MULTIPLE MEMORY SYSTEMS AND EXTINCTION

A Thesis

by

AMANDA GABRIELE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Psychology

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Approved as to style and content by:

Mark Packard (Chair of Committee)	Barry Setlow (Member)
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ABSTRACT

Multiple Memory Systems and Extinction. (May 2005)

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Several lines of evidence suggest that initial acquisition of learned behavior involves multiple memory systems. In particular, lesions of the hippocampus impair the acquisition of cognitive or relational memory, but do not impair the acquisition of stimulus-response habits. Extinction behavior also involves new learning, and therefore it is possible that multiple forms of memory may also underlie extinction.

We examined this hypothesis by training rats in a task in which extinction behavior could putatively be acquired by either a cognitive or habit memory system. Adult male Long-Evans rats were initially trained to run in a straight alley maze for food reward. Following training they were placed into one of two extinction conditions. In one condition rats were allowed to run to an empty goal box (i.e. response extinction). In a second condition rats were placed into an empty goal box without making a running response (i.e. latent or non-response extinction). Prior to each daily session of extinction training, rats received intra-hippocampal infusions of either the local anesthetic bupivacaine (0.75% solution/0.5 ul), or saline.

Rats receiving saline infusions displayed extinction behavior in both the response and non-response conditions. In contrast, rats receiving intra-hippocampal infusions of

bupivacaine extinguished normally in the response condition, but did not display non-response extinction. This latent extinction effect was enhanced by decreasing the amount of time between the last extinction trial and the probe trial. Additionally, administering extinction training and probe trials in different contexts did not appear to prevent latent extinction, however large variability may be masking this effect. The new context administered during extinction prevented latent extinction in some animals, but not others. These findings suggest that, similar to initial acquisition, the learning that occurs during extinction also involves multiple memory systems. Specifically, the hippocampus may selectively mediate extinction under conditions in which new stimulus-response learning is prevented.

ACKNOWLEDGMENTS

I would like to thank Dr. Mark Packard for directing this research, Dr. Barry Setlow and Dr. William Griffith for serving on my committee, and Audrea Elliott, Zane Lybrand, Daniel Griffith, Siegfried Meier, and Kara Keuthan for all of their help.

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INTRODUCTION

The study of memory has always been extremely important and relevant in understanding how the brain functions. Increasing evidence indicates that rather than one overlying memory system there are, in fact, multiple memory systems responsible for many different types of memory**.** However, most multiple memory systems research has been devoted to studying the acquisition of new learning. Extinction, or the reduction of a response due to lack of reinforcement, is also believed to be "new learning" so it follows that if information is acquired through multiple memory systems, that information may also be extinguished through these same systems.

Evidence for Multiple Memory Systems

Evidence of multiple memory systems has been shown in human lesion patients who, based on their lesion, had deficits in one area of memory, but not others. The most famous being H.M., a patient who received a temporal lobectomy which included removal of the hippocampus, amygdala and surrounding areas as a treatment for seizures, and as a result experienced severe anterograde amnesia. Although H.M. was unable to form new declarative memories, he could still learn procedural tasks without any recollection of doing so (Scoville & Milner, 1957). This striking difference between impairment of conscious recollection (or cognitive learning) and task memories (or stimulus-response habit learning) implies that perhaps there are at least two separate memory systems responsible for each of these two types of memories.

This thesis follows the style and format of *Behavioral Neuroscience*.

Animal brain lesion studies were utilized to further explore this apparent dichotomy. Evidence for multiple memory systems is provided by studies using dissociation methodology. A double dissociation shows functional independence by illustrating that a lesion in brain area A impairs task C and not D, and that a lesion in brain area B impairs task D and not C. Studies done by Packard, et. al. (1989) have shown a double dissociation between the hippocampus and the caudate-putamen in two radial arm maze tasks. Further studies have also found a similar dissociation in a plus-shaped maze (Packard & McGaugh, 1996). Rats were placed in a plus-shaped maze and trained to start in the south arm and locate a food reward in the west arm. Rats were later probed by placing them in the north arm. Rats who made a right body turn and entered the west arm were classified "place" learners since they learned the task by remembering the spatial location of the food. Rats who made a left body turn and entered the east arm were classified "response" learners since they remembered the response of the left body turn they made during training. It was found that if rats were probed early in training (day 8) they would exhibit place learning whereas if they were probed late in training (day 16) they would exhibit response learning. This study found that if lidocaine, a sodium channel blocker, is injected into the hippocampus, place learning is blocked in the early probe trial while response learning in the late probe trial remains intact. Differentially, when lidocaine is used to inactivate the caudate-putamen, place learning remains intact in the early trial and response learning in the late probe trial is blocked. This suggests that place learning is hippocampus dependent and response learning is caudate dependent. Similar results showed that glutamate injections into the

hippocampus enhanced place and not response learning while injections into the caudate enhanced response and not place learning (Packard, 1999).

Other studies have found similar dissociations between the hippocampus and the caudate (Kesner, et. al., 1993). Literature examining monkeys and humans has resulted in similar findings. Monkeys with hippocampal-amygdala lesions showed impairments in object discrimination but were unaffected in motor skills tasks that required complex manipulation of tools in order to retrieve a food reward (Zola-Morgan & Squire, 1984). These impairments mirror those of H.M. (Scoville & Milner, 1957). Similarly, Parkinson's patients with neostriatal damage show impairments in a weather-predicting probabilistic classification task but were able to accurately answer a questionnaire based on declarative memories from the classification task. Conversely, amnesic patients were able to learn the probabilistic classification task but were impaired in the questionnaire (Knowlton, et. al., 1996). These findings support the hypothesis that memory for the initial acquisition and the subsequent expression of information is organized in multiple memory systems. It is not known whether these different memory systems also play a selective role in extinction of previously acquired information. Examining this question is the goal of the proposed research.

Extinction Is "New Learning"

Extinction is defined as the reduction of a response that occurs because the response is no longer followed by the reinforcer. Originally, extinction was thought to be simply the "forgetting" of an association and original learning is degraded (McClelland & Rumelhart, 1985). However, more recent evidence has shown that instead extinction

is new learning about the original association. The most prominent evidence that supports the "new learning" theory are the post-extinction relapse mechanisms of reinstatement, renewal, spontaneous recovery, and reacquisition (Bouton & Swartzentruber, 1991). Reinstatement is the restoration of a conditioned response when the US is once again presented. Context is especially important in the reinstatement phenomenon. Once the US has been re-presented, the CS will elicit the CR in the original conditioning context. Renewal is also extremely context dependent. Renewal occurs when the extinction training is not in the same context as the original conditioning. If replaced in the conditioning context the CS should once again elicit a CR. Spontaneous recovery is simply the CS eliciting the CR once extinction training is complete. Reacquisition occurs when the CS and the US are paired again after extinction, causing the CS to elicit the CR. These relapse mechanisms would not be possible if the CS-US association was destroyed, giving rise to the idea that extinction involves forming the association that the CS no longer predicts the US rather than "forgetting" this association all together (For review see Bouton, 2002).

Further evidence that the CS-US association is maintained during extinction is the fact that simultaneous excitatory and inhibitory associations are possible between the CS and the US. Tait and Saladin (1986) demonstrated with backward conditioning that a single CS concurrently had an excitatory association with a shock which produced conditioned lick suppression and an inhibitory association with the shock which produced retarded eyeblink conditioning. This shows that as extinction training progresses, an inhibitory association may be forming in addition to the already present

excitatory association. These two simultaneous associations provide evidence for the "new learning" theory and further explain the presence of relapse mechanisms. Additionally, when glucose or oxotremorine (a muscarinic receptor agonist) is given either systemically or intra-amygdala it will, in much the same manner than it affects learning, facilitate consolidation of conditioned place preference extinction (Schroeder & Packard, 2003; Schroeder & Packard, 2004).

The Hippocampus and Extinction: Lesion Studies

Early research on the hippocampus has indicated hippocampal lesions lead to impairments in runway extinction (Gaffan, 1972; Raphelson et. al., 1966; Jarrard et. al., 1964) resulting in the theory that the hippocampus was necessary for response inhibition. These studies were done in the late 60s and early 70s and the lesions were performed using radical ablation techniques, many of which were conducted prior to acquisition, that often times removes more than the hippocampus. The permanence of this lesion technique may play a role. The possibility should be considered that the animal, forced to learn the task with an S-R strategy in absence of the hippocampus, may be more resistant to extinction due to this difference in acquisition since S-R tasks are more difficult to extinguish (O'Keefe & Nadel, 1978; Osborne & Markgraf, 1988) Since the hippocampal lesion is primarily affecting acquisition that, in turn, affects extinction. Lesioning the hippocampus just prior to extinction could then lead to differing results.

Impairments in extinction following hippocampal lesions may be dependent upon what type of learning is being extinguished. Generally, studies examining extinction in classical conditioning tasks (Schmaltz & Theios, 1972) and one-way active avoidance

(Thomas & McCleary, 1974) found no deficits following hippocampal lesions. In contrast, extinction in two-way active avoidance tasks (Isaacson, et. al., 1961) is impaired by hippocampal lesions, as is extinction in conditioned taste preference (Reilly et. al., 1993).

A significant portion of the research studying the hippocampus and extinction has focused of the extinction of conditioned fear. Contextual fear conditioning, which involves associations between a context and foot shock, is hippocampal dependent (Rudy et. al., 2002). However, hippocampal lesions given prior to both Pavlovian fear conditioning where a light was paired with foot shock and operant bar-press conditioning were found to impair reinstatement but not extinction (Wilson et. al. 1995). In contrast, hippocampal lesions have been found to impair extinction in appetitive Pavlovian conditioning where a tone was paired with food (Benoit et. al., 1999). Extinction has been shown to allow a CS to simultaneously be involved in an excitatory and an inhibitory association with the US (Chan et. al., 2001). This indicates that perhaps hippocampal lesions impaired the ability to form inhibitory associations necessary for extinction of Pavlovian conditioning (Benoit et. al., 1999). Studies investigating the hippocampus and context extinction have found that the hippocampus may be necessary for this simultaneous inhibitory association to occur (Chan et. al., 2001). While the previous studies seem to conflict, it is evident that the involvement of the hippocampus in extinction may be task dependent and further research is needed to elucidate the role the hippocampus plays. Similarly, research studying hippocampal involvement in contextually mediated fear extinction has been contradictory. While

Wilson et. al. (1995) and others (Frohardt et. al., 2001) have found that hippocampal lesions do not affect renewal of contextual fear conditioning, contrasting results have been found. Corcoran and Maren (2001) established that when hippocampal inactivation occurred just prior to the test period, renewal was impaired. The discrepancy between the results of these studies may be attributed to differences in the time course of the hippocampal lesion. In studies that found no effect on renewal, the hippocampus was permanently lesioned prior to acquisition. This indicates that perhaps hippocampal lesions impair retrieval. However, when extinction is learned without a hippocampus, other areas may compensate (Corcoran & Maren, 2001). While the hippocampus is crucially involved in contextually mediated extinction in intact rats, it is not necessary for this extinction to occur (Myers & Davis, 2002).

Generally speaking, much of the literature on the role of the hippocampus in extinction is contradictory, focusing more on stimulus-response tasks, whereas studies on complex maze learning are lacking. It is general agreement that the hippocampus is involved in spatial learning **(**O'Keefe & Nadel, 1978**)** so to better understand the role of the hippocampus in extinction, it is useful to examine the effects of hippocampal lesions on extinction where spatial information is critical. A procedure that involves this is latent extinction.

Latent Extinction

Latent extinction was first discovered by Seward and Levy in 1949. The idea of latent extinction was based on the theories of Tolman and colleagues that animals acquire learned expectancies, or the idea that they learn "what leads to what" (Tolman,

1932). At that time, Tolman, who is thought of as the forefather of cognitive learning, challenged the current S-R theory by using words such as "purposive" and "planning" to describe animal behavior. According to this cognitive view of animal learning, if an animal is trained to run to a goalbox for food reward and subsequently finds the goalbox empty, it only needs to recall its emptiness for extinction to be possible (Tolman, 1932). Seward and Levy hypothesized that these requirements can be met without the animal actually performing the running response, simply by placing the animal in an empty goalbox. Consistent with this idea, after training animals to run a straight alley maze for a food reward, those animals who received confinement in the goalbox of the maze showed significantly longer running latencies than animals who were confined off of the maze. This phenomenon was termed latent extinction (Seward & Levy, 1949).

This finding was met with a great deal of controversy. At the time, stimulus-response (S-R) learning theories were prominent, and the idea that an animal can extinguish a running response without the running response being made is directly in contrast to S-R theory. Proponents of the S-R theory challenged the findings of Seward and Levy and the idea of latent extinction. In an attempt to question the latent extinction finding, it was found that a replication of Seward and Levy's experiment did not produce similar results (Bugelski et. al., 1952). It was later determined that there was a difference in extra maze cues between the two experiments, with the experiment conducted by Bugelski et al. containing fewer cues (Denny & Ratner, 1959). This latter study also demonstrated that latent extinction is spatially dependent, and requires exposure to extra maze cues to occur. Placing a curtain around the goalbox during latent extinction

essentially removed the latent extinction effect and animals in this group performed no different that controls who had not received latent extinction on the maze. In contrast, animals with a view of extra-maze stimuli showed a strong latent extinction effect (Denny & Ratner, 1959).

Another attempt to explain latent extinction using S-R theory was offered by Moltz (1957). According to Moltz, the latent extinction effect can be explained as a reduction in the "fractional anticipatory response" in which the animal's anticipatory consummatory response to the food reward is extinguished during latent extinction (Moltz, 1957). However, according to this fractional anticipatory response theory, once the animal has completed the latent extinction and proceeds to regular extinction trials, the animal should continually increase running times. It was found that this is not the case. By the end of five regular extinction trials, the difference in latencies between animals that received latent extinction and controls disappears (Dyal, 1962.) Overall, the S-R theorists were unable to explain the latent extinction effect adequately.

Many further studies were done to examine factors that affect latent extinction. It was found that a distinctive goalbox resulted in an enhanced latent extinction effect when recording running latencies. When training in a T-maze for food reward, animals who received latent extinction in a black and white striped goalbox showed an enhanced latent extinction effect as compared to both controls and animals that received latent extinction in a goalbox that was an extension of the alley (Hughes et. al., 1960). Latent extinction has also been ascertained in a U-maze discrimination task. Animals that received latent extinction in this U-maze task showed significantly fewer correct choices

as compared to controls (Deese, 1951). Also, overtraining was found to cause resistance to the latent extinction effect. Animals that received either 28 or 77 training trials on a straight alley showed a robust latent extinction effect, while those that received 210 training trials were no different than controls post latent extinction (Dyal, 1963). However, it has also been found that if animals are given a single latent extinction trial, a facilitation of goal running occurs. This has been interpreted as the single latent extinction trial produces an increase in frustration, which leads to increased running (Jones et. al., 1970). Interestingly, it was also found that the interval between latent extinction trials and test trials is important. The latent extinction effect was significant at an interval of 60 seconds or one hour and weakened at an interval of 24 hours (Dyal, 1964). Studies on latent extinction have indicated that this type of extinction is spatial in nature (Denny & Ratner, 1959). The animal does not need to make a running response to determine the goalbox no longer contains the food reward. If extinction is indeed "new learning" it follows that if spatial information is learned with the hippocampus (O'Keefe & Nadel, 1978), then the hippocampus should also be involved in extinguishing associations that are learned spatially. In view of what we know of the role of the hippocampus in spatial learning, it is possible to examine the neural basis of latent extinction, which has yet to be explored. The following experiments begin to investigate the role the hippocampus plays in latent extinction.

Predictions

The first experiment examines whether latent extinction is hippocampal dependent. If the hippocampus is reversibly inactivated using a sodium channel blocker

bupivacaine during latent extinction, extinction behavior should be impaired. Previous studies on latent extinction have found that the latent extinction effect degrades with the length of time following the last latent extinction trial and the probe trial. For example, the latent extinction effect is stronger one hour after extinction training as opposed to 24 hours. (Dyal, 1964). Shortening the period between latent extinction training and the probe trials increases the magnitude of the latent extinction effect in controls. This should increase the likelihood of observing an effect of intra-hippocampal bupivacaine. Also, more probe trials following the latent extinction training were given in order to determine the effect of latent extinction over several probe trials. Animals in experiment 2 received training identical to experiment 1 but the interval between the last extinction trial and the probe trial was decreased from 24 hours to 90 minutes. In order to determine whether the results from experiments 1 and 2 are due to a drug effect on latent extinction and not simply extinction in general, animals in experiment 3 were given "response" extinction. In response extinction, animals were allowed to run the length of the maze as in training, to find the goalbox empty. Based on our current hypotheses, it is predicted that in experiments 1, 2, and 3, animals receiving intra-hippocampal administration of bupivacaine will not display latent extinction but will extinguish normally in the response condition. Rats receiving saline infusions will display extinction behavior in both the response and non-response conditions to demonstrate the latent extinction in the runway task. Also in experiment 4, response extinction was analyzed using the experimental paradigm from experiment 2. Animals were allowed to make the running response during extinction but given the 90-minute interval before the

probe trials. It is predicted that the latent extinction effect will be stronger in experiment 2 in control animals than in experiment 1 due to the shorter latency between extinction training and probe trial. The stronger latent extinction effect should allow for a clear assessment of the bupivacaine effect. In order to determine whether latent extinction is truly spatial in nature a control group were given latent extinction in a different environmental context (i.e. a different room) than acquisition, but probed in the same context as initial training. It is predicted that since latent extinction is spatially mediated, animals receiving latent extinction in a new context will not show the latent extinction effect when probed in the initial training context. Overall, the hypothesis of the proposed research is that extinction involves multiple memory systems and the hippocampus mediates the acquisition of latent extinction. According to our hypothesis, if the hippocampus is reversibly inactivated, it will selectively block latent extinction.

GENERAL METHOD

Apparatus

The maze was a clear plexiglass open straight alley maze that is 70 in. long, 4.5 in. wide and 8 in. tall. A small reward cup 1 in. in diameter was located at one end of the maze. The maze was elevated 34 in. off of the ground and placed in a room with several extra- maze cues.

Subjects

Subjects were male Long-Evans rats (275-300 g). Rats were individually housed on a 12:12 hour light-dark cycle, with lights on from 8:00 a.m. to 8:00 p.m. All animals received food and water *ad libitum*.

Surgery

Animals were anesthetized with sodium pentobarbital (60 mg/kg) and bilateral guide cannula (10 mm long) were inserted in the dorsal hippocampus using standard stereotaxic techniques. Coordinates were anterior-posterior (AP) = -3.1 mm from bregma, medial-lateral (ML) = +/- 2.0 mm from bregma, and dorsal-ventral (DV) = -2.0 mm from skull surface. These coordinates were chosen based on prior research in our lab (Schroeder, et. al., 2002). Behavioral testing began two weeks after surgery.

Histology

The animals were anesthetized with 1 cc of sodium pentobarbital (60 mg/kg) and perfused with a physiological saline solution, followed by a 10% formol-saline solution. Brains were sectioned at 20 um and stained with cresyl violet. Cannula implantations were verified with Paxinos and Watson (1986). Histological results are shown in Figure

1. Infusion needles tips were located -2.80 to –3.30 anterior-posterior from bregma.

Drugs and Infusions

A 0.75% bupivacaine solution (Abbott Laboratories) was used to produce reversible inactivation of the hippocampus. This percentage has been found to lead to memory impairments in both the hippocampus and amygdala (Schroeder et. al., 2002; Hsu et. al., 2002). Bupivacaine is a local anesthetic that causes a reversible inactivation of neural tissue, and like the structurally similar lidocaine, prevents the conductance of action potentials through a blockade of sodium channels. However, bupivacaine possesses a longer duration of action (lidocaine has an approx. 10-30 min range, bupivacaine has an approx. 30-50 min. range) (Caterall & Mackie, 1986). Saline was used for control animals. 0.5 uL/side was administered using a microsyringe pump with 10 uL Hamilton syringes connected to polyethylene tubing. Infusions were administered over a period of 54 s, and left in place for an additional 60 s to allow for diffusion. This volume has been found to lead to satisfactory memory impairment (Schroeder et. al., 2002).

Behavioral Procedures

Prior to training, rats were reduced to 85% of initial body weight and maintained at this weight throughout training. On day 1, animals were habituated to the straight alley maze in a single two-minute trial with no food. Animals then received 15 Noyes sucrose food pellets in their home cage to introduce them to the food reward. On day 2 of training, animals were shaped to approach the goal box by placement of pellets along the length of the maze. Animals were given ten days of training (6 trials per day) for a

reward of 1 sucrose pellet per trial in the goal box. Latencies to reach the food cup were recorded by the experimenter. If the animal failed to transverse the maze in 60 seconds, it was removed from the maze. During each 30-second intertrial interval, animals were placed in an opaque holding box adjacent to the maze. Following training, animals were given three days of extinction training (6 trials per day). Drug infusions (either saline or bupivacaine) were given immediately pre-training for all three days of extinction training.

Statistics

A two-way one-repeated measures ANOVA was used to analyze running latencies during initial training to determine that are no differences in groups prior to extinction training in terms of acquisition. Probe trial latencies were analyzed using a one-way ANOVA. A one-way ANOVA will also be used to analyze differences between the last training trial and the probe trial for groups receiving latent extinction.

EXPERIMENT 1

Method

14 naïve rats were used in experiment 1. Animals were rank-ordered and matched based on latencies during the last three days of training (all animals had asymptoted by day 7 of training) to form two groups and then assigned a drug condition: bupivacaine or saline (n = 8, n = 6 respectively). Animals were given bilateral hippocampal cannulation surgeries and behavioral acquisition training on the straight alley maze as described in the general methods section above. Following food-rewarded training, animals were given three days of latent extinction training (6 trials per day) by confining them in the goal box of the maze without reward. Each trial lasted 60 seconds. As in initial training, during each 30 second intertrial interval, animals were removed from the maze and placed in an opaque box adjacent to the maze. Drug infusions (either saline or bupivacaine) were given immediately pre-training for all three days of extinction training. 24 hours after completion of the final extinction trial, animals were given a drug-free probe trial in which they were placed in the straight alley as in initial training and latency to reach the goal box was recorded.

Results

One-way ANOVA indicated intra-hippocampal infusions of bupivacaine did not selectively block latent extinction as compared to controls on the probe trial ($F_{1,13}$ = 1.201, p = 0.295) (Figure 2). However, further analyses revealed that while there was a significant difference between the last trial on day 10 (the last day of acquisition training) and the probe trial for controls ($F_{1,11}$= 4.098, p = 0.070) there was no

difference in animals that received bupivacaine ($F_{1,15} = 0.878$, $p = 0.365$) indicating that intra-hippocampal bupivacaine produced similar latencies on the probe trial as on the last training trial (Figure 3). Additionally, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{4.25, 12} = 17.554$, $p < 0.001$) indicating significant differences in latencies between training days. There was not a significant main effect for treatment ($F_{1,12} = 0.139$ $p = 0.715$) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{4.25,12} = 1.073$, $p = 0.388$). This study found that, using some measures, hippocampal inactivation blocks latent extinction.

EXPERIMENT 2

Method

14 naïve rats were used in experiment 2. Animals were rank-ordered and matched based on latencies during the last three days of training (all animals had asymptoted by day 7 of training) to form two groups and then assigned a drug condition: bupivacaine or saline (n = 7). The methods are identical to experiment 1, with the exception that four probe trials were given 90 minutes following the last latent extinction trial.

Results

One-way ANOVA indicated intra-hippocampal infusions of bupivacaine selectively blocked latent extinction as compared to controls as indicated by significant differences in group means on the probe trials ($F_{1,13} = 11.209$, $p = 0.006$) (Figure 4). However, further analyses revealed that while there a significant difference between the last trial on day 10 (the last day of acquisition training) and the first probe trial for controls ($F_{1,11} = 4.218$, $p = 0.067$) there was also a difference in animals that received bupivacaine ($F_{1,13} = 10.159$, $p = 0.008$) (Figure 5). This indicates that while animals who received intra-hippocampal infusions of bupivacaine during extinction had significantly shorter latencies on the probe trials than controls, intra-hippocampal bupivacaine did not produced similar latencies on the probe trial as on the last training trial. Additionally, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{3.97, 12} = 45.528$, $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for treatment ($F_{1,12} = 0.153$ $p = 0.702$) indicating no significant differences in latencies

between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{3.97,12}$= 0.992, p = 0.421). This experiment found that hippocampal inactivation blocks latent extinction.

EXPERIMENT 3

Method

16 naive rats were used in experiment 3. Animals were rank-ordered and matched based on latencies during the last three days of training (all animals had asymptoted by day 7 of training) to form two groups and then assigned a drug condition: bupivacaine or saline (n = 8). Animals were given bilateral hippocampal cannulation surgeries and behavioral acquisition training on the straight alley maze identical to experiment 1. Following training, animals were given three days of "response" extinction training (6 trials per day) by allowing them to run the length of the maze as in training, but being removed from the goal box without reward. Again, if an animal failed to reach the food cup in 60 seconds, it was removed from the maze. As in initial training, during each 30 second intertrial interval, animals were removed from the maze and placed in an opaque box adjacent to the maze. Drug infusions (either saline or bupivacaine) were given immediately pre-training for all three days of extinction training. 24 hours after completion of the final extinction trial, animals were given a single drug-free probe trial in which they were placed in the straight alley as in initial training and latency to reach the goal box was recorded.

Results

One-way ANOVA indicated no difference in response extinction on the probe trial for those animals receiving intra-hippocampal infusions of bupivacaine as compared to controls ($F_{1,15} = 2.011$, $p = 0.178$). Additionally, a repeated measures ANOVA found no differences in groups based on drug treatment for acquisition of extinction, no

significant main effect for extinction day ($F_{2, 14} = 0.225$, $p = 0.800$), drug treatment ($F_{1,14} = 0.009$ $p = 0.924$) or interaction between extinction day and drug treatment ($F_{2,14} = 0.305$, $p = 0.740$) were found (Figure 6). Also, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{3.09, 14} = 21.629$ $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for treatment ($F_{1,14} = 0.069$ $p = 0.796$) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{3.09,14} = 0.793$, $p = 0.507$). This experiment found that hippocampal inactivation does not block response extinction.

EXPERIMENT 4

Method

13 naïve rats were used in experiment 4. Animals were rank-ordered and matched based on latencies during the last three days of training (all animals had asymptoted by day 7 of training) to form two groups and then assigned a drug condition: bupivacaine or saline (n = 7, n = 5 respectively). One saline animal was removed due to incorrect cannula placement. The methods are identical to experiment 3, with the exception that four probe trials were given 90 minutes following the last latent extinction trial.

Results

One-way ANOVA indicated no difference in response extinction for group means on the probe trial for those animals receiving intra-hippocampal infusions of bupivacaine as compared to controls ($F_{1,11} = 0.020$, $p = 0.892$). Additionally, a repeated measures ANOVA found no differences in groups based on drug treatment for acquisition of extinction, a significant main effect for extinction day was found ($F_{2, 10} = 4.464$, $p = 0.025$), but the main effect for drug treatment ($F_{1,10} = 1.645$ $p = 0.229$) and the interaction between extinction day and drug treatment ($F_{2,10} = 1.280$, $p = 0.300$) were not significant (Figure 7). Also, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{3.89, 10} = 31.074$ $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for treatment ($F_{1, 10} = 0.001$ $p = 0.976$) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed

between day and treatment ($F_{3.89,10} = 0.648$, $p = 0.627$). This experiment found that hippocampal inactivation does not block response extinction.

EXPERIMENT 5

Method

13 naïve rats were used in experiment 5. Animals were rank-ordered and matched based on latencies during the last three days of training (all animals had asymptoted by day 7 of training) to form two groups and then assigned an extinction condition: new context or same context (n = 6, n = 7 respectively). Animals were given behavioral acquisition training and latent extinction on the straight alley maze identical to experiment 2 with the exception of extinction context. Animals in the "same context" extinction group were given latent extinction training in the acquisition context. Animals in the "different context" extinction group were given latent extinction training in a new context by placing the maze in a new room with different extra-maze cues. Each trial lasted 60 seconds. As in initial training, during each 30 second intertrial interval, animals were removed from the maze and placed in an opaque box adjacent to the maze. Both groups received four probe trials 90 minutes following the last latent extinction trial in the initial training context and latencies to reach the goalbox were recorded.

Results

One-way ANOVA indicated latent extinction in a new context did not block the latent extinction as compared to controls as indicated by differences in group means on the probe trials ($F_{1,12} = 3.098$, $p = 0.106$) (Figure 8). However, further analyses revealed that while there a significant difference between the last trial on day 10 (the last day of acquisition training) and the probe trail for same-extinction context controls ($F_{1,11} = 4.218$, $p = 0.067$) there was not a difference in animals that received latent extinction in

the new context ($F_{1,11} = 3.133$, $p = 0.107$) (Figure 9). This indicates that latent extinction training in a new context produces similar latencies on the probe trial as on the last training trial. Additionally, in a repeated measures ANOVA examining acquisition, a significant main effect for day was found ($F_{3.49, 11} = 53.098$, $p < 0.001$) indicating significant differences in latencies between days. There was not a significant main effect for extinction treatment ($F_{1,11} = 0.466$ $p = 0.509$) indicating no significant differences in latencies between treatment groups. Also, a significant interaction effect was not observed between day and treatment ($F_{3.49,11} = 0.847$, $p = 0.491$). This experiment indicated that administering extinction training and probe trials in different contexts did not appear to prevent latent extinction, however large variability may be masking this effect.

SUMMARY AND CONCLUSIONS

Conclusions

The present studies examined the effect of reversible lesions of the hippocampus on latent extinction. These studies compared latent extinction, in which the animals are confined in the goalbox and no response is made, and response extinction, in which the animals are allowed to run the length of the maze. The overall goal was to show a hippocampal dissociation between these two types of extinction, with the hippocampus selectively mediating latent extinction, indicating that a multiple memory systems approach to extinction is feasible. Previous research on multiple memory systems has shown a double dissociation between the hippocampus and the caudate for acquisition of tasks that can be acquired both spatially and by stimulus response associations (Packard & McGaugh, 1996). In experiment 1 significant statistical trends indicated that the animals that received saline had higher latencies on the probe trial than those that received bupivacaine treatments. Large variability in the saline group likely contributed to the lack of significance. However, if latent extinction is truly blocked, then bupivacaine treated animals should perform on the probe trial with latencies similar to the last training trial run during acquisition. Interestingly, this was found to be the case. Animals that received intra-hippocampal infusions of bupivacaine were found to have no differences in latencies between the last acquisition trial and the probe trial. Conversely, saline controls had significantly higher latencies on the probe trial than on the last acquisition trial. This indicates that using this measure, hippocampal inactivation impairs latent extinction. Overall, the findings from experiment 1 indicate that hippocampal

inactivation blocks latent extinction using some measures, but the variability in the saline animals may be masking the drug effect. Furthermore, extinction-probe trial intervals greater than 24 hours have been found to reduce the latent extinction effect (Dyal, 1964), which may have contributed to the lack of significant differences. Therefore, in order to further examine the effect of hippocampal inactivation on latent extinction, the time period between the last extinction trial and the first probe trial was shortened. Also, the large amount of variability in the probe trials could also be due to the fact that animals were only given a single probe trial, so abnormalities in a single probe trial could not be accounted for by averaging into several trials. By administering four probe trials, post-extinction behavior could be analyzed more clearly.

In experiment 2, using a longer time period between latent extinction training and the probe trials demonstrated that intra-hippocampal infusions of bupivacaine reliably blocked latent extinction. Importantly, as groups were matched for acquisition performance on the straight alley task prior to extinction, the differences between groups were due to treatment conditions applied during extinction. This finding indicates that the hippocampus is part of the neural circuitry underlying latent extinction.

However, these results do not indicate whether the effect of hippocampal inactivation on latent extinction is selective. This question was examined in experiments 3 and 4. In both studies, hippocampal inactivation did not block response extinction. This demonstrates that hippocampal inactivation is not affecting motivation, or causing motoric or sensory defects. Instead, latent extinction is selectively blocked by hippocampal inactivation, providing evidence for a dissociation of hippocampal function

in extinction. The level of extinction of control animals that received latent exposure was similar to control animals that received response extinction. This finding indicates that, using the present training parameters; latent extinction produces an effect similar in magnitude to response extinction. These findings suggest that the hippocampus selectively mediates extinction under conditions in which new stimulus-response learning is prevented. Similar to initial acquisition of learned behavior (Packard, et. al., 1989; Packard & McGaugh, 1996; Kesner, et. al., 1993), the learning that occurs during extinction likely involves multiple memory systems.

Experiment 5 found that latent extinction was not blocked when extinction training and probe trials were applied in different contexts. Animals that received latent extinction in the new context had shorter, but not significantly different, latencies than control animals that received latent extinction in the acquisition context. The results of this experiment were extremely variable. The latent extinction effect was blocked in two animals that received extinction in a different context. This experiment should be replicated using a larger "n" in order to account for the large amount of variability in the present study.

Summary

These findings provide more elucidation for the role the hippocampus plays in extinction. The hippocampus may mediate spatial extinction in a similar manner to spatial acquisition. Experiments 1 and 2 indicated that hippocampal inactivation blocks latent extinction, while experiments 3 and 4 indicated that the hippocampus does not play a role in response extinction. According to experiment 5, context may play a role in

latent extinction but additional studies are needed. Similar to initial acquisition, the mechanisms involved in extinction likely involve multiple memory systems. Taken together, these studies indicate that the hippocampus plays an important role in extinction by selectively mediating latent extinction and further studies are needed to determine exact nature of this role.

Spatial Acquisition and Extinction: Cognitive or Stimulus-Response Learning Mechanisms?

Lattal and Abel have argued that the mechanisms underlying spatial extinction are different from those fundamental to spatial acquisition (2001). While acquisition of a spatial water maze task requires protein synthesis; extinction of this task is not protein synthesis dependent (Lattal & Abel, 2001). Further exploration of spatial extinction has found several similarities to Pavlovian extinction. Pavlovian extinction principles such as renewal and spontaneous recovery have been demonstrated in the water maze task indicating that the original associations are not lost during spatial extinction (Lattal et. al., 2003). Therefore, theories based on Pavlovian learning principles may also apply to spatial extinction.

Additionally, while is has been argued that spatial acquisition is different from other types of learning due to the uniqueness of place cells and their ability to fire in response to spatial locations (O'Keefe & Dostrovsky, 1971), Lattal and Abel further contend that acquisition in a water maze task has many similarities to principles of Pavlovian conditioning as well, such as latent inhibition and blocking. Stimulus pre-exposure of adjacent landmarks has been found to cause latent inhibition in the water

maze task while pre-exposure to landmarks with salient similarities enhanced acquisition in the water maze (Prados et. al., 1999). Additionally in the water maze, animals were found to use a landmark paired with the platform rather than a stable landmark for escape strategies. (Roberts & Pierce, 1998). Once an animal has been trained with several landmarks, addition of a new landmark has not been found to influence escape strategies, demonstrating the blocking effect in the water maze (Rodrigo et. al., 1997). These results have been used as evidence to argue against the cognitive map theory and instead for an associative theory of spatial learning (Rodrigo et. al., 1997). However, contrasting evidence shows that the associative theory does not accurately account for all spatial behavior. According to the associative theory, the animal associates goal stimuli or configural sets of stimuli with the reward and therefore uses a set of associations working backwards to determine their path to the goal (Rodrigo et. al., 1997). This explanation cannot account for novel goal strategies (Poucet, 1993) such as Tolman's 1930 study on introduction of a reward. Hungry animals that were run on T-maze for food reward showed improvement in their accuracy in speed in completing the maze, while non-rewarded animals remained the same, running the maze without a great deal of choice accuracy or speed. However, when a food reward was introduced onto the maze to the previously unrewarded group on day 11 of acquisition, their latencies and choice accuracy improved immediately to match those of the rewarded animals. The association theory of spatial learning cannot account for this behavior since the animals learned about their spatial location in the absence of a reward (Tolman & Honzik, 1930).

While Lattal et. al. use the presence Pavlovian principles in both acquisition and extinction of spatial tasks to argue that spatial extinction is Pavlovian in nature, it is important to note that simply because Pavlovian principles are evident in spatial extinction, that does not imply that extinction of spatial behavior uses Pavlovian S-R mechanisms. Latent inhibition and blocking can also be interpreted with the framework of cognitive learning theory, as animals acquire expectancies about the goal. Furthermore, reinforcer devaluation prior to extinction of an instrumental response leads to a significant decrease in responding as compared to animals that had not received devaluation (Rescorla, 1993). This indicates that perhaps during extinction, response-outcome relationships are not extinguished, and instead inhibitory S-R associations cause the decrease in responding (Domjan, 2003). Additionally, previous studies on multiple memory systems have shown that tasks such as a plus-maze task can be acquired using both spatial and S-R learning strategies simultaneously (Packard, 1999). This indicates that perhaps extinction can be learned both spatially and with S-R strategies, leading to Pavlovian principles in seemingly spatial tasks. The present studies focus on latent extinction, which has been found to be spatially dependent (Denny & Ratner, 1959). The animal does not make a response during latent extinction, and S-R theorists have been unable to explain latent extinction in S-R terms (Dyal, 1962). The present dissociation further shows that latent extinction is not based on S-R associations. Since the hippocampus is known to be unnecessary for S-R behavior, if latent extinction was S-R based then hippocampal inactivation would have no effect. This was not found to be the case, illustrating that latent extinction is truly a spatial form of extinction.

Molecular Basis of Extinction: The Hippocampus

The present studies used reversible lesion techniques to study extinction. However, this approach does not provide evidence for the molecular mechanisms that underlie extinction. Evidence has shown that protein synthesis required for learning occurs in the hippocampus during extinction training. Specifically, infusions of protein synthesis inhibitors (anisomycin) prevent extinction in inhibitory avoidance from occurring, indicating that the hippocampus is directly involved in the acquisition of extinction. (Vianna et al., 2001). The extinction of inhibitory avoidance was found to depend on both protein synthesis and gene expression in the hippocampus (Vianna et. al., 2003). However, other studies have found that intra-hippocampal infusions of anisomycin and puromycin (protein synthesis inhibitors) enhanced extinction of conditioned fear while intra-hippocampal infusions of cytochalasin D and latrunculin (actin rearrangement inhibitors) blocked extinction of conditioned fear where a single exposure of a context and tone are paired with foot shock, indicating that perhaps protein synthesis in the hippocampus is not required for all types of extinction (Fischer et. al., 2004). Additionally, Lattal & Abel (2001) found that while systemic administration of anisomycin blocked acquisition of fear conditioning where context was paired with a foot shock and the Morris water maze task, extinction was acquired normally in the absence of protein synthesis indicating that perhaps the molecular mechanisms that underlie extinction of hippocampal-dependent tasks may be protein synthesis independent. The inconsistency in the results of these studies indicates that perhaps the role of protein synthesis in extinction is more specific than has been previously

examined. Further studies are needed to elucidate the function of protein synthesis in hippocampal-dependent extinction. Perhaps the latent extinction paradigm developed here can be useful in these investigations. The present studies demonstrated a dissociation for response and latent extinction when the hippocampus is inactivated. Since the effect of protein synthesis on extinction appears to be task dependent, latent extinction provides a spatial extinction task that is hippocampal dependent.

Further studies on the molecular mechanisms of extinction behavior have found that infusions of AP5 (an NMDA antagonist), Rp-cAMPs (a PKA inhibititor), KN-62 (a CaMKII inhibitor) and PD098059 (a MAPK kinase inhibitor) into the hippocampus all produced impairments in contextually based extinction in the inhibitory avoidance task (Szapiro et. al., 2003). This indicates that the same mechanisms necessary for acquisition of contextual fear are also involved in the extinction of that fear (Szapiro et. al., 2003). An additional study on recombinant inbred mice with significant reductions of the hippocampal commissure (HC) found that the HC is necessary for the extinction of conditioned fear where a single exposure of a context and tone are paired with foot shock and the short-term plasticity associated with that extinction (Schimanski et. al., 2002).

Neurochemical Basis of Extinction

NMDA glutamate receptors have been implicated in extinction of conditioned fear, specifically in the amygdala. Intra-amygdala infusions of AP5 (an NMDA antagonist) blocked conditioned fear extinction (Falls et al. 1992). Santini et al. (2001) further explored the role NMDA receptors play in extinction though systemic NMDA

receptor antagonist injections during consolidation of extinction learning. Rats were given extinction training of conditioned fear where a tone was paired with foot shock and then after either an hour or 24-hour break, extinction training was repeated. When the NMDA receptor antagonist (CPP) was given just prior to initial extinction training in the one hour break group, animals were able to learn extinction but unable to recall it 24 hours later. Second, when CPP was given prior to extinction in the 24-hour break group, extinction was both learned and recalled 48 hours later. Third, when CPP was given during the break in the 24-hr break group, no CPP effect was observed. Fourth, if CPP was given both prior to extinction and during the break of the 24-hr break group, extinction recall is blocked. These results suggest that while NMDA receptor antagonists do not affect short-term memory, NMDA receptors are essential for consolidation that leads to long-term memory of extinction. However, it is also evident that extinction memory can have delayed consolidation. Additionally, systemic injections of D-cycloserine (a partial NMDA agonist) were found to enhance conditioned fear extinction (Santini et al. 2001). Further studies also found that this enhancement was blocked by administration of an antagonist (HA-966) that acts on the same site on the NMDA receptor, which give further evidence that the facilatory effect of C-cycloserine is through the NMDA receptor (Walker et. al., 2002). While most research on the role of NMDA receptors in extinction has focused on either systemic or intra-amygdala infusions (Falls et. al., 1992), it is clear that NMDA receptors are necessary for at least some types of extinction. Since NMDA receptors play such a significant role in

hippocampal plasticity during LTP, further investigations into NMDA receptors in the hippocampus during extinction are warranted.

There has also been a great deal of research investigating the role of GABA in extinction. McGaugh et. al. (1990) examined the effect of systemic picrotoxin (a GABAergic antagonist) injections on conditioned fear where a tone was paired with a footshock. Animals who received post-extinction trial injections of picrotoxin showed enhanced extinction as compared to controls. Pereira et. al. (1989) additionally found that GABA agonists (diazepam) administered prior to shuttle-avoidance extinction had no effect on extinction formation, but impaired retention. Harris and Westbrook (1998) also studied GABA transmission in conditioned fear where a tone was paired with footshock. A ß-carboline (FG 7142), which binds to the benzodiazepine site on the GABA receptor compound and acts to antagonize the inhibitory effects of GABA, was used. When FG 7142 was injected subcutaneously prior to the test period, it was found to impair extinction retention in the extinction context. These findings suggest that perhaps there is a difference in the way GABA acts pre and post-extinction. Myers & Davis (2002) have offered the proposal that perhaps GABA is acting to inhibit the formation of extinction by counteracting NMDA induced plasticity whereas post-extinction, GABA is acting to inhibit other excitatory neurons and therefore preserve extinction. These findings support the hypothesis that GABA is responsible for reduction in fear through its inhibitory influence.

Multiple Forms of Extinction: Clinical Implications

Further understanding the role the hippocampus plays in extinction has important implications for behavioral therapy. Much of behavioral therapy involves the extinction of unwanted or potentially dangerous responses. However, the limitations of extinction learning itself provide limitations on behavioral therapy based on extinction. Since extinction learning is based on forming a new inhibitory association simultaneously with the previous excitatory association, extinction learning never fully reverses acquisition (Domjan, 2003). However, if the neural mechanisms underlying extinction are more fully understood, then perhaps this extinction learning could be enhanced, increasing the likelihood of success. Additionally, one of the largest problems with extinction when it is used for drug addiction is contextually induced relapse in the form of renewal. Renewal occurs when the extinction has been learning in a different context then acquisition. When the subject returns to the original learning context, the CS again elicits the US (Bouton & Swartzentruber, 1991). Such is the case with drug addiction. When a drug addict enters rehab treatment, the extinction occurs in the rehab center. However, once the addict returns home, there is a high likelihood of relapse since the addict will most likely still interact with the same people and potentially be in the same place where drugs were used. If this occurs, the extinction that occurred in the rehab center will be overpowered by the renewal effect of the context of drug use. Hippocampal lesions have been found to impair renewal (Corcoran & Maren, 2001) and reinstatement (Wilson et. al., 1995) and conversely, hippocampal stimulation is found to induce drug-seeking relapse behavior in animals that had been previously extinguished

for cocaine self-administration (Vorel et. al., 2001) indicating a possible role for the hippocampus in the termination of extinction of drug use.

The treatment of Obsessive Compulsive Disorder (OCD) also may involve the extinction of undesirable responses. OCD is most commonly treated with response prevention behavioral therapy, which is based on stimulus-response theory. However, according to the multiple memory systems hypothesis, extinction using only the S-R system will not result in complete extinction of the behavior. The hippocampal based cognitive system is still active and can lead to relapse of the behavior. Further directions of behavior therapy could perhaps use a multiple memory systems approach to either extinguish using both systems, or potentially enhance extinction using the cognitive system.

The current studies provide evidence that extinction may be acquired through multiple memory systems, which would provide a new way of thinking about extinction. The hippocampus has been found to selectively mediate latent extinction indicating that perhaps the role of the hippocampus in spatial extinction is similar to that in acquisition. Using latent extinction, the present dissociation of hippocampal involvement in latent and response extinction learning provides new evidence on how extinction behavior is acquired and paves the way for future studies. The latent extinction paradigm can now be applied to look at the underlying neurochemical basis of extinction.

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APPENDIX

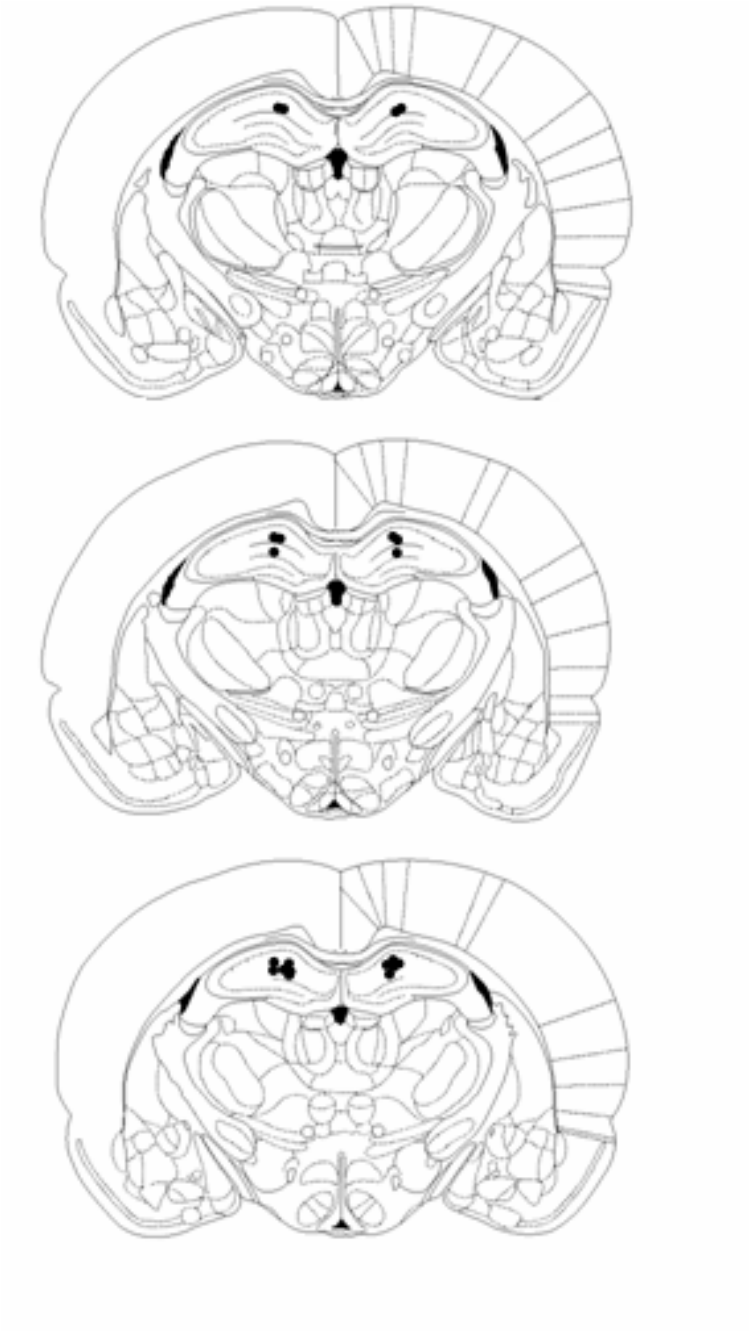

Figure 1. Coronal sections verifying hippocampal injection needle placement. The placements range from -3.30 (top) to -2.80 (bottom) anterior-posterior to bregma. (Adapted from Paxinos & Watson, 1997).

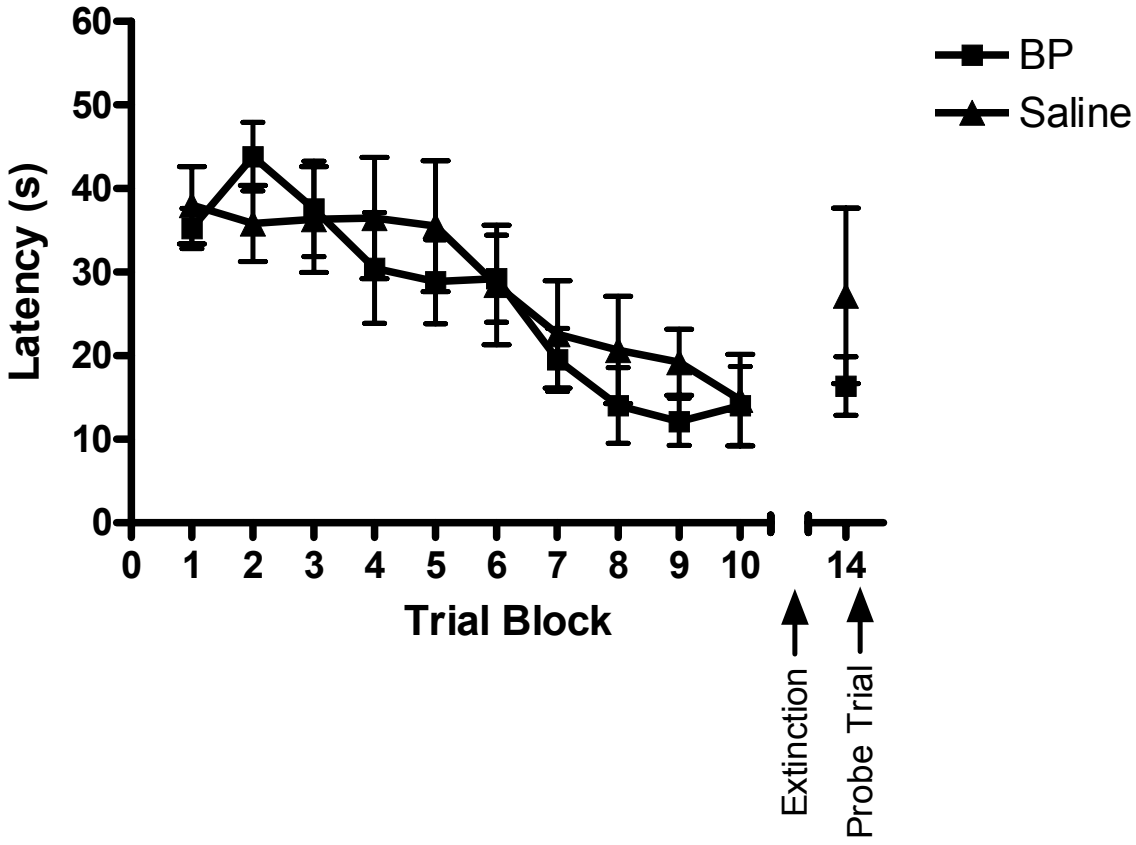

Figure 2. The effect of hippocampal inactivation on latent extinction (experiment 1). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group.

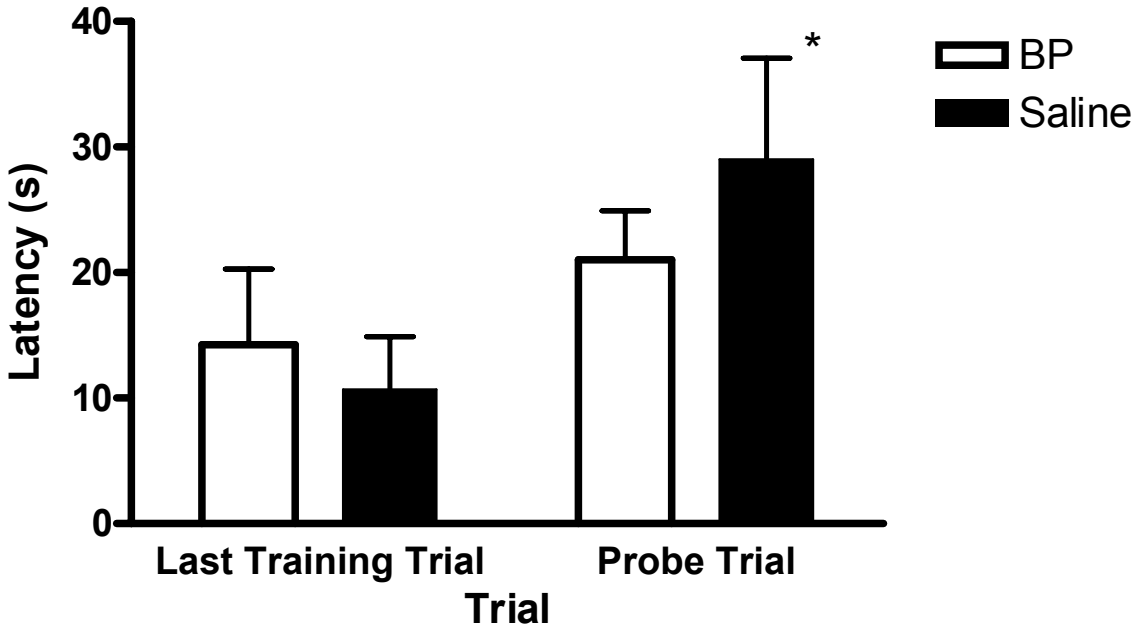

Figure 3. The effect of hippocampal inactivation on the difference between the last training trial and the probe trial (experiment 1). The mean (+ SEM) of the latency (in seconds) of the last trial of initial acquisition and the probe trial. Asterisk indicates a significant difference in the saline treated group ($p < 0.10$).

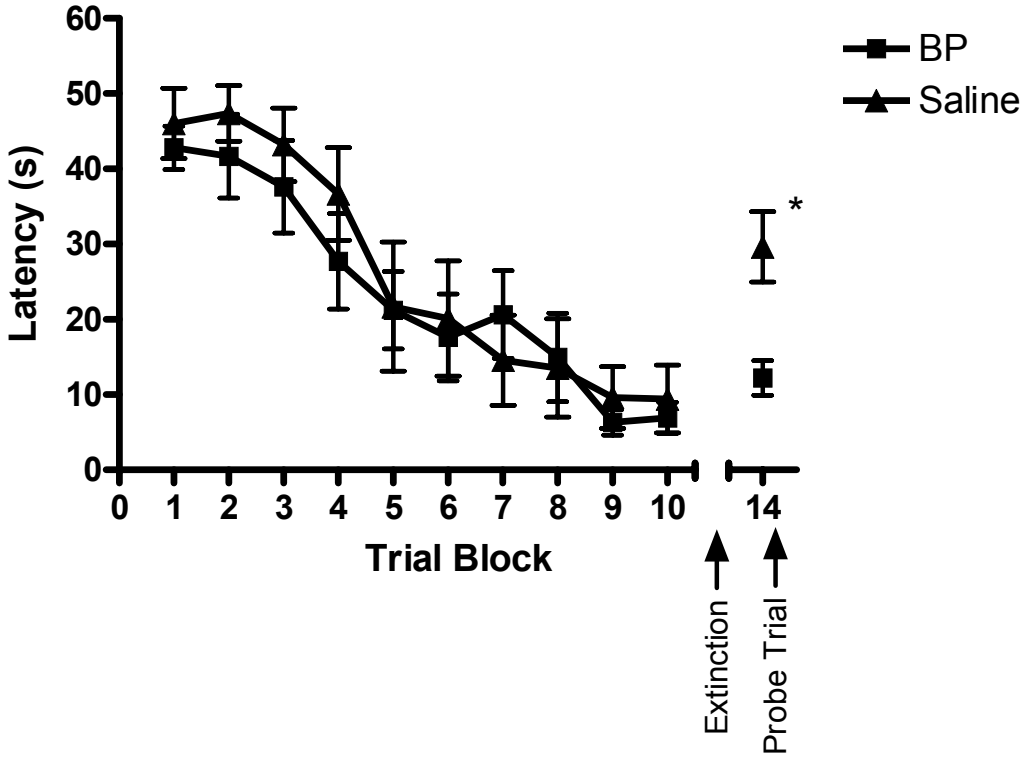

Figure 4. The effect of hippocampal inactivation on latent extinction (experiment 2). Mean (± SEM) of latency (in seconds) to reach the goal over training days by group. Asterisk indicates a significant difference between group means for probe trial latency ($p < 0.01$).

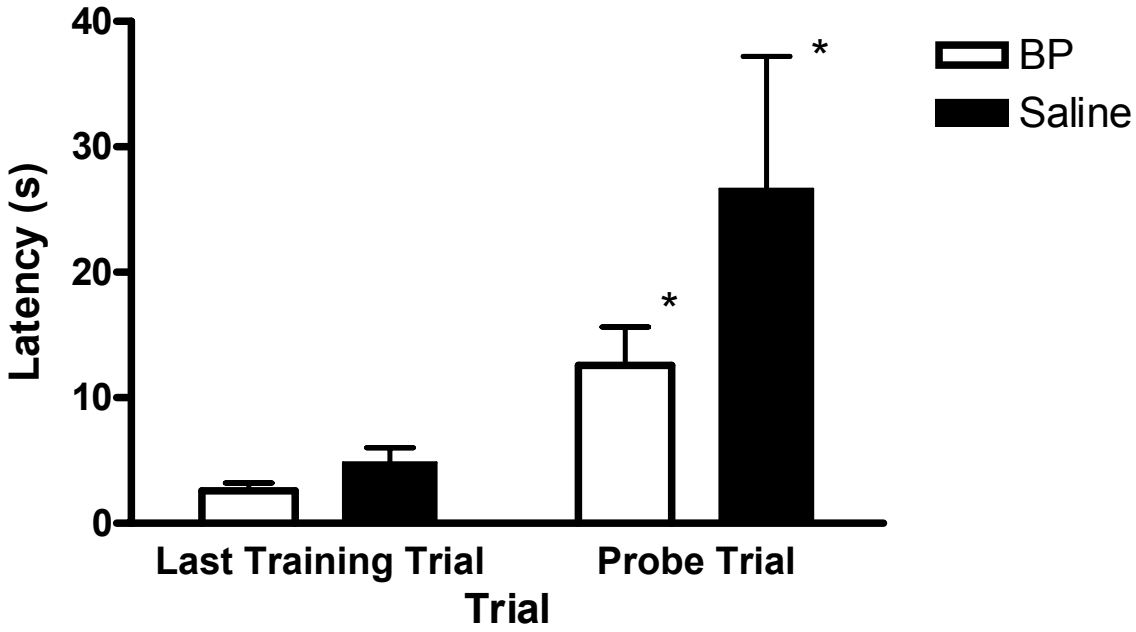

Figure 5. The effect of hippocampal inactivation the difference between the last training trial and the first probe trial (experiment 2). The mean (+ SEM) of the latency (in seconds) of the last trial of initial acquisition and the probe trial. Asterisk indicates a significant difference in both the saline and bupivacaine treated groups ($p < 0.10$).

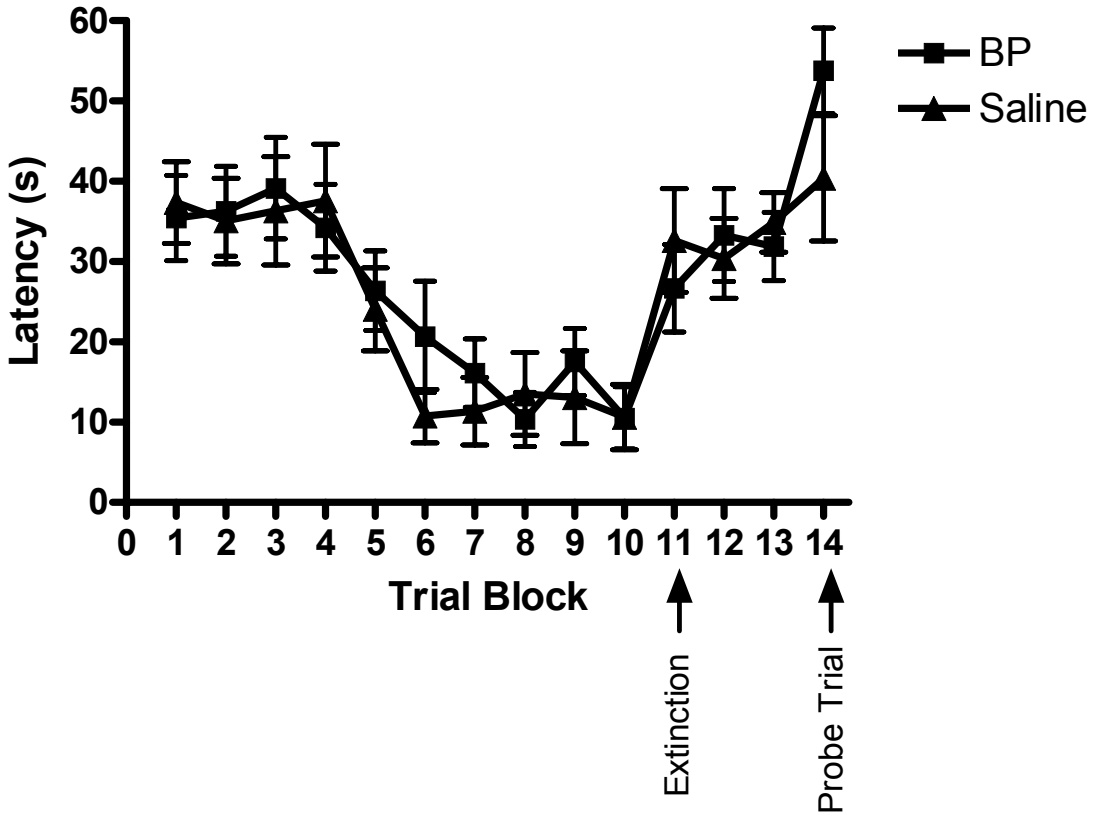

Figure 6. The effect of hippocampal inactivation on response extinction (experiment 3). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group.

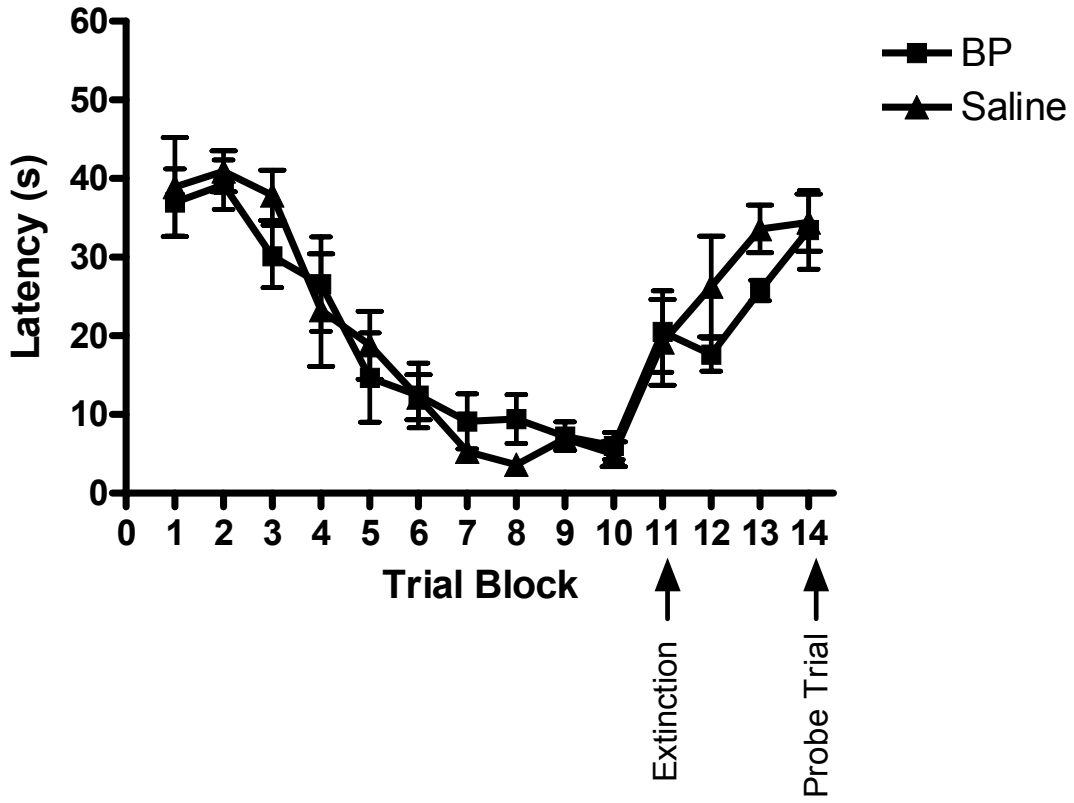

Figure 7. The effect of hippocampal inactivation on response extinction (experiment 4). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group.

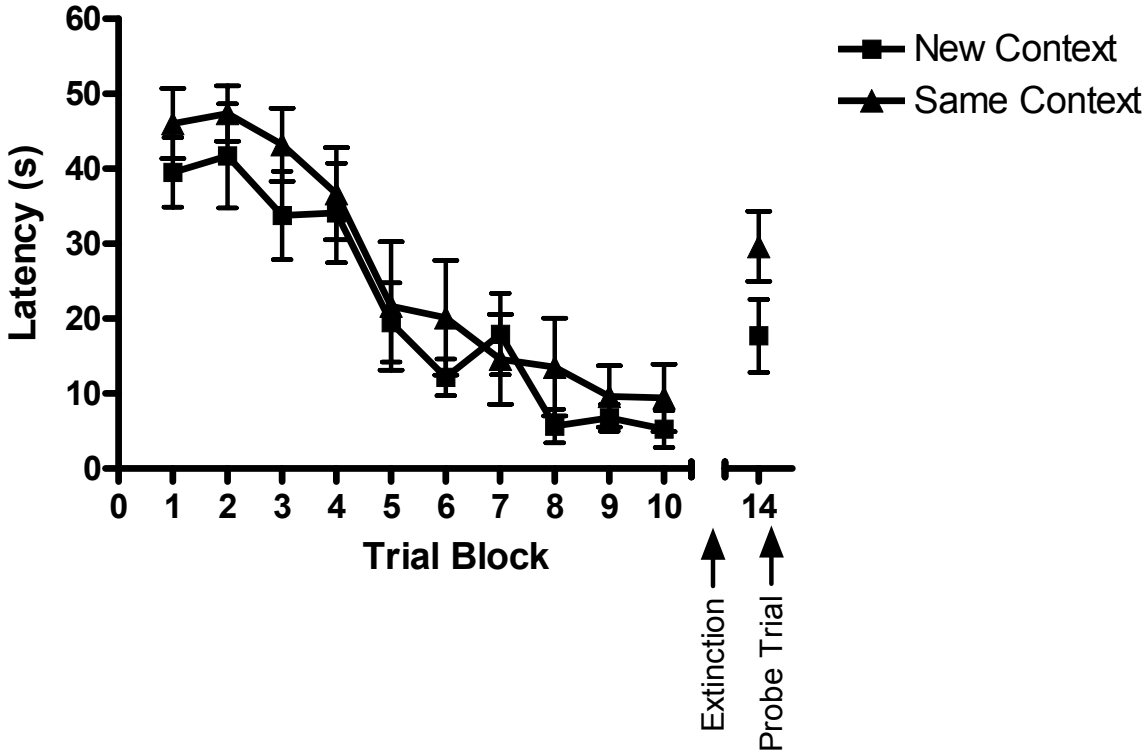

Figure 8. The effect of a new extinction context on latent extinction (experiment 5). Mean (± SEM) of latency (in seconds) to reach the goal over trial block by group.

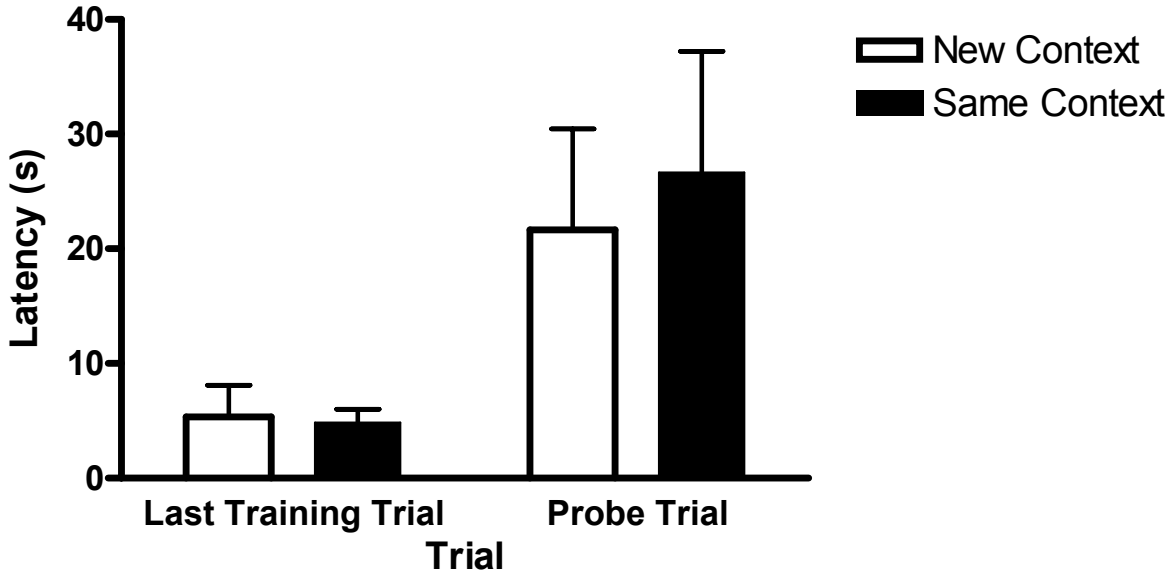

Figure 9. The effect of a new extinction context on the difference between the last training trial and the first probe trial (experiment 5). The mean (+ SEM) of the latency (in seconds) of the last trial of initial acquisition and the probe trial.

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ARTICLES

Gabriele, A., & Packard, M.G. (2005). Multiple memory systems and extinction. Manuscript submitted.