to be presented in terms of ABB vs. ABC responding. Data are presented as difference scores (CS – Pre CS) and display mean magazine responses during the 15 sec stimuli as well as during a 20 sec post stimulus period during which food had been delivered during initial training.

Data from the delayed non-matching to place task are presented below in figure 9. Mean percent correct choice responding is shown as a function of delay (60 sec vs. 120 sec) and infusion condition (saline or muscimol). The figure shows that subjects performed worse (i.e., with reduced accuracy) on muscimol than vehicle sessions. This was true for both the 60 sec and 120 sec delay tests. The data were analyzed using an Infusion x Delay repeated measures ANOVA and revealed that subjects performed worse following muscimol infusions compared to its saline vehicle regardless of the delay interpolated between sample and choice runs, $F\,(1, 15) = 22.08$, $p < 0.01$. A supplementary ANOVA with group assignment during the ABC renewal task as a factor, revealed that subjects in the vehicle

and muscimol conditions during ABC renewal both showed the delayed nonmatching to place impairment.

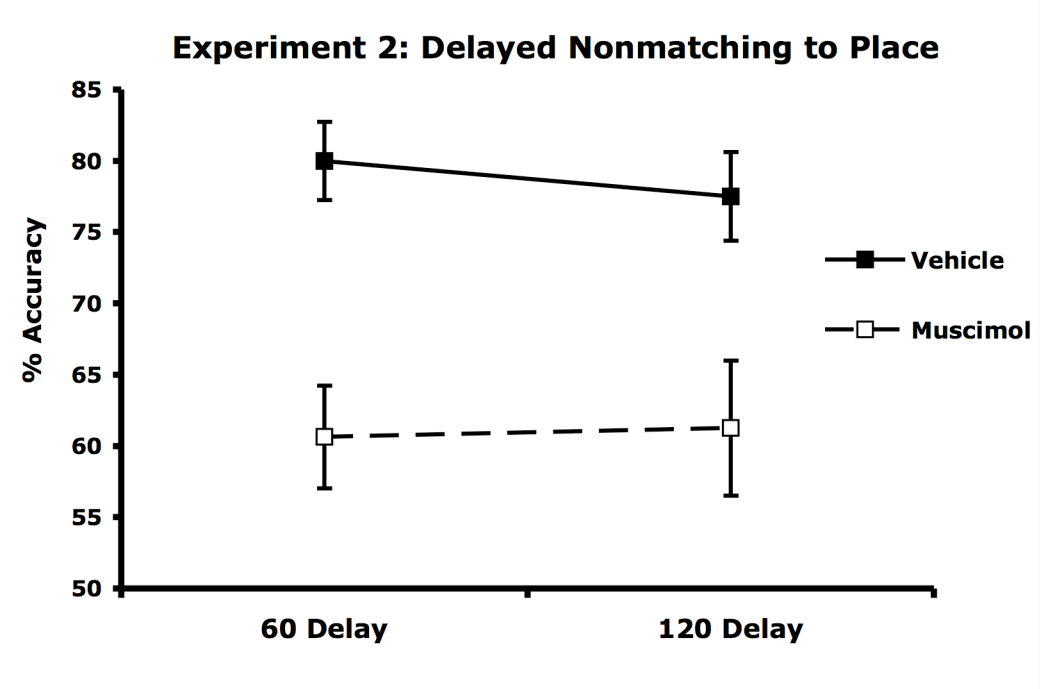



**Figure 9:** Data from the delayed non-matching to place task in Experiment 2 are presented in figure 9. The figure plots mean percent correct on trials following vehicle and muscimol infusions.

Histological results consisting of intracerebral injection sites for animals included in this study can be found in figure 10 below. The injections sites were located and referenced as described above. Generally the injections occurred at the boundary of the DH and the cortex with very few sites being found ventrally beyond this area.

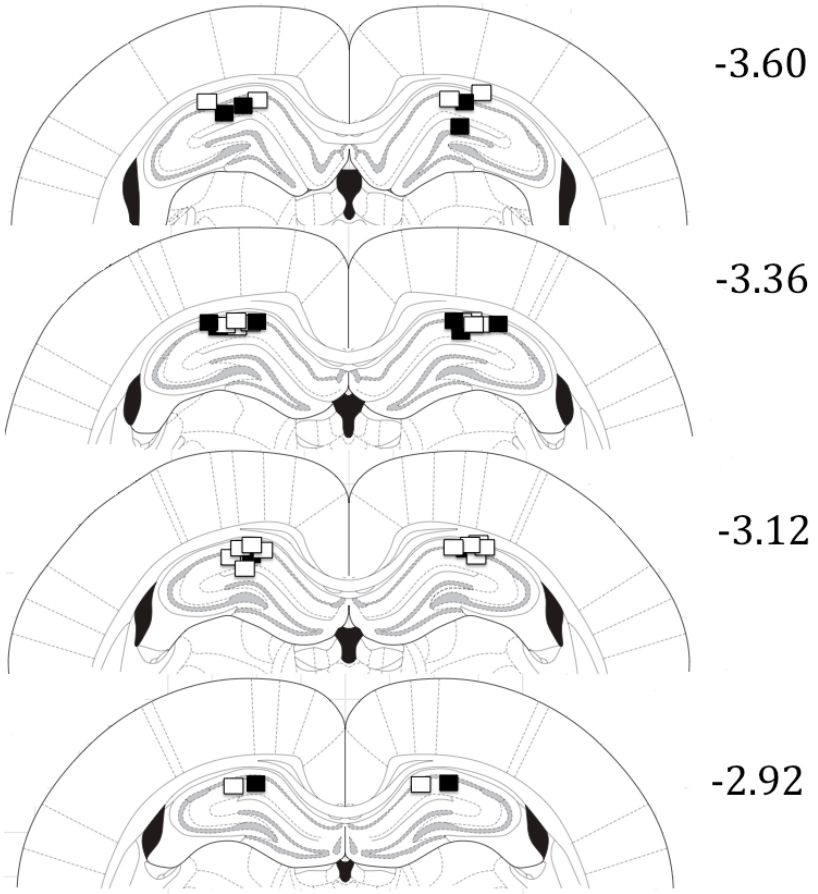



**Figure 10:** Figure 10 depicts the intracerebral infusion sites for subjects included in the Experiment 2. Group designations reflect assignment during the renewal tests with vehicle animal sites represented by white squares and muscimol animals in black. The coordinates from which the atlas figures derive are found to the right of each atlas figure and are relative to bregma.

## Discussion

Subjects in both groups showed more magazine approach CRs when a CS was tested outside of its extinction context than within the extinction context. Because this effect was seen in animals treated with infusions of muscimol into the DH prior to renewal testing as well as in control subjects, this suggests that the DH is not needed for ABC renewal of an

appetitive response. This finding is contrary to what has been reported in studies evaluating the importance of the DH in ABC renewal of conditioned fear. Corcoran and Maren (2001; 2004) and Maren and Hobin (2007) reported that muscimol infusions into the DH impaired ABC fear renewal by reducing fear to a stimulus tested outside of the extinction context. One possible reason why the current study failed to find an effect of muscimol on renewal may be that extinction occurred very quickly in this study. Responding was very quickly reduced in the first session of extinction and fell no lower as a result of the following sessions. This point is of interest because some findings have suggested that while contextual memories may be formed in the hippocampus, they migrate to extrahippocampal structures over time (e.g., Kim & Fanselow 1992; Nadel & Moscovitch 1997). Therefore, if extinction was quickly learned in the current study it is possible that the DH was no longer needed to express the extinction memory as it may have migrated to an extrahippocampal area. While the length of the extinction phases of Experiments 1 & 2 were the same, the subjects in Experiment 2 may have acquired extinction more rapidly than those in Experiment 1 because the extinction contexts used in Experiment 2 were not previously acquisition contexts. In other words, residual excitation activated by the context after initial training may have slowed extinction in Experiment 1, but not in Experiment 2.

Caution should be used in interpreting why responding was so strongly attenuated during the first session of extinction. A perhaps simpler explanation is that the low levels of CRs seen during extinction may be attributable to the external inhibition produced by the novel situation presented during extinction. Subjects were placed in novel contexts and then experienced CS presentations for the first time in these new environments. These

conditions could have masked the full extent of excitatory conditioning to the CS resulting from the initial training phase.

Despite these possibilities, one implication of the design used for Experiment 2 is that the ABC renewal effect observed in the current study likely reflects a conditional learning process in which the extinction context 'sets the occasion' for retrieval of the extinction memory and hence reduced CRs. The results suggest that the DH is not needed for conditional control of extinction by physical contexts in an appetitive task. It remains to be seen whether the DH would be important if assessed after more limited extinction.

Efficacy of the DH inactivation technique is shown in the subsequent T-maze task, as in Experiment 1. Subjects performed significantly worse on this task following infusions of muscimol compared to its saline vehicle. This was true at both test delays (i.e., 60 sec and 120 sec). Furthermore injection sites for all subjects included in the study presented in figure 8 confirm the accuracy of the infusions.

**Experiment 3: The Effect of Post Training Lesions on ABA and ABC Renewal of Magazine Approach**

Ji and Maren 2005 reported that electrolytic lesions of the DH eliminated ABA fear renewal. However, as noted above, an earlier finding showed that DH inactivation with muscimol failed to influence ABA renewal of fear (Corcoran & Maren 2004). These authors suggested that while some hippocampal cells might be spared inactivation in infusion studies, lesions may be more thorough in their damage than an infusion technique. To address this possibility, the present study was performed to determine whether destruction of the DH would produce a behavioral deficit in ABA and/or ABC renewal. There were four groups in this study, group ABA was trained as was described for Experiment 1, while group ABC was trained as in Experiment 2. The groups in this study were treated identically to their Experiment 1 and 2 counterparts with a couple of exceptions. One of the exceptions was that instead of inactivation, subjects were given lesions of the DH, which occurred after extinction had taken place. Groups ABA and ABC were each divided into 2 subgroups, one received lesions and the other received control surgeries to form a total of 4 groups in the experiment. Additionally, NMDA lesions were used because they only destroy cell bodies in the zone of infusion and not fibers of passage – unlike electrolytic lesions. The other difference was that the maze task was run slightly differently from the prior experiments, as will be described below.

| | Acquisition | Extinction | Surgery ↓ Test 1 | Rest | Test 2 |
|---|---|---|---|---|---|
| **GP ABA** | CX 1: S1+<br>CX 2: S2+ | CX 1: S2-<br>CX 2: S1- | CX 1: S1-, S2- ? | 5 Days | CX 2: S1-, S2- ? |
| **GP ABC** | CX 1: S1+, S2+ | CX 2: S1-<br>CX 3: S2- | CX 2: S1-, S2- ? | 5 Days | CX 3: S1-, S2- ? |

**Table 3:** Table 3 presents the design used in Experiment 3. CX 1, 2 & 3 were distinct chambers used in earlier studies. S1 and S2 were the 15 sec stimuli (tone or light). Plus signs indicate reinforcement with food, while minus signs indicate nonreinforcement. The asterisk indicates the point in the experiment where half of the animals from each group were given DH lesions while the other half received sham surgery. Following surgery animals were given a 2 week recovery period. Testing began after the recovery period.

## Methods

### Subjects

Subjects were 52 experimentally naïve Long-Evans rats (26 males and 26 females). Females were included in this study in order to increase the sample size since additional males were unavailable. Additionally, the study was run in two replications, the first had 24 subjects and the second had 28. The subjects were housed in standard stainless steel hanging cages within a colony room on a 14:10 light:dark schedule. The subjects were reduced to 85% of their free feeding body prior to starting the study. Subjects weighed between 189 g and 391 g at the start of the study and were maintained within a +/-5 g range of their target weights with supplemental feedings of standard chow following the last session of each day. Subjects had free access to water while in their home cages. Data from this experiment were collected in two replications with 20 subjects run in the first replication and the remaining 32 in the second replication. From the total number of subjects, 24 served in the ABA groups while the other 28 were part of the ABC groups.

### Surgeries

Subjects were anaesthetized using a 1% body weight injection of sodium pentobarbital at a 50 mg/ml concentration resulting in a dose of 50 mg/kg. Subjects were treated with a 1.35 % body weight injection of atropine sulfate concentrated at 0.4 mg/mL 10 min following injection of the sodium pentobarbital in order to aid respiratory function. The scalp was then shaved and the rat placed in a stoelting stereotaxic apparatus. Lubricant was placed on the cornea to maintain moisture and the head swabbed with a 10 % ethanol solution. An incision was made over the midline of the skull and the fascia pushed aside to reveal bregma and lambda. Once bregma and lambda were confirmed to lie on the same horizontal plane, the unit was calibrated by placing the tip of a 1μL Hamilton syringe onto bregma and recording the coordinates. Four holes were drilled over the dorsal hippocampus (1 on each side of the midline at each anterior-posterior coordinate), two holes at 3.8 mm posterior to bregma, 1.6 mm lateral to the midline. The other two holes were drilled 4.2 mm posterior to bregma and 2.6 mm lateral to the midline. The injector was lowered 3.3 mm below the surface of the skull for the anterior sites and 3.0 mm for posterior sites. Once at the appropriate depth .4μL of NMDA (20mg/mL) was infused at each site at a rate of .1μL/min, the injector remained in place for another 4min following each infusion. For sham surgeries no neurotoxin was infused during this time. For these surgeries the needle was simple lowered into place at the injection site, a procedure commonly employed in the literature, (e.g., see Maren, Aharonov & Fanselow, 1997). Additionally, it should be noted that the sham procedure used by Ji and Maren (2005) consisted of lowering the electrode used to create lesions to the appropriate depth and did not deliver any stimulation, which is analogous to the what was done in the current surgeries. The infusion was automated using a KD scientific pump. Once all infusions were

made the wound to the scalp was sutured and bacitracin applied to the area. Subjects were placed in a recovery area until locomotor activity was evident and then returned to their home tub for 2-week recovery with food freely available.

## Histology

Histological procedures were done as described above.

## Apparatus

Rats were trained in the same contexts as in Experiments 1 and 2. The T-maze used in the follow up study was also the same as described above.

## Magazine Training, Acquisition and Extinction

Subjects in groups ABA and ABC received magazine, acquisition and extinction training as was described for Experiments 1 and 2 above.

## Tests

In contrast to Experiments 1 and 2, subjects did not undergo testing the day following extinction. Instead, they received stereotaxic surgery at this point. Following a 2-week recovery period from surgeries, rats received two tests separated by 5 days as described above for Experiments 1 & 2. The order of stimulus presentations on test 1 was T, L, T, L, L, T, L, T, L, T, T, L, T, L, T, L and this was reversed for test 2. Because animals had been lesioned, no infusions took place prior to tests in this experiment. Groups were matched using the same process as described for Experiments 1 & 2.

## Delayed Non-Matching to Place Task

Initial training on the T-maze was the same as described above. However, due to the nature of the neural manipulation used in this study, subjects were not trained to a specific acquisition criteria. They were simply given 4 sessions of zero delay training prior to the test session as previously described. During the test, a delay of 3 min was introduced between the sample and choice.

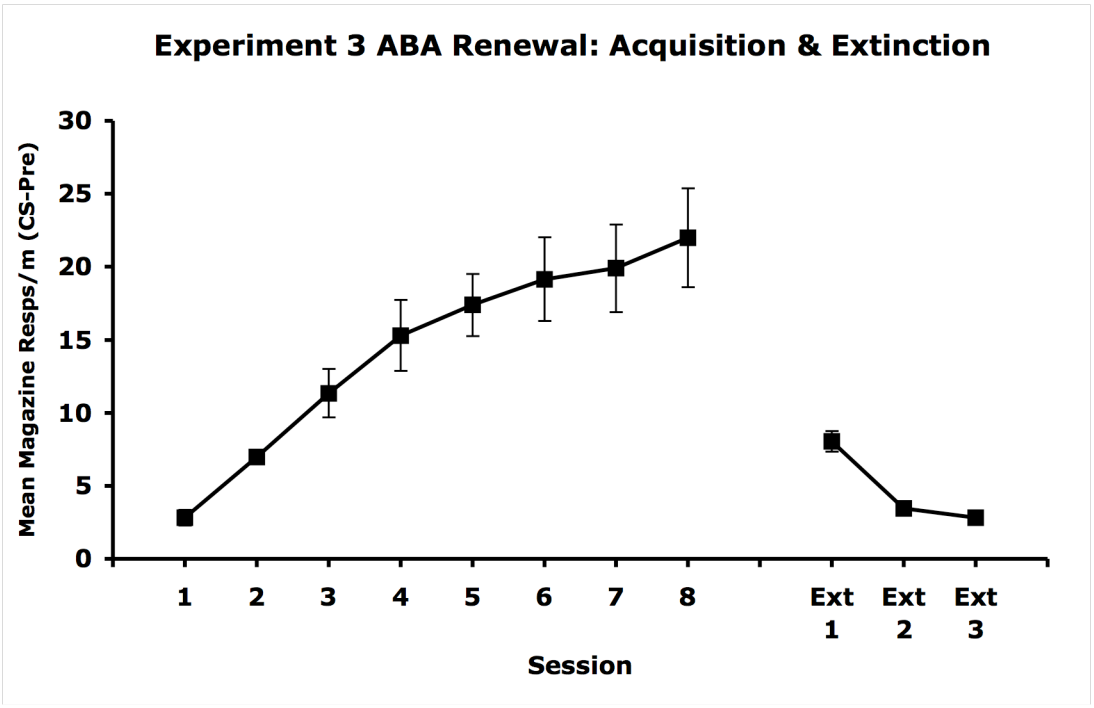
## Results

Out of the 52 animals that started the study a total of 7 were excluded from analysis. One of these rats died during the course of training and the remainder were excluded due to insufficient damage to the DH. A further three animals that were included in the renewal tasks were excluded from the delayed non-matching to place task, as they were perfused prior to completion of the maze task due to illness.

Magazine response rate data from the acquisition and extinction phases are presented below in figure 11 in the form of difference scores (CS-Pre CS). Data for subjects trained on the ABA task can be seen in the upper panel while data for subjects trained on the ABC task are found in the lower panel. Because stimulus did not interact with surgical condition the data were collapsed across this factor. Additionally, no significant effects were found with Gender as well as replication, therefore, the data were collapsed across these factors. Subjects in both the ABA and ABC training conditions showed increased CRs to the stimuli over the acquisition phase. Conditioned responding was attenuated during the extinction phase for subjects in all conditions as well. However, subjects trained on the ABC tasks

showed a greater reduction in CRs on day 1 of extinction than the ABA trained subjects replicating the effect seen during extinction in Experiment 2.

The data for each training phase were analyzed using separate Task x Session repeated measures ANOVAs. The analysis of acquisition data revealed a significant main effect for session confirming the impression that as training proceeded subjects came to respond increasingly more during the stimuli than during the pre-stimulus period, $F$ (7, 301) = 49.851 $p < 0.05$. Session did not interact with the Task factor indicating that acquisition was the same for subjects in all conditions, $F$ (7, 301) = 0.653 $p > 0.05$. Data from the extinction phase were analyzed similarly, and revealed that responding during the stimuli was attenuated over the extinction phase, $F$ (2, 86) = 12.103 $p < 0.05$. However, a significant Task x Session interaction confirmed the impression that for subjects trained on the ABC task, conditioned responding was more quickly reduced in the first extinction session than for subjects trained on the ABC task, $F$ (2, 86) = 6.845 $p < 0.05$.



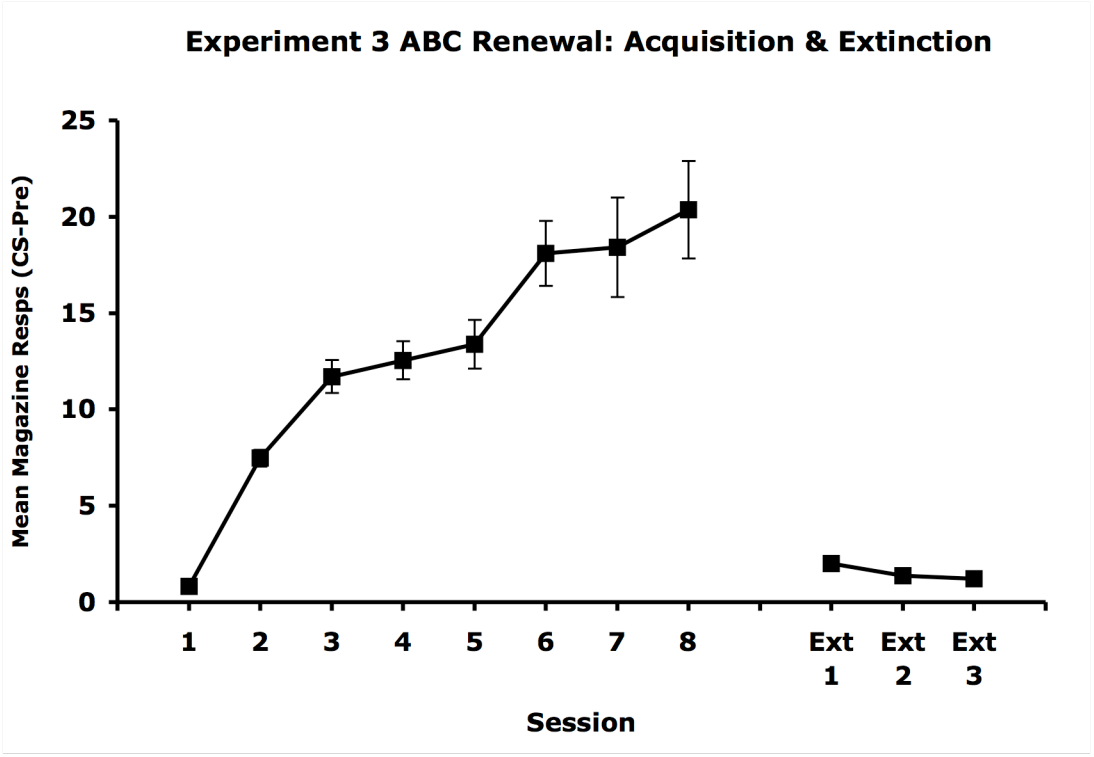
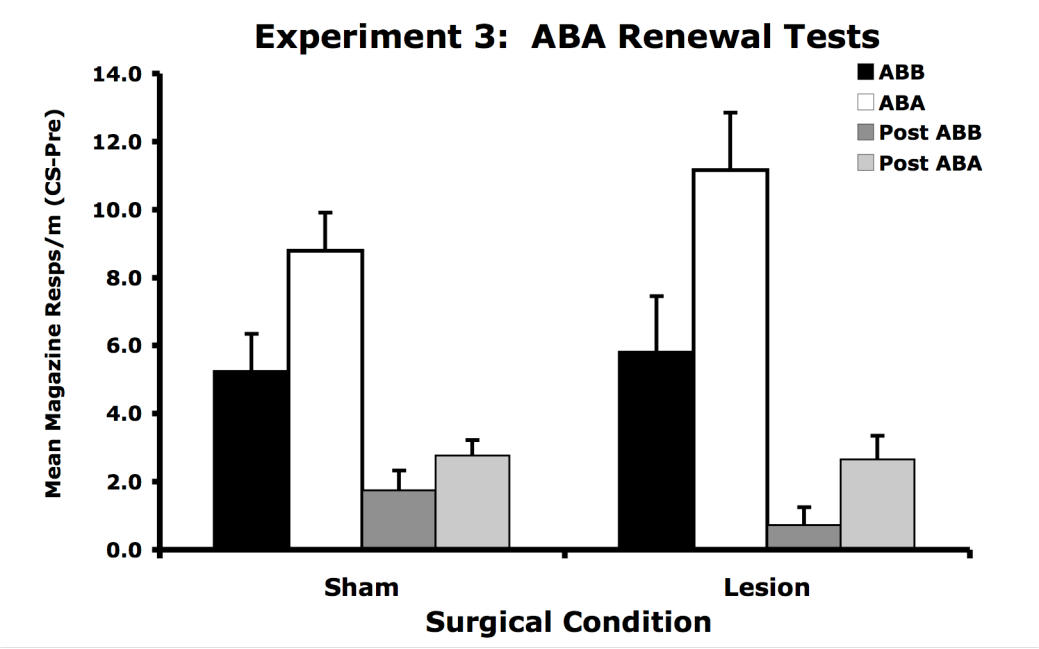




**Figure 11:** Figure 11 depicts mean magazine responses during acquisition (left panel) and extinction (right panel) in terms of difference scores (CS-Pre) in Experiment 3.

Magazine entry data (CS-Pre CS) from the test phase are presented in figure 12 in the upper panel for the two groups trained on the ABA task and the lower panel for the groups trained with the ABC task. Preliminary analyses detected a main effect of Test Session. However, this did not interact with any other factor, therefore, the data were collapsed across this factor. Because replication and gender did not interact with Surgical Condition (Sham or Lesion) or Task (ABA or ABC renewal), the data were collapsed across these factors as well. For the subjects trained on the ABA task responding during the test phase was expressed in terms of whether a CS was tested in its acquisition or extinction context (i.e., ABA vs ABB). For the subjects that were trained on the ABC task, responding was expressed in terms of whether a CS was tested in its own or in a different CS's extinction context (i.e., ABB vs ABC). An analysis on Pre CS response rates revealed no significant effects. For the sham operated subjects trained on the ABA task, the Pre CS rates were 2.1 (Pre ABB) and 2.7 (Pre ABA), for the ABC trained shams the values were 2.8 (Pre ABB) and 3.0 (Pre ABC). For the lesioned subjects trained on the ABA task, the Pre CS rates were 3.9 (Pre ABB) and 3.6 (Pre ABA), for the ABC trained shams the values were 2.2 (Pre ABB) and 2.1 (Pre ABC). Sham operated subjects trained on the ABA task showed more magazine CRs when stimuli were tested in their acquisition compared to extinction context (figure 12 upper panel). This same pattern of responding was seen in the lesioned animals trained on the ABA task further suggesting that the DH is not involved in ABA renewal of appetitive learning. Similarly, lesioned subjects trained on the ABC task showed a similar pattern of responding, although, the overall magnitude of renewal appeared reduced compared to ABA renewal (figure 12 lower panel). More CRs were observed for these subjects when stimuli were tested outside of compared to within their extinction context. However, sham

operated subjects trained on the ABC task did not appear to show any difference in responding as a function of the test location. One aberrant subject in this group skewed these data. This sham operated subject responded in the ABB condition well above the levels seen for all other subjects in the study except one and did not show much responding in the ABC condition. When excluding this one subject the means for the sham group trained on the ABC task become, ABB: 4.7, ABC: 6.6, PO ABB: 2.1 and POABC: 3.1.

Test data were analyzed using a Stimulus x Recording Interval x Surgical Condition x Task split plot ANOVA. This analysis revealed a significant main effect of Stimulus, $F$ (1, 41) = 8.78, $p < 0.01$, which did not interact with Task or Surgical Condition. This analysis included the one aberrant sham subject trained on the ABC task. The same conclusions applied if that subject was excluded from the analysis. This effect indicates that subjects in all surgical conditions showed an increase in magazine approach CRs when extinguished stimuli were tested outside of their extinction contexts. A significant main effect for Recording Interval was also found, $F$ (1, 41) = 38.87, $p < 0.001$, indicating that more responding was seen during the stimuli compared to the post stimulus period. No other significant main effects or interactions were obtained.



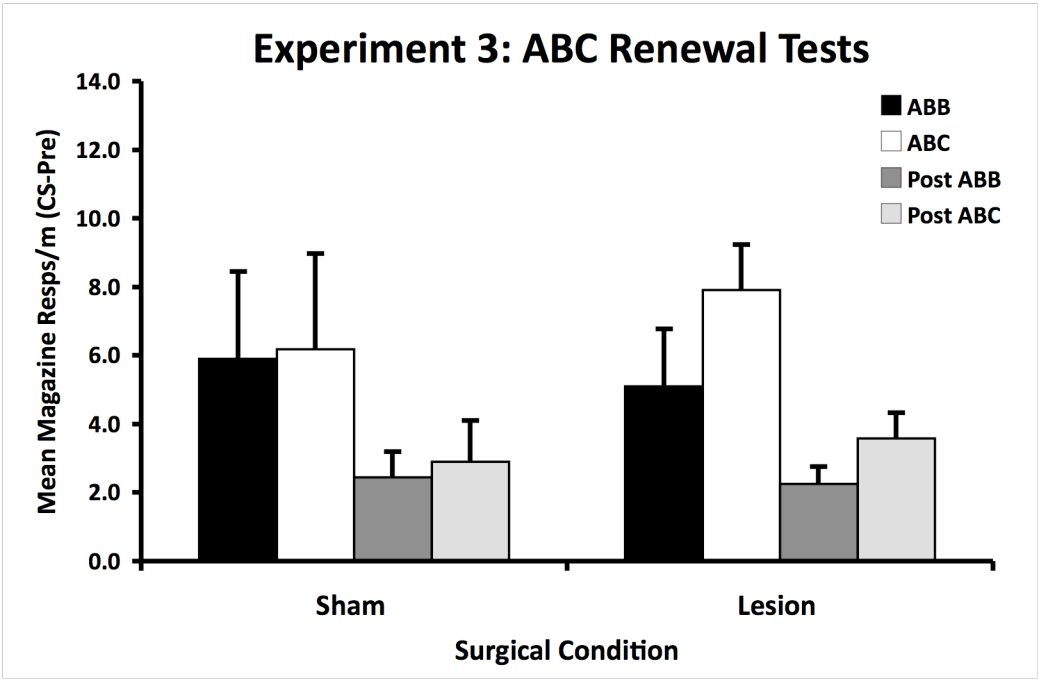




**Figure 12:** Figure 12 displays data from both renewal tests in Experiment 3 collapsed across stimulus to be presented in terms of ABB vs. ABA responding. Data are presented in difference score format and represent mean magazine responses during the 15sec stimuli as well as during a 20sec post stimulus period during which food had been delivered during initial training.

Data from the non-matching to place task are presented in figure 13 below. The figure shows mean percent correct during the test. The left panel depicts the data from subjects trained on the ABA task, while the right panel displays similar data from subjects trained with the ABC renewal task. The figure suggests that DH lesioned subjects from both renewal training conditions performed with less accuracy than sham operated subjects. The data were analyzed with a Surgical Condition x Renewal type (ABA, ABC) factorial ANOVA. The only significant effect obtained was a main effect for surgery, $F$ (1, 38) = 6.955, $p < 0.05$, confirming that hippocampally lesioned subjects performed worse than sham subjects. Supplementary analyses showed no significant interactions between the factor of Surgery and Gender as well as Replication.

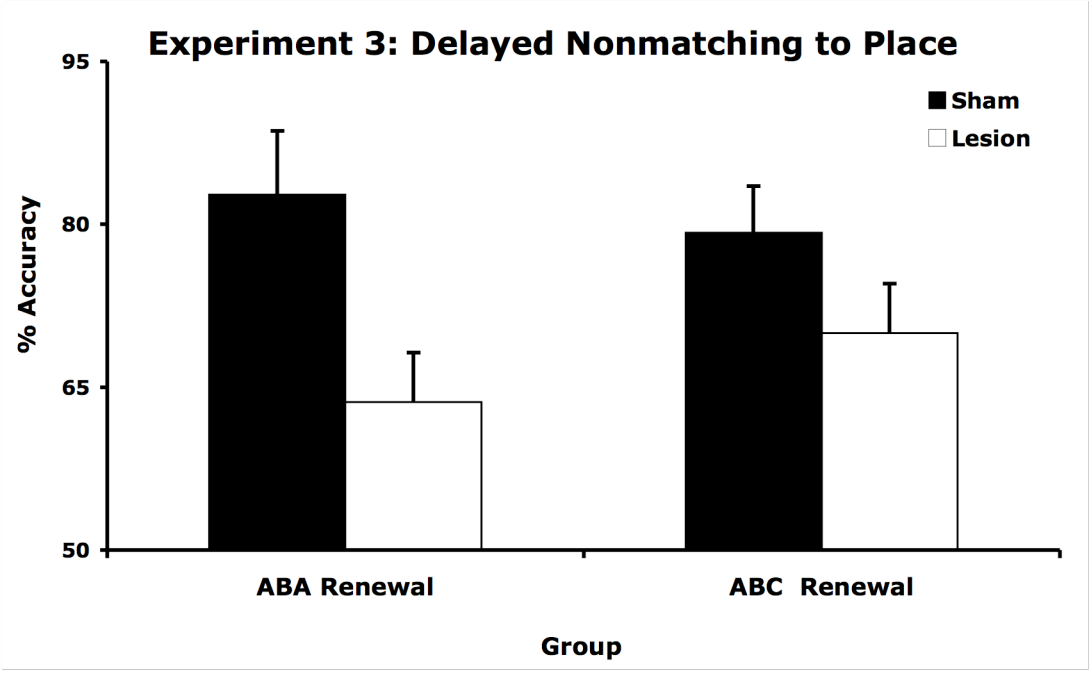



**Figure 13:** Data from the delayed non-matching to place task from Experiments 3 & 4 are presented in figure 13 as percent accuracy (the left panel corresponds to animals from Experiment 3 and the right panel Experiment 4). Animals were tested with a 3 min delay between sample and choice runs.

Histological results are presented in figure 14 below. This figure depicts the extent of damage induced in this study throughout the DH. The shading corresponds to the damage observed during histological evaluation. Subjects for whom portions of CA fields or the dentate gyrus remained intact despite undergoing experimental surgery were excluded from all analyses. The minimum (black) and maximum (grey) acceptable damage for inclusion in the study are shown on the left half of each atlas slide while the right side shows the target structure (DH) itself. While the extent of the lesion continued caudally, the ventral hippocampus was spared, with the dorsal damage conforming to the minimum maximum specifications presented in figure 14.

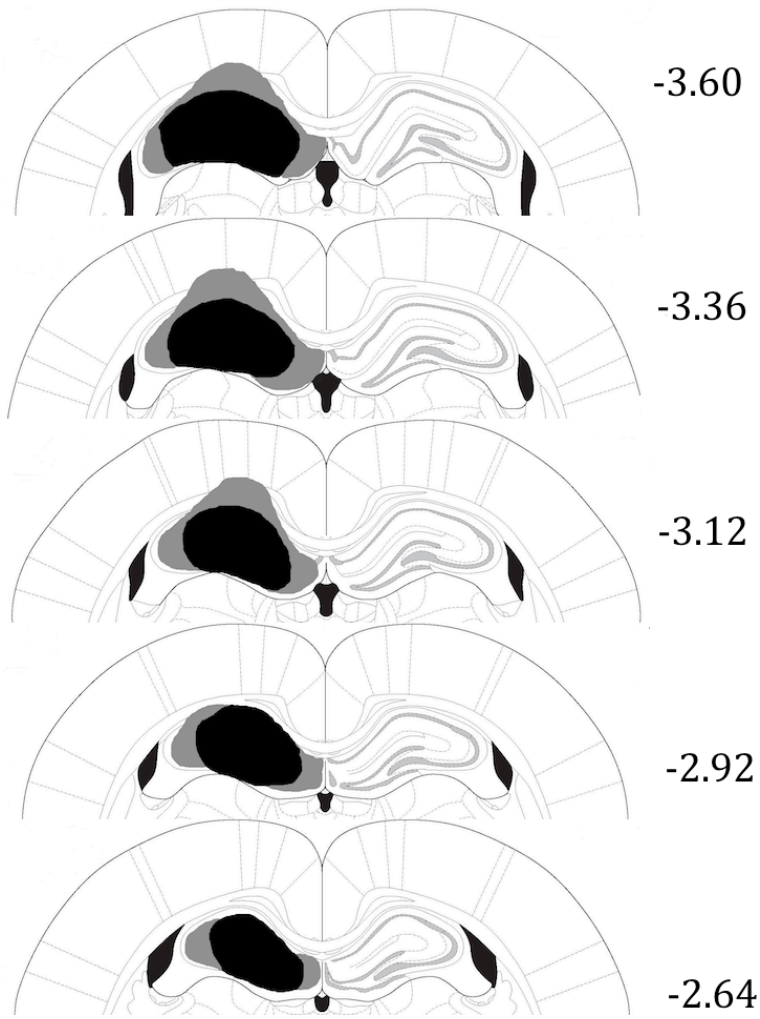



**Figure 14:** Figure 14 shows the minimum amount of acceptable damage on the left hemisphere in black and the maximum extent in grey for lesions in Experiments 3 & 4. The coordinates from which the atlas figures derive are found to the right of each atlas figure and are relative to bregma.

## Discussion

The results of Experiment 3 provide further evidence that the DH is not necessary for appetitive ABA or ABC renewal effects. Subjects with DH lesions did not differ from sham operated subjects in showing greater responding when a CS was presented in its acquisition than its extinction context (e.g., ABA renewal) or when a CS was tested in a context where another CS was extinguished (e.g., ABC renewal) compared to its own extinction context. The failure of DH lesions to produce an impairment in both ABA and

ABC renewal is somewhat surprising because previous studies in aversive learning have suggested that the DH is involved in both of these forms of renewal (Corcoran & Maren 2001; 2004; Ji & Maren 2005). It is not likely that the lack of a lesion induced impairment on renewal reflects ineffective neural manipulations since non-matching to place performance was significantly impaired, replicating the effects of DH lesions on this task seen by others (Hock & Bunsey, 1998). Furthermore, histological results confirmed that the structure had been destroyed.

One potential source for the difference between the current ABA findings and those of Ji and Maren (2005) may be the type of lesion used to destroy the DH. Ji and Maren (2005) used an electrolytic technique while the present study used a chemolytic technique to ablate tissue. Because the electrolytic approach destroys axons that pass through the area where current is applied it can affect other structures outside the DH, which excitotoxic lesions do not impact. This difference in experimentally induced damage may be a reason for the discrepant findings discussed here. However, an alternative explanation of these discrepant results may relate to experimental design differences between studies. It may be the case that the current studies promote a different psychological mechanism than do the aversive renewal studies, with the latter being hippocampally dependent and the former not. Because the test contexts in the current studies have been equated for their histories with the US, summation-based accounts of the renewal effect are not likely applicable here. The renewal effects observed in Experiment 3 are better explained by the occasion-setting process suggested Bouton (1993; 1994). This mechanism is distinct from a summation mechanism that may explain renewal when test contexts have different associative relationships to the US. While this difference has not been addressed in ABA fear renewal

studies, Hobin and Maren (2007) used a design that equated tests contexts in an ABC fear renewal study (this design was identical to that used for the current ABC experiment). Using this design Hobin and Maren (2007) reported that DH inactivation impaired renewal of fear conditioning, contrary to what was found in the current ABC study which used lesions instead of inactivation. This suggests that the failure to obtain DH impairments in renewal may simply reflect differences in the neural control of aversive and appetitive learning, this point will be further explored in the general discussion.

**Experiment 4: The Effects of DH Inactivation on Spontaneous Recovery of Magazine Approach**

Experiment 4 investigated whether or not the spontaneous recovery of magazine approach CRs are dependent upon DH function. Spontaneous recovery refers to the reemergence of CRs seen when sufficient time is interpolated between extinction and test (e.g., Rescorla, 2004). Spontaneous recovery's dependence on the DH is of interest because it has been proposed that spontaneous recovery is a form of renewal in which time comes to act as a contextual cue and that these two phenomena may be dependent on the same underlying mechanisms (Bouton, 1993; 1994; Bouton et al. 2006). In other words, while changing the physical context where testing occurs removes the subject from the extinction context, the passage of time following extinction serves to remove the animal from the temporal context in which extinction occurred. This idea is supported by findings which show that changes in physical contexts and temporal contexts are additive (Rosas & Bouton 1998). In other words, removing subjects from either the extinction context or the time when extinction occurred alone would not produce as strong of a recovery effect as when subjects are removed from both the location and time of extinction.

In another line of evidence, the DH has been shown to be involved in some instances where time plays an important role. For example, Esclassan, Coutureau, Di Scala and Marchand (2009) have shown that DH inactivation and lesions impair trace conditioning in rats. Using mice, Misane, Tovote, Meyer, Spiess, Ogren and Stiedl (2005) showed that APV (an NMDA receptor antagonist) infusions into the DH prior to training impaired acquisition of long delay but not short delay auditiory trace fear conditioning. Furthermore, Chowdhury, Quinn and Fanselow (2005) reported that long delay trace fear conditioning

but not short-delay trace fear conditioning was impaired by neurotoxic DH lesions in mice. While spontaneous recovery and trace conditioning appear to be distinct phenomena, it seems likely that temporal factors play a role in both. If the DH is important in temporal control more generally, then it may play a role in spontaneous recovery. It is worth pointing out that while Wilson, Brooks and Bouton (1995) found no effect of lesions to the fornix on spontaneous recovery, the current study evaluated the impact of temporary inactivation of the DH itself on spontaneous recovery.

In the present study the role of the DH was determined by inactivating the structure prior to a test for spontaneous recovery to two stimuli. The design used here (see table 4 below) was suggested by Rescorla (2004) and ensures that both stimuli are tested in the same session, eliminating potential within-subject differences at the time of test (e.g., maturation). Additionally, it keeps the interval between acquisition and extinction equal for both stimuli tested. One of these stimuli (S1) was conditioned for 3 days and then extinguished in a single session. Following a three-day break period a second stimulus (S2) was conditioned for 3 days. Following the extinction session for S2 subjects were removed from the chambers and infused, then 15 min later they were returned for a session in which S1 and S2 were tested. Control subjects are expected to show more recovery to S1 than S2 because the test occurs outside of the temporal extinction context of S1 (e.g., one-week following S1 extinction), but within the temporal extinction context of S2 (e.g., immediately after the S2 extinction session. If the DH is important for temporal modulation of extinction, subjects with compromised DH function would be anticipated to show low levels of responding to both S1 and S2 during the test. While Experiments 1-3 have shown that the DH is not involved in the conditional control of extinguished magazine approach

CRs by physical contexts, the present study investigates the potential role of the DH in conditional control by temporal contexts.

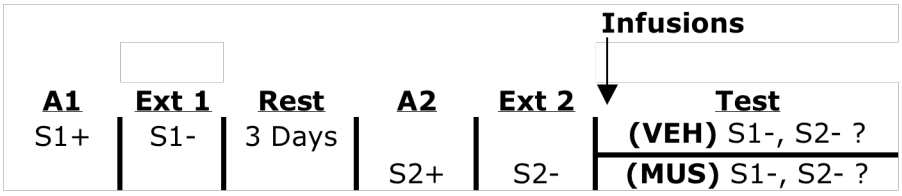



**Table 4:** Table 4 presents the design used in Experiment 4. S1 and S2 were the 15 sec stimuli (tone or light) used in the previous studies. Plus signs indicate reinforcement with food, while minus signs indicate nonreinforcement. Groups specified with MUS and VEH, received either muscimol or its saline vehicle into the dorsal hippocampus 15 min prior to tests. All phases of this study were conducted in the same physical context.

## Methods

### Subjects

Subjects were 32 experimentally naïve Long-Evans male rats weighing between 295 g and 346 g at the start of the study. The subjects were bred and housed as described above for Experiment 1. The study was run in two replications with 16 animals in each.

### Surgeries

Surgeries were preformed prior to any experimental treatments as described above for Experiment 1.

### Infusions

Infusions into the dorsal hippocampus were done one hemisphere at a time. A 25 μL Hamilton syringe attached to PE50 tubing was connected to a 28 g injector (Plastics One), which extended 1 mm beyond the tip of the guide cannula. For subjects in the first

replication, the syringe was automated by a KD scientific pump, however, the syringe was operated manually in the second replication. This was done because problems with the pump resulted in infusions having to be redone, necessitating subjects to be run in very small groups for the final extinction and test session (with the aim of keeping the time between extinction and test equal). In both cases, 1 μL of solution was infused into each hippocampus at a rate of 0.32 μL/min using the pump, or over a 40 sec period when infused by hand. Subjects were restrained during this process and remained connected to the infusion line for an additional 2 min following each infusion. Muscimol was concentrated at 1 mg/mL in physiological saline. Order of infusion was counterbalanced with regard to cerebral hemisphere.

## Histology

Histology was done as described above.

## Apparatus

Rats were trained in the chambers used in Experiment 1. There were no inserts present in these chambers for this study.

## Magazine Training

Magazine training was done as previously described for Experiment 2.

## Acquisition & Extinction

S1 was trained on days 1-3. For half of the subjects S1 was a the Tone CS used in Experiments 1-4, while for the other half S1 was the Light CS used in Experiments 1-4. In each session the 15 sec CS was presented 20 times and reinforced with two food pellets presented at the offset of the stimulus. All sessions had an intertrial interval that averaged 3 min, with a range of 1-5 minutes. On day 4 the S1 stimulus was extinguished. Session parameters were identical to the training phase, except that the stimulus was presented without reinforcement. There was then a 3-day break period during which time the rats remained in their home cages. On days 8-10 the excitatory training of S2 occurred, followed on day 11, by the extinction of S2, which was accomplished exactly as described for S1. Immediately after this session subjects were infused and then tested.

Test

There was a single test session that occurred on day 11. Immediately following the end of the 2nd extinction session for S2, animals were removed from the training chambers and given either vehicle or muscimol infusions into the DH (see above for details). Fifteen minutes following the infusions subjects were returned to the chambers and tested for spontaneous recovery to both S1 and S2. During this session, each stimulus was tested under extinction 8 times using the same average intertrial interval and range as the previous phases of the study. The order of stimulus presentations during the test session was T, L, T, L, L, T, L, T, L, T, T, L, T, L, T, L. Group assignments were made on the basis of average responding to S1 and S2 on the last day of training for each stimulus. Data from extinction were not used for matching due to the need to quickly remove, infuse and return animals to the conditioning chambers after the extinction of S2.

Results

Out of the 32 subjects that started this study, 7 were not included in the data. Five of the subjects reacted in a highly abnormal way to the infusions. Specifically, rats either rolled over onto their backs repeatedly or they became very slow in their movements and were generally sluggish. Two subjects from the control group and 3 subjects from the muscimol group were excluded due to these problems. The other two animals were excluded due to inaccurate cannulae placement.

Magazine entry data from the acquisition and extinction phases are presented in figure 17 below in the form of difference scores (CS-Pre CS) for S1 and S2. The data are collapsed across replication because preliminary analyses showed that this factor did not significantly interact with CS or Block factors. While CRs increased over blocks during training for both CSs, responding to S2 was higher. This was very likely due to some form of stimulus generalization from prior training of S1 since S2 was trained second. This was confirmed by a CS (S1, S2) x Block (1-6) repeated measures ANOVA revealing significant main effects for both Block, $F$ (5, 120) = 34.51, $p < 0.01$, and CS, $F$ (1, 24) = 5.21, $p = 0.032$. No significant CS x Block interaction was observed. During extinction, responding to both stimuli declined over blocks. The extinction data were analyzed in the same way as acquisition data. The analysis revealed a significant main effect for Block, $F$ (1, 24) = 18.849, $p < 0.05$. Once again, no significant CS x Block interaction was observed, additionally no significant main effect for stimulus was seen during extinction.

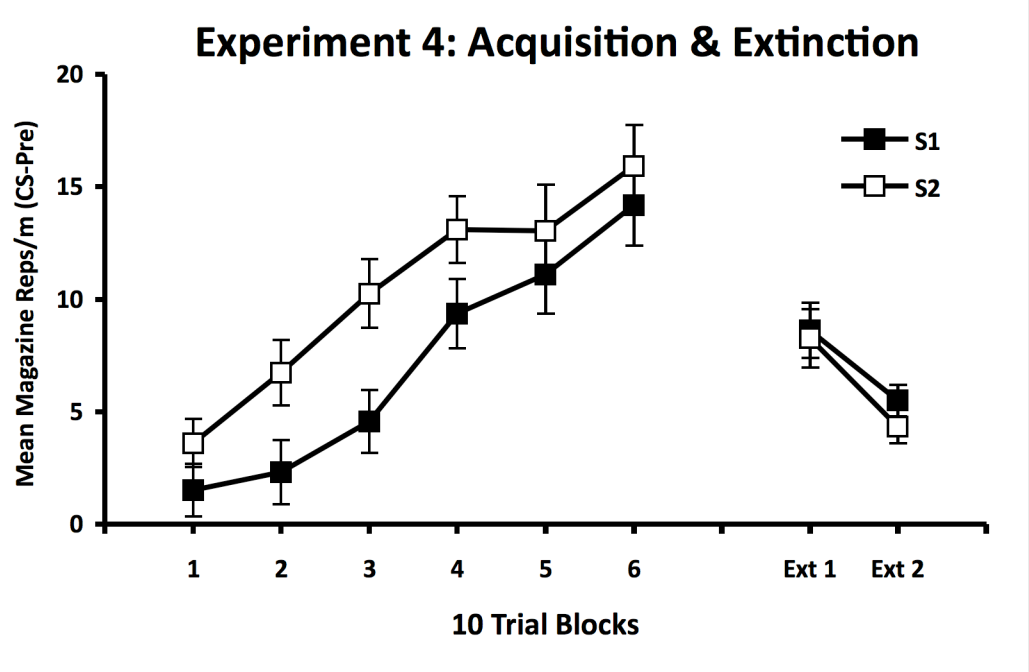



**Figure 17:** Figure 17 depicts mean magazine responses during acquisition (left panel) and extinction (right panel) for S1 and S2 in Experiment 4 in difference score form (CS-Pre).

Magazine entry data from the test session are presented below in figure 18 in the form of difference scores (CS-Pre CS). Preliminary analyses showed no effect of replication, therefore the data were collapsed across this factor. An analysis of Pre CS responding revealed no significant effects. For subjects infused with Vehicle, the Pre CS rates were 0.3 (S1) and 0.8 (S2), while for subjects infused with muscimol the rates were 2.0 for S1 and 1.4 for S2. The subjects infused with vehicle prior to testing showed more spontaneous recovery of magazine approach CRs to S1 than to S2 during the stimuli. The recovery effect for S1 persisted into the post CS period (when the US had normally been delivered). Animals infused with muscimol did not show spontaneous recovery to S1 as responding was equally low in all recording intervals.

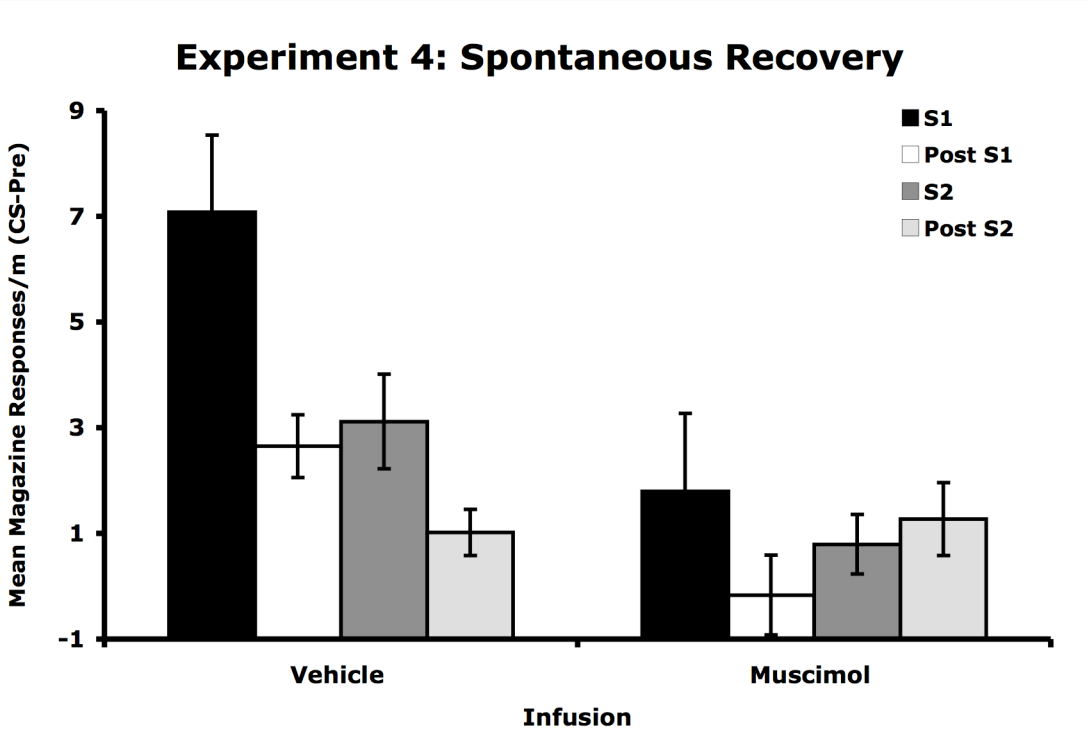



**Figure 18:** Figure 18 depicts mean magazine responses during S1 and S2 for control subjects on the left and muscimol treated subjects on the right during the test session in Experiment 4.

The data were analyzed using a CS (S1, S2) x Recording Interval (CS, Post CS) x Infusion (Mus, Veh) split plot ANOVA. This analysis yielded a marginally significant effect of stimulus, $F$ (1, 23) = 4.169, $p$ = 0.053 and a significant Stimulus x Infusion interaction, $F$ (1, 23) = 5.754, $p < 0.05$. To assess the basis of this interaction separate one-way ANOVAs were conducted for each group with a pooled error term. Analysis of vehicle subject data revealed a significant overall main effect, $F$ (3, 36) = 12.012, $p < 0.05$, and post hoc tests (Rodger, 1974) showed that these subjects displayed more spontaneous recovery to S1 than S2, $F$ (3, 36) = 5.96, $p < 0.05$. A one-way analysis on the data from subjects infused with muscimol did not show a significant overall effect, therefore, no further tests were conducted on these data. Additionally, subjects infused with muscimol appeared to respond at a generally lower level than control subjects, this was revealed by a significant main effect of infusion, $F$ (1, 23) = 7.812, $p < 0.05$.

Results of the histological analysis are presented in figure 19 below, which shows the locations of the infusion sites for the subjects included in this study. The injection sites were mostly located around -3.36 posterior to bregma at the dorsal most portion of the hippocampus, with few sites found ventral to that boundary.

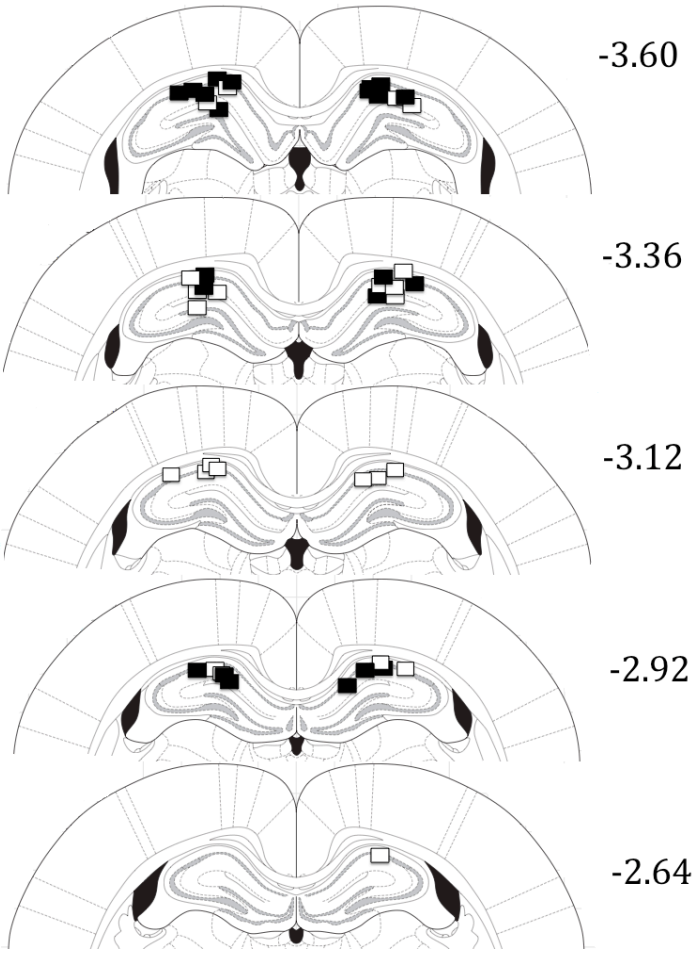



**Figure 19:** Figure 19 depicts the intracerebral infusion sites for subjects included in the Experiment 4. Sites for subjects in the vehicle group during the renewal tests are represented by white squares and muscimol by black. The coordinates from which the atlas figures derive are found to the right of each atlas figure and are relative to bregma.

## Discussion

Subjects infused with the saline vehicle prior to the test session displayed more recovery of magazine approach CRs to S1 than to S2. This result is consistent with previous findings of spontaneous recovery in this within-subjects experimental design (see Rescorla 2004). Subjects infused with muscimol, however, displayed low levels of responding to both S1 and S2 stimuli (i.e., no spontaneous recovery to S1). These results suggest that the DH plays a role in spontaneous recovery. However, the exact role is unclear. One popular approach towards understanding spontaneous recovery is to regard it as a form of renewal. In other words, because extinction for S1 and the test session occurred 1 week apart, these two events might be processed as having occurred in different temporal contexts compared to S2 for which extinction and test occurred in the same temporal context (i.e., 15 minutes apart). The design of Experiment 4 could be construed as an ABB vs ABC renewal design using temporal instead of physical contexts. One possibility is that the DH is important in determining how temporal contexts modulate extinction learning in appetitive conditioning. This is similar to the suggested role of the DH in the renewal of extinguished fear conditioning using physical contexts (Corcoran & Maren, 2004). Alternatively, it may be that the DH is important for recognizing that the temporal context at the time of test is different from that of extinction for S1. DH inactivated subjects may have failed to recognize the time of the test session as different from the time of extinction for S1. From that perspective the test would be occurring in the extinction context for those subjects and would be expected to result in no recovery to this CS, which is exactly what was observed. This issue will be revisited in the general discussion.

## General Discussion

The results of Experiment 1 demonstrated ABA renewal of magazine approach responding and further showed that DH inactivation did not impair this effect. Experiment 2 demonstrated ABC renewal of magazine approach, and that inactivation of the DH did not impair this effect. The results of Experiment 3 replicated the ABA and ABC renewal effects seen in Experiments 1 and 2, respectively, and also revealed no effects of post-training neurotoxic DH lesions. However, in all of these studies, the neural manipulations did result in impaired performance on the delayed non-matching to place task conducted after renewal testing concluded, suggesting that these manipulations did have a functional impact. The results of Experiment 4 demonstrated that control subjects showed more spontaneous recovery to a CS which had been extinguished well before being tested, compared to another CS, which had been extinguished just prior to the test session. However, muscimol-treated subjects did not show this difference, suggesting that the DH may be important for the control of extinction when time is an important determinant of responding. These results differ in some respects to those found in fear conditioning studies and so some discussion will follow concerning any similarities and discrepancies.

In fear conditioning, Corcoran and Maren (2004) similarly showed that ABA renewal was unimpaired by DH inactivation. However, because Corcoran and Maren (2004) used test contexts that differed in their associative history with the shock US, the nature of the psychological process underlying the behavioral results remains unclear. On the other hand, the design for Experiment 1 of the current paper did equate the conditioning history of the test contexts. Because each context was the acquisition context for one stimulus and the extinction context for the other, simple summation-based accounts do not adequately

account for renewal under these conditions. Bouton's (1993; 1994) modulatory account of extinction offers a more plausible explanation of renewal using such a design. Because Experiment 1 did not find an effect of DH inactivation on renewal, this suggests that the DH is not involved in the conditional control (by context) of extinguished appetitive learning in an ABA renewal design.

Ji and Maren, (2005), however, reported that electrolytic DH lesions did impair ABA renewal of fear conditioning. Therefore, it was unexpected to find that ABA renewal of magazine approach was not disrupted by DH lesions in Experiment 3. One potential reason for the discrepancy between Ji and Maren (2005) and the ABA renewal findings in Experiment 3 of the current paper may be that aversive and appetitive extinction learning processes are fundamentally different from one another and involve different brain structures. However, other research suggests that this idea may not be entirely correct. For example, lesions of the infralimbic region of the vmPFC have been reported to increase spontaneous recovery in aversive (Quirk et al. 2000) and appetitive conditioning preparations (Rhodes & Killcross, 2004). This suggests that at least some of the underlying circuitry in appetitive and aversive extinction learning overlaps. Another difference between the findings of Ji and Maren (2005) and those of Experiment 3 worth noting is the technique used to lesion the DH. Electrolytic lesions are known to destroy axonal fibers in the vicinity of the electrode tip leaving open the possibility that communication between structures in other parts of the brain may have additionally been compromised. The NMDA lesions used in Experiment 3 are known to selectively destroy cell bodies in the vicinity of the diffusion zone without affecting fibers of passage. It may be the case that collateral damage in the Ji and Maren (2005) study could have resulted in impaired ABA renewal of

fear conditioning. Finally, another difference between the two studies is that the experimental design used in Experiment 3 likely assessed the contribution of conditional control processes in renewal whereas simple context-US summation processes could have controlled performance in the Ji and Maren (2005) study. Any one of these differences could account for the discrepant findings and future work will be required in order to resolve the discrepancy.

The findings of Experiments 2 & 3 are contrary to those reported by Maren and colleagues (Corcoran & Maren 2004; Maren & Hobin 2007), in which ABC renewal of fear conditioning was impaired by DH inactivation. The design used by Corcoran and Maren (2004) was quite different from the design used in Experiments 2 and 3 and was amenable to an analysis in terms of context-US summation processes. Therefore, the present results are not, strictly speaking, in direct conflict with these particular findings. However, Maren and Hobin (2007) used a nearly identical design to the ABC design used in the current paper. Therefore, the failure to observe impaired ABC renewal in DH-inactivated subjects in the present situation was unexpected. The results of the delayed non-matching to place task suggests that the neural manipulation was effective, so the failure to find an effect on renewal was not due to a poor neural manipulation. However, two procedural differences between our experiments may have played an especially important role. Maren and Hobin (2007) used a fixed ITI throughout training and extinction, whereas variable ITIs were used in the present tasks. In addition, during the conditioning phase trials strictly alternated in Maren and Hobin's (2005) study whereas they were pseudo randomly interspersed in the present studies. As a result of these differences, subjects in Maren and

Hobin's (2007) experiment may have used the time between stimuli as an effective "context" cue that overshadowed learning about physical contexts.

During training, Maren and Hobin (2007) used a fixed ITI of 62 sec and two different 10 sec stimuli were trained in an alternating manner. This resulted in each individual CS being presented every 134 sec. During extinction, each CS was extinguished in a distinct extinction context and was presented every 60 sec. Thus, each CS was separated by a longer ITI during training than during extinction. The longer ITI could have set the occasion for the target stimulus being reinforced, while the shorter ITI could have set the occasion for nonreinforcement of the target stimulus. In the current ABC renewal studies, stimuli did not strictly alternate during acquisition, and variable ITIs were used. Therefore, subjects could not have as easily used the ITI to differentially cue acquisition and extinction trials. Bouton and Hendrix (2011) showed that subjects trained to associate food with a 10 sec tone following a 16 min ITI but not after a 4 min ITI readily acquired this discrimination. This task is conceptually similar to the conditions produced by the training and extinction phases used by Maren and Hobin (2007). Preliminary data from our lab suggests that DH lesions made prior to training on this task impair the acquisition of this discrimination. It may be that the process of temporal control was impaired by DH inactivation in Maren and Hobin's (2007) study, and because time could not act as an occasion-setting stimulus in Experiments 2 and 3 no effect of DH inactivation or lesions were observed in those studies.

Of course it is also possible that appetitive and aversive learning differ in the role of the DH in ABC renewal. Future work will be needed to further examine this possibility. Another difference, as discussed more thoroughly earlier, is the rapid loss of responding seen during extinction in the present ABC renewal studies compared to prior work.

The possibility that the DH may play a role in the control of extinction by temporal factors was investigated in Experiment 4. As noted above, Bouton (1993: 1994) suggested that renewal and spontaneous recovery might be fundamentally similar phenomena, both involving conditional control mechanisms. While changes in physical locations are responsible for renewal after extinction, the passage of time involves a change in temporal context, which promotes spontaneous recovery. Within this framework, the design of Experiment 4 can be thought of as an ABC renewal design. S1 training occurred in the 'A' temporal context, S1's extinction in the 'B' temporal context, and testing occurred in a 'C' temporal context, resulting in recovery to S1. There is no recovery to S2 because testing occurred within S2's temporal extinction context (analogous to an ABB control condition). DH-inactivated subjects in this study showed equally low levels of CRs to S1 and S2. These findings suggest that muscimol treated subjects were not able to use the shift from the temporal context of S1 extinction (context B) to the test context to control responding. Other data suggest that discrimination of time, per se, is not influenced by hippocampal manipulations (Kyd, Pearce, Haselgrove, Amin & Aggleton, 2007). This would make it unlikely that subjects could not recognize the time of test as different from when S1 had been extinguished - the control of extinction by temporal context as occasion-setting stimuli is the more likely process to have been impaired given these findings. If this analysis of spontaneous recovery is correct then the results make sense of the discrepant findings noted above concerning the effects of DH inactivation on ABC renewal where physical contexts were used. That analysis requires that the fixed ITIs used by Maren and Hobin (2007) were functionally equivalent to temporal occasion setting cues.

The results reported here can be interpreted to mean that renewal and spontaneous recovery do not share the DH as a common substrate since DH manipulations failed to impair ABA and ABC renewal in Experiments 1-3, but inactivation did eliminate spontaneous recovery in Experiment 4. However, these findings do not rule out the possibility that a similar psychological mechanism may be at the root of both of these recovery phenomena. In other words, spontaneous recovery and renewal may both be dependent on the removal of the subject from an extinction context in order to promote response recovery. However, the DH may be involved in modulating extinction when the context is temporal in nature and not physical (as in renewal). This idea is supported by findings from Iordanova, Burkett, Aggleton, Good and Honey (2009) who showed that hippocampal lesions impaired the use of time cues in memory retrieval. These authors trained rats to associate context 1 with a tone and context 2 with a clicker in the morning and in the afternoon these relationship were reversed (i.e., context 2 with tone and context 1 with clicker). In a 3$^{rd}$ context between the morning and afternoon sessions, the tone was later paired with shock. In subsequent tests for context fear, rats froze more in the contexts where tone had been presented only at the times of day when tone had been presented in that context (i.e., context 1 in the morning and context 2 in the afternoon). Rats with hippocampal lesions showed fear in each test context and at each time of testing. This result offers support for the idea that the hippocampus is important in using time as a conditional stimulus.

The present findings have two main implications for our understanding of the neural circuitry mediating extinction learning. As was discussed above in reference to figure 2, studies in aversive learning have found that coordination between the vmPFC, amygdala,

and DH is crucial for extinction. In this model, the hippocampus has been suggested as the source of contextual modulation. The current studies find that the DH plays this role in appetitive extinction as well, but only for special kinds of contexts, i.e., temporal ones. Additionally, the findings of Corcoran and Maren (2004) taken together with the present findings suggest that the DH may also be involved in renewal when summation processes are involved. It is currently not known whether the form of appetitive renewal that depends upon Context-CS summation would also be affected by DH inactivation. While the neural mediation of fear extinction has been frequently investigated, only a limited number of studies have evaluated whether the findings are applicable to appetitive extinction learning. There is some evidence that the infralimbic region (IL) of the vmPFC may be similarly involved in both appetitive (e.g., Rhodes & Killcross 2004) and aversive extinction (e.g., Quirk et al. 2000). As noted above the amygdala has also been found to be involved in fear conditioning and extinction (e.g., Chhatwal, Myers, Ressler, & Davis, 2005; Fanselow & Poulos, 2005) as well as in appetitive processes (e.g., Holland & Gallagher, 2004). The current paper provides the first analysis of the role of the hippocampus in the extinction of appetitive learning.

More recently, Rhodes and Killcross (2007) found that lesions of the IL enhance renewal of appetitive learning. While this finding has not been demonstrated in aversive learning, this result would also be anticipated by the neural model discussed above. For example, responding is low when an extinguished CS is tested where it was extinguished, according to this model, because the extinction context activates the DH, which in turn, stimulates the IL. The IL then sends excitatory signals to the BLA where inhibitory interneurons as well as inhibitory intercalated cells will suppress the central nucleus of the amygdala and inhibit

the CR (see figure 2). On the other hand, when the CS is tested outside of the extinction context responding is renewed because the IL pathway is not as strongly activated by the DH. This results in more net excitation in the amygdala and, ultimately, the performance of CRs.

While in the aversive literature, it is commonly assumed that this neural model mediates renewal when conditional control processes are involved, the present data suggest that this view may not be entirely correct. Renewal studies in this paper have shown that when renewal is driven by conditional learning to the context the DH is not involved. Instead, The DH seems to be important when temporal contexts are the conditional stimuli which control responding. The neural model described above (see figure 2) may be more applicable to renewal when context-CS summation underlies renewal. For instance, the amygdala has been shown to be the site of convergence for the CS and US in fear conditioning (Ledoux, 2000, Fanselow & Polous 2005). On the one hand, the plasticity that occurs in the amygdala during training may be the neural correlate of the excitatory CS-US association that develops during training. On the other hand, the pathway from the DH and IL to the BLA may be analogous to an inhibitory context-US association. The neural circuit presented in figure 2 may then underlie renewal when context-CS summation is the determinant of responding. Alternatively, when renewal is dependent upon conditional control processes with temporal, but not physical, contexts, a different, unknown circuit may be involved. This circuit likely includes the DH since studies have found that the DH is involved in conditional control of extinction by temporal contexts (Experiment 4).

More work is clearly needed to determine whether the interactions of the structures found to be important for fear extinction is the same in appetitive learning. For example,

electrophysiological recordings in the BLA show context specificity following extinction. When the DH is inactivated, the specificity is eliminated (Maren & Hobin, 2007). Future studies showing a similar finding in appetitive conditioning would be very strong evidence that the circuit is operating in the same manner across both motivational domains.

A recurring point in the current paper has been the suggestion that more than one possible psychological mechanism may underlie the renewal effect. One of these mechanisms could be conditioned inhibition acquired to the extinction context during extinction. However, Bouton and King (1983) as well as Bouton and Swartzentruber (1986; 1989) failed to find evidence that the extinction context acquires inhibitory properties during extinction, suggesting that this summation-based account is unlikely. Bouton, (1993; 1994) proposed an alternative model of extinction in which the extinction context gates the expression of inhibitory CS-US learning. It has been suggested on multiple occasions that this model more adequately accounts for the results of the studies reported in the current paper. However, because the issue of whether or not the extinction context becomes a conditioned inhibitor has received recent attention, it is worth addressing possible summation based accounts of the renewal findings in the current paper as well as in other research designs.

Using summation and retardation tests, Polack, Laborda and Miller (in press) reported that extinction can result in the context becoming a conditioned inhibitor. In their study, however, massed trials during the extinction phase were shown to be more effective at producing this inhibition than spaced trials. Because the ITI used throughout training and extinction in Experiments 1-3 was, on average, 3 min it is unclear whether or not that would qualify as a spaced or massed arrangement. However, even if the extinction context

does, in fact, become a conditioned inhibitor in the present circumstances it is difficult to see how this could explain renewal in Experiments 1-3. Because these studies controlled for the training histories given to the different contexts, any differences in responding during the test cannot readily be explained by simple summation because both test contexts should have become equally inhibitory. However, one might attempt to explain the present findings in such terms when recognizing that a conditioned inhibitor is more effective at inhibiting the response to a CS with which it was trained than to a so called "transfer" stimulus. This phenomenon is known as incomplete transfer of conditioned inhibition (Rescorla, 1982). Rescorla (1982) demonstrated that this phenomenon likely involves the development of within compound associations between an inhibitor and the excitor used to establish it. Rescorla (1982), trained pigeons to associate two visual stimuli (A & B) with food in an autoshaping experiment. Then A was used to establish another visual cue (X) as a conditioned inhibitor by intermixing trials in which A was reinforced with trials in which AX was nonreinforced (i.e., A+, AX-). During the test phase it was found that responding to AX was lower than responding to BX. In other words, the transfer of inhibition from X to B was incomplete when an excitor other than that with which the inhibitor was originally trained was tested. Rescorla (1982) interpreted this incomplete transfer effect by noting that the inhibitor could associatively activate the memory of its training excitor (A) during the transfer test (BX). This would result in more net excitation on BX trials compared to AX trials because the memory of A plus B on BX trials produces more net excitation than A alone on AX trials. In another study, this idea was tested by extinguishing the within compound association formed between X and A (i.e., both the inhibitor and its training excitor were presented separately). Rescorla (1982) found that

after this treatment the ability of the inhibitor to transfer inhibition on BX test trials was enhanced, and this suggested that an important part of the incomplete transfer is due to the integrity of this within compound association.

Applied to the ABC renewal experiments in this paper, this reasoning can possibly explain the effects seen in those studies. For example, S1 was extinguished in CX2 and S2 was extinguished in CX3 in these studies. This could have resulted in CX2 and CX3 becoming inhibitory, as well as in the formation of separate CX2-S1 and CX3-S2 within compound associations. When S2 was tested in CX2, the within compound association between CX2 and S1 may have become active and whatever excitation S1 possessed would have combined with that remaining to S2 to result in more net excitation present on this test trial for the putatively inhibitory context to suppress. This would result in increased responding on that test trial (i.e., ABC renewal). A potential problem with this account, however, is that the within compound associations between the extinction contexts and the CSs undergoing extinction may have been extinguished. Because the presumed inhibitors (i.e., the extinction contexts) were repeatedly presented alone during the ITI in extinction, this could have served to extinguish the within compound association between these contexts and the CSs extinguished therein. Thus, it is not so obvious that incomplete transfer of inhibition should have occurred through this mechanism.

This explanation is more difficult to apply to the ABA renewal studies in this paper. In these studies, each context had the opportunity to associate with both CSs since each CS received training in one of these contexts and extinction in the other. Therefore during the test sessions, when either CS was presented, the context should have associatively activated the memory of the absent stimulus and comparable amounts of excitation and

inhibition should have occurred on each trial. However, if one assumes that the strength of the association between the context and the extinguished CS was greater than that between the context and the CS trained there, this reasoning could apply. This possibility is unlikely, though, because there were many more training trials (64) than extinction trials (24). Therefore, the incomplete transfer of inhibition is probably not the best explanation for the renewal effects seen in this paper.

More recently, Schmajuk and colleagues (Larrauri & Schmajuk 2008) offered another view of extinction. They also suggested that extinction results in inhibitory Context-US associations that partly contribute to renewal, and, additionally, that attention (or novelty) plays an important role. Specifically, these authors asserted that attention to the context and CS decreases during extinction. When the CS is tested outside of the extinction context, there is a return of attention to the CS because it is presented unexpectedly. This increased processing given to the CS results in renewal of conditioned responding. In the designs used in Experiments 1 & 2, attention to both contexts (as well as the CSs) during the extinction phase would be low as a result of extinction. When the CS extinguished in a particular context is tested in that context, attention remains low because the CS is expected in that context, and it is, therefore, poorly processed. When the other CS (the one that had been extinguished elsewhere) is presented in the same context, novelty increases. This is because presentations of that CS are not expected in the other CS's extinction context. This means that renewal should be observed in ABA and ABC test conditions in the current paper. One potential problem for this account of the ABA renewal studies is that there are grounds for both CSs to be expected in each of the two test contexts. This is due to the fact that each context served as an acquisition context for one CS and an

extinction context for the other. Laraurri and Schmajuk's (2008) account of renewal can only apply if the context-CS associations formed during the acquisition phase are assumed to be weaker that the context-CS associations formed during the extinction phase. Since there were a total of 64 acquisition trials and only 24 extinction trials it is not obvious that this assumption is justified.

Overall, the results of the experiments in this paper suggest that the DH is not involved in the conditional control of responding by physical contexts after extinction, at least in appetitive learning. Instead, the DH appears to be involved in controlling extinction of appetitive learning when temporal factors are important indicators of CS-US relationships. One feature of the current studies to highlight is that the designs used to study renewal in Experiments 1-3 were very effective at equating the training histories of the test contexts. This important attribute allows for more certainty with regards to the psychological mechanism being attributed to brain structures found to augment responding in renewal when they are manipulated. More work is needed to determine whether or not these findings reflect fundamental differences in the neural control of extinction between aversive and appetitive conditioning or whether the precise role of the DH in these recovery designs is much more limited than has commonly been assumed.

**Future Directions**

The studies in this dissertation add to a relatively new body of data which evaluates the possibility that the neural structures found to be important for mediating fear extinction are also involved in extinction of appetitive learning. While there is evidence that the role of some of these structures is the same across both domains (Rhodes & Killcross 2004), the current studies found, contrary to reports in fear conditioning (Corcoran & Maren 2004), that DH manipulations do not influence renewal of appetitive learning when physical contexts are used. Possible interpretations of these findings were offered in the preceding section and further work will be needed to evaluate those ideas. For example, one issue worth exploring is the possibility that physical-context-based renewal effects which depend on summation processes engage the DH while those based on occasion-setting like process do not. In order to investigate this question lesion or inactivation studies comparing different renewal designs within the same motivational domain would be required. While Maren and Hobin (2007) used a design more amenable to occasion-setting processes to study ABC renewal and Corcoran and Maren (2004) used a design amenable to summation process to study ABC renewal, the possible confound discussed above regarding the use of temporal factors by Maren and Hobin (2007) as occasion-setting stimuli prevent such a comparison between these studies.

Another question of interest regards the role of the DH when time plays the role of an occasion-setting stimulus. If the interpretation offered for the results of Experiment 4 is correct than instances in which temporal stimuli are used to signal CS-US relationships should be influence by hippocampal manipulations. For example, Bouton and Hendrix (2011) used the intertrial interval (ITI) as a conditional stimulus to signal whether or not a

target cue (a tone) would be reinforced. Specifically, if the tone followed a 16 min ITI the tone would be reinforced, however, if the tone followed a short 4 min ITI no reinforcement occurred. If the DH is important for using time as an occasion-setting stimulus than manipulations of the DH would be expected to impair acquisition of such a discrimination.

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