

**THE HIPPOCAMPUS, RETROGRADE AMNESIA, AND MEMORY
DECONSOLIDATION**

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Abstract

There are numerous clinical and experimental accounts of retrograde and anterograde amnesia resulting from damage to the hippocampus (HPC). Several theories on the HPC hold that only certain types of recent memories should be affected by HPC damage. These theories do not accurately predict the circumstances within which memories are vulnerable to HPC damage. Here I show the HPC plays a role in the formation and storage of a wider range of memories than is posited in contemporary theories. I will demonstrate that an important factor in eliciting retrograde amnesia is the number of similar learning episodes. Exposure to multiple problems in the same task context leads to retrograde amnesia that is not observed when only one problem is learned under otherwise identical parameters. When multiple discriminations are learned, the output of the HPC blocks recall from and future use of the extra-HPC memory system.

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List of Abbreviations

ANOVA	Analysis of variance
C	Context
CAS	Configural association system
HPC	Hippocampus
LSD	Least significant difference test
MTL	Medial temporal lobe
NMDA	N-methyl-D-aspartic acid
PFA	Paraformaldehyde
P	Problem
SAS	Simple association system

Chapter One

Introduction: The hippocampus and its role in learning and memory

A continuing central challenge for neuroscience is explaining the neural mechanisms responsible for learning and memory. It is clear that there is no single mechanism or structure that is responsible for all types of memory. One view on this is that there are several memory systems and each has a specific and unique function (McDonald & White, 1994; Packard, Hirsh & White, 1989; White & McDonald, 2002). One of these systems has as its central structure the HPC, a structure that has received a great deal of attention. Situated in the medial temporal lobe, the HPC is a relatively large structure (Figure 1.1) with reciprocal connections to many regions including prefrontal and parahippocampal cortices, as well as with several subcortical regions (Amaral & Witter, 1995). The HPC is well situated to participate in memory processes since it receives highly processed information from nearly all cortical areas. It is the HPC that will be the focus of the studies carried out here. Specifically, my aim is to further clarify the function of the HPC as it relates to certain processes of learning and memory in the rat.

The exact role that the HPC performs in learning and memory has been the subject of intense scrutiny, but there is still no conclusive answer. The confluence of numerous disciplines into the field of neuroscience has provided numerous methods and

levels of studying how the nervous system acquires and stores memories including the use of functional neuroimaging, gene transcription, electrophysiology, and behavioural analysis. One of the key methods for studying the function of a given structure such as the HPC has been to study the aspects of the rat's behaviour, in this case of learning and memory, that are lost or retained following removal of that structure.

Historically, surgical removal of the HPC inadvertently initiated a long lasting investigation into the functional relevance of the HPC to learning and memory. Exploration of the HPC as a critical learning and memory centre stems in large part from the amnesic patient H.M. Arguably the most famous neuropsychological case, H.M. has received a great deal of attention from many researchers (Corkin, 2002; Corkin, Amaral, Gonzalez, & Johnson, 1997; Scoville & Milner, 1957; Smith, 1988). William Scoville performed a bilateral medial temporal lobe (MTL) resection as an experimental therapy for the epileptic seizures from which H.M. suffered (Scoville, 1954). The tissue removed by Scoville included parts of several structures in the MTL including the uncus, amygdala and, large portions, but not complete removal, of each HPC (the damage has since been more accurately analyzed using functional magnetic resonance imaging and is reviewed by Corkin et al., 1997). Remarkably, following the operation the seizures that had incapacitated him were largely reduced but H.M. now suffers from severe amnesia. Both retrograde amnesia (a loss of memory for events that occurred prior to brain damage) and anterograde amnesia (inability to acquire new memories following brain damage) were apparent. H.M.'s retrograde amnesia does not uniformly affect prior memories, but instead spans a period of several years immediately preceding the operation. In contrast, his memory for events that had occurred before those years

remained intact. His anterograde amnesia on the other hand has not diminished to this day. Despite the severity of the amnesia from which H.M. suffers, he does retain the ability to form some types of new memories. Retention of procedural learning, such as the mirror drawing task (Milner, Corkin, & Teuber, 1968) are intact, despite an inability to declare that he had previously performed the task. The fact that medial temporal lobe damage did not cause a general learning or memory deficit showed that certain areas of the brain were specialized for the formation and storage of certain types of memory. H.M.'s case provided the first clear human neuropsychological demonstration of localization of memory to a certain structure. Previous work had suggested that memory could not be disrupted by focal lesions. Karl Lashley believed that this was the case because the memory was distributed throughout the neocortex (Lashley, 1950).

With a heightened interest in the medial temporal lobe, a large and diverse literature was quickly generated exploring this area as a critical memory site. The focus quickly narrowed from the MTL to the HPC after reports that similar surgical ablations of the MTL that did not include the HPC left patients without amnesia (Scoville & Milner, 1957). It is clear that the HPC is important if not critical to some forms of learning and memory, but it has also been widely accepted by most researchers that the HPC is not required for all types of learning and memory. Numerous studies have described tasks for which the HPC is critical. These include spatial navigation, (Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland, Kolb & Whishaw, 1982; Sutherland Whishaw & Kolb, 1983) conditioning to context, (Kim & Fanselow, 1992; Kim, Rison, & Fanselow, 1993) and formation of configural representations (Rudy & Sutherland, 1995; Rudy & Sutherland, 1989; Sutherland & Rudy 1989; Sutherland, Rudy, McDonald, & Hill, 1989).

Conversely an equally abundant literature describes learning and memory tasks that can be performed normally without a contribution from the HPC. These include some forms of procedural skill learning (Cohen & Squire, 1981; Milner, Corkin, & Teuber, 1968) and elemental associative learning, (Alvarado & Rudy, 1995; Whishaw & Tomie, 1991). As of yet, there is no clear consensus on why certain tasks require the HPC.

Many researchers have attempted to define alternative classification schemes to predict what types of memory are dependent on the HPC and therefore vulnerable to HPC damage. As was touched upon earlier in relation to H.M., a potential distinction was made between declarative and procedural memory (Cohen & Squire, 1981). This scheme attributed declarative memories (memory for facts and events that can be consciously recalled) to the HPC. Procedural memory (memory for skills, priming, etc.) on the other hand, is not dependent on the HPC. However, the dissociation between declarative and procedural memory can at times be difficult to make. Since declarative memories are those that can be consciously recalled it becomes a restrictive distinction that is not easily applied to the study of rat models of human memory. Thus, this classification system confuses the issue as much as it clarifies it. When studying memory, especially in the rat, this distinction provides little more than an alternative that is semantically equivalent to HPC dependent versus HPC independent. Numerous similar dissociations were introduced (for review see Squire, 1987) but all seemed to suffer the same pitfalls as the declarative/procedural distinction.

Cognitive Map Theory

Many theories have attempted to identify which specific aspects of learning and memory are contributed by the HPC. O'Keefe and Nadel (1978) suggested that the HPC

is needed to create and store a topographical representation of the rats environment. According to their cognitive map theory, any task that requires the use of topographical relationships among cues or places should be dependent on the HPC, but tasks that could be solved using local or landmark cues could be solved by other systems. The HPC does appear to be requisite for navigating to a spatial location of a goal if a constellation of distal cues must be used (Morris et al., 1982). Furthermore, rats with HPC damage can locate a hidden goal when navigation by distal cues is unnecessary: they are able to navigate by means of a landmark cue. However, the cognitive map theory fails to predict HPC dependence that is sometimes seen in non-navigational tasks such as the transverse patterning problem (Alvarado & Rudy, 1995), contextual conditioning (Kim & Fanselow, 1992), or learning non-spatial cue relationships (Sutherland, McDonald, Hill, & Rudy, 1989).

Configural Association Theory

Configural association theory (Rudy & Sutherland, 1995; Rudy & Sutherland, 1989; Sutherland & Rudy, 1989) explains the role of the HPC in spatial as well as non-spatial learning. This theory states that the HPC makes a critical contribution to tasks that require a configural solution. That is, the solution requires a representation that is compiled from multiple elements that alone do not provide the solution to the problem. In contrast, Sutherland & Rudy (1989) state that if the solution involves a simple or elemental discrimination the HPC is not required for acquisition or retention of the memory. Configural theory assumes that there are two active memory systems, a configural association system (CAS) and a simple association system (SAS). The CAS is able to solve configural problems because of the way that it encodes multiple elements

into a configural representation, unlike the SAS which encodes the elements independently of each other. An example of a configural task is the transverse patterning problem. Three pairs of cues are presented as follows: A+/B-, B+/C- and, C+/A-. The goal of the task is to choose the correct cue in each case. The solution cannot be solved with an elemental solution because each element has an equal probability of being either correct or incorrect. The solution to the problem requires a configural representation of each reinforced element in relation to the corresponding non-reinforced element. For each pair of cues it is not sufficient to know the elemental solution because each cue can be both reinforced and non-reinforced depending on which cue it is paired with. The solution requires the subject to know, for example, that A is the correct cue only if presented with B, but not if it is paired with C.

There is a considerable disagreement in this literature about which tasks are dependent on the HPC and which tasks are not. At least some of this debate can be traced back to a distinction between studies that probe retrograde amnesia versus those that examine anterograde amnesia. Rat experiments that examine the interaction between different types of memory and amnesia have taken two basic forms. Anterograde studies examine ability to learn a new task following HPC damage. Retrograde studies test the effect of HPC damage on the retention of a previously acquired task. The majority of studies utilize anterograde amnesia to study HPC contributions to learning and memory. It is important to acknowledge the dissociation between what is demonstrated by the results of retrograde amnesia and anterograde amnesia. Behavioural analysis of a rat with retrograde amnesia will illustrate what the intact HPC is necessary for. In contrast, anterograde amnesia will only show what the rest of the brain can do when the HPC is

not intact. Of interest here is the study by Alvarado and Rudy (1995). Rats with HPC damage were tested exclusively in the anterograde direction. The result was impaired performance on configural but not elemental tasks. This does not necessarily eliminate the possibility that the intact HPC makes an important contribution to elemental learning. In fact, a number of studies of retrograde amnesia (Sara, 1981; Sutherland et al., 2001; Weisend et al., 1996) have reported that the HPC is involved in a wider range of tasks than has been seen in the anterograde direction. The simplest prediction here is that HPC damage should impair performance on the same tasks in the anterograde direction as it does in the retrograde direction. There is reason however, to speculate that this might not be the case. The brain shows a remarkable ability to compensate for the loss of a particular structure. Anterograde amnesia may appear to affect a narrower range of memory functions because in the absence of the HPC other structures in the brain are capable of compensating for an action that would normally be executed by the HPC. On the other hand, retrograde amnesia should more accurately illustrate what the contribution of the HPC was when it was functioning normally in the intact brain. This approach to studying memory loss eliminates the confounding variable produced by compensatory actions or certain forms of redundancy between structures.

The Standard Model of Memory Consolidation

Patient H.M. lost memories from a period of several years prior to the surgery, but he is able to recall information from more remote time periods. Ribot (1882) first described this pattern of memory loss and stated that recent memories are more vulnerable to disruption than remote memories. Originally known as Ribot's law of regression, this phenomenon is now referred to as temporally graded retrograde amnesia.

Numerous studies report evidence of temporal gradients following HPC damage in tests of contextual fear conditioning, (Anagnostaras, Maren, & Fanselow, 1999), socially transmitted food preference (Winocur, McDonald, & Moscovitch, 2001; Clark, Broadbent, Zola, & Squire, 2002) and, in human neuropsychological findings (Reed & Squire, 1998; Rempel-Clower, Zola, Squire, & Amaral, 1996). However there is contradictory evidence from both human and non-human research supporting non-graded retrograde amnesia or, an equal memory disturbance at all time points. Numerous rat studies have shown that retrograde amnesia can alternatively appear in a non-graded fashion. Sutherland et al. (2001) demonstrated a flat gradient for up to 15 weeks on both configural and non-configural tasks in the rat. Similarly, Bolhuis, Stewart, and Forrest (1994) demonstrated a flat gradient for 14 weeks following HPC damage in the rat. Cipolotti et al. (2001) described patient V.C. as having extensive but limited bilateral HPC damage. In contrast to the traditional view, V.C. has a very extensive retrograde amnesia characterized by a flat temporal gradient. Another patient, N.T. has been described as having a temporally non-graded retrograde amnesia despite extensive and generally contained HPC damage (Chan, Revesz, and Rudge, 2002). Although the traditional description of a temporary role for the HPC in memory storage is extremely popular, there is enough evidence to the contrary to prevent accepting it without debate. This debate is critical to our understanding of how the HPC functions under normal conditions because of what is indicated by the appearance of either a flat or a temporal gradient. Assume for a moment that a certain memory task has been identified to be dependent on the HPC and rats are then trained on this task either weeks or hours prior to HPC damage. If the rats showed an equal impairment at both of these post-

training/surgical time points then they have exhibited a flat gradient. Conversely, if the rats are impaired when damage occurred within hours of training but not when the training occurred several weeks prior then the rats displayed a temporally graded retrograde amnesia. Presumably in the case of a temporal gradient, the task required initial HPC processing, but after a given amount of time, the memory can be supported without HPC involvement. This is not the case if a flat gradient was found. In such a situation, the HPC would appear to perform a crucial and possibly permanent role. This could indicate that the HPC circuitry is needed for proper recall of the memory or even that the HPC is a permanent storage site for that particular type of memory.

In order to explain the phenomenon of temporally graded retrograde amnesia the theory of between systems consolidation was proposed. Two versions of this theory exist. In the first, the HPC is initially needed in order to stabilize the organization of the memory trace (Squire, Cohen, & Nadel, 1984). The HPC is not the permanent location of the long-term memory, but temporarily stores the memory while it helps to bind together the separate cortical elements of the memory into a coherent and self-supporting unit. This process is dependent on the HPC but only until the memory is stable. An earlier variant of this theory proposes that the short-term memory is initially stored only within the HPC circuitry. With the passage of time the memory comes to be stored in remote cortical areas at which point the HPC is no longer needed for recall (Marr, 1971). The essential aspect of both explanations is that the memory is only dependent upon the HPC for a limited period. During this time (but not after) if the HPC is damaged the result will be an impaired ability to recall that memory.

A temporal gradient is not always observed with retrograde amnesia but, where there is evidence of temporal gradients, there are also strikingly large discrepancies in the length of the consolidation period. Memory consolidation has been reported to take between days (Winocur, 1990) and years (Rempel-Clower et al., 1996) depending on the task and species that is tested. In a study of human amnesiacs, Squire & Zola (1996) even suggest the consolidation period may take up to 25 years to complete. Such an explanation seems implausible based on the time and resources that would apparently be required to carry out such a lengthy process.

Reconsolidation Theory

A conceptually similar theory to the standard model is reconsolidation theory (Misanin, Miller, & Lewis, 1968). Like the standard model, this theory postulates that memories are initially but temporarily dependent on the HPC. Over time the memory moves from this labile HPC dependent state to be stored in the neocortex. Unlike the standard model however, it is believed that each subsequent retrieval of the memory causes it to once again become dependent on the HPC. The process of consolidation to neocortex takes place again essentially producing a new memory. This theory also states that at short training-surgery intervals the memory will be disrupted but it will not be at longer training-surgery intervals. As was described above this is not always found to be the case and because of this, reconsolidation theory fails in many cases to account for the existing data.

Multiple Trace Model

Lesion size and location have been identified as problematic factors in studies of amnesia. Temporal gradients could be explained by the use of only partial lesions. In

many studies only the dorsal or ventral HPC is damaged (Anagnostaras et al., 1999; Kim & Fanselow, 1992) leaving the possibility that the temporal gradient is a result of a within-system consolidation process and not necessarily due to between-systems consolidation. Memory acquisition, consolidation and long-term storage could all be HPC dependent processes that are carried out either in a dispersed manner throughout the HPC or in different sub-fields of the structure. If so, partial HPC damage could result in a temporal gradient if the area damaged was the area required for consolidation but not for storage. Alternatively, multiple similar copies of a memory may be produced within the HPC such that the number of copies increases with time (Moscovitch & Nadel, 1998; Nadel and Moscovitch, 1997). Partial HPC damage might produce retrograde amnesia initially after a memory is learned because there is only a single copy of the memory. However, at later time points there could be several copies of the memory distributed throughout the HPC. Due to these inconsistencies it has not been possible to reject or accept a time limited role of HPC function.

The two main factors described to this point, memory age and type, are widely accepted to be the most accurate predictors of retrograde amnesia following HPC damage. Nevertheless, there remains a high degree of variability between studies. Age and type of memory alone or in conjunction, fail in many cases to accurately predict whether HPC damage will result in retrograde amnesia. The purpose of the following experiments is to develop an analytic series of experiments using a simple memory paradigm to determine which are valid predictors of retrograde amnesia. The results of several experiments will be detailed that provide evidence against the theory of between-systems consolidation. Instead, evidence is provided for a permanent role for the HPC in

learning and memory. Furthermore, the experiments show that the HPC is involved in a greater range of memory tasks than is currently accepted. Finally a new theory of HPC function will be outlined that will more accurately predict the appearance of HPC damage dependent retrograde amnesia. Specifically, the novel theory will account for studies that show retrograde amnesia at all training-surgery intervals and will allow for dissociation between anterograde and retrograde amnesia. This theory, unlike any current model, will make the novel prediction that retrograde amnesia will appear when multiple discriminations (but not a single discrimination) are acquired in the same context.

Chapter Two

General experimental materials and methods

SUBJECTS:

All experimental procedures were carried out in accordance with the University of Lethbridge Animal Welfare Committee and the Canadian Council on Animal Care guidelines for the use of experimental rats. The subjects used in the following experiments were 96 male Long-Evans rats obtained from the University of Lethbridge Canadian Centre for Behavioural Neuroscience breeding colony. Rats were pair-housed in standard cages with *ad libitum* access to food and water. A light/dark cycle was maintained in the colony room with lights turned on and off at 7:30 am and 7:30 pm respectively. A constant temperature of 20°C was maintained in the vivarium. At the start of training, all rats were between 80 and 100 days old. Prior to behavioural training, rats were handled for several days to familiarize them with the experimenter.

DISCRIMINATION TRAINING:

Discrimination learning was carried out in the visual water task (Prusky, West, & Douglas, 2000). The apparatus (figure 2.1) was a trapezoidal shaped, water filled tank with two computer monitors at one end. A barrier divided one end of the pool into two arms. The monitors, one at each arm, displayed images one of which was the reinforced

cue and was always paired with a hidden platform. A representative sample of the cues used in these experiments can be seen in figure 2.2. The escape platform was constructed of clear Plexiglas and was submerged below the surface of the water. Using a pseudo-random pattern (Left-Right-Left-Left-Right-Left-Right-Right) I moved the platform location and the corresponding reinforced cue after each trial. This procedure prevented the rats from forming a side bias. In order to increase the salience of the visual cues and to ensure that the rats could not see the platform, the only sources of light in the testing room were the three computer monitors. A trial was incorrect if the body of the rat crossed the plane perpendicular to the end of the central barrier on the side of the non-reinforced cue. In the case of an incorrect trial the rat was required to continue swimming until it found the platform. If the rat responded by swimming directly to the reinforced cue without first entering the non-reinforced arm then the trial was scored as correct and the rat was immediately removed from the platform (figure 2.3). Following both correct and incorrect trials the rat was removed from the pool only once it had found the platform. The apparatus used here provided strong cue control and rats quickly learned to respond based on the computer-generated cues. On a few occasions, typically during the early stage of training, rats developed a side bias. This was corrected by biasing the platform location to the opposite side using an alternate pattern (Left-Left-Right or Right-Right-Left). Rats were run in groups of two at a time such that the inter-trial interval was approximately one minute. Water temperature was maintained at a constant temperature of $20 \pm 1^\circ\text{C}$. At the end of each training session the rats were returned to their home cages.

SURGICAL PROCEDURES:

A variety of techniques can be used to cause damage to the rodent HPC. In addition to non-selective techniques such as aspiration, radiofrequency, and electrolytic lesions, numerous toxins have been identified that can be used to destroy HPC tissue. Some of these toxins such as kainic acid and colchicine can at some concentrations cause damage only to selective subfields of the HPC. Others such as N-Methyl-D -aspartic acid (NMDA) cause non-selective cellular damage and can be used to damage the entire HPC. Here I used NMDA to induce damage throughout the HPC by injecting small amounts at multiple sites within the HPC. NMDA is a potent glutamate agonist that exerts its effects by causing a rapid and toxic influx of calcium into the cells surrounding the injection site. A side effect of NMDA administration is the associated excitotoxic seizure activity. Because of this, 2 mg/kg of diazepam was injected intraperitoneally 10 minutes prior to surgery to reduce seizure activity.

Anaesthesia was induced by placing rats in a Plexiglas induction chamber into which 4% isoflurane was delivered in oxygen at a rate of 2 L/min. Once the rat was anesthetized, the top of the head was shaved and then the rat was positioned in a stereotaxic frame (David Kopf Instruments). Once a surgical plane was established the anaesthesia was maintained at 2% isoflurane in 2 L/min of oxygen. The head was then cleaned with three alternating applications of hibitane (4% chlorhexidine gluconate) and 70% alcohol.

Next, a midline incision was made in the scalp and the skin and periosteum were retracted using hemostatic clamps to expose the surface of the skull. The location of bregma was recorded and the injection coordinates were calculated relative to bregma.

Burr holes were made stereotaxically at these 10 sites. NMDA (Sigma) was injected at a concentration of 10 mg/mL in 0.9% saline into five sites per HPC (see table 2.1) through 30-gauge cannulae attached to 10 μ L Hamilton syringes driven by a Harvard instruments pump. At each site, 0.5 μ L of NMDA was infused at a rate of 0.125 μ L/min over a four-minute period. Following infusion, the cannulae were left in place for an additional four minutes to allow diffusion of the NMDA away from the tips of the cannulae. Once all injections were complete, the skin was closed with absorbable suture material. Sham operated rats had their scalps incised, retracted, and sutured but no holes were drilled in the skull. All other treatments were the same between groups.

Rats were treated with an additional dose of diazepam (2 mg/kg) upon waking from the anaesthetic to continue the suppression of possible seizure activity. Despite an effort to prevent it, some rats still developed seizures and in those cases further doses of diazepam were given as needed. Rats were monitored for any complications for at least two hours before being returned to the colony room. Buprenex (Buprenorphine HCL) was injected sub-cutaneously (1.5 mg/kg) as an analgesic before the rats were returned to their home cages. For the first post-operative day, the rats were housed individually for further monitoring. A one-week recovery period was given following surgery during which all rats were handled daily to reduce the hyperactivity sometimes associated with HPC damage (Maren & Fanselow, 1997).

HISTOLOGY:

After the completion of all testing the rats were sacrificed to assess the damage produced in the HPC and extra-HPC regions. A deep anaesthesia was induced using 100

mg/kg Euthansol (Sodium pentobarbital, 100 mg/ml). Rats were then perfused transcardially with 0.9% saline followed by a 4% v/v solution of paraformaldehyde (PFA). The brains were then extracted and stored in a solution of 30% sucrose in 4% PFA for post fixation and cryoprotection. The tissue was later sectioned into 50 μ m slices on a cryostat microtome. Every fifth section was mounted on glass slides, stained with cresyl violet, cover-slipped, and examined for evaluation of damage. The lesions typically produced extensive damage to all principle subfields of the HPC and extra-HPC damage was minimal in most cases. Although not quantified here, Sutherland et al. (2001) found that using the identical surgical procedure resulted in loss of 75%-95% loss of HPC tissue. Some variation was seen in this study in regards to the size and exact location of the lesion. Typically however, if sparing of HPC tissue occurred, it was isolated to the most posterior areas of the ventral HPC. Representative HPC damage can be seen in figure 2.4.

Chapter Three

Experiment 1: Retrograde effects of HPC damage on the retention of a single elemental visual discrimination.

INTRODUCTION:

The vast majority of theories posit that HPC damage should not affect elemental discrimination learning. That is, tasks that only require a rat to form a simple association between the reinforced cue and the reinforcer should not depend on the HPC. The first experiment described here was designed to address issues raised in several studies (Sara, 1981; Sutherland et al., 2001, Weisend, Astur, & Sutherland, 1996) regarding whether effects of HPC damage are different in the retrograde direction compared with the anterograde direction. Elemental discrimination learning proceeds at a normal rate in rats with HPC damage (Alvarado & Rudy, 1995; Rudy & Sutherland, 1989), but it may be the case that if the discrimination is learned prior to damage subsequent recall will be impaired. I first tested this idea by training rats on a single visual discrimination problem. Using a between-subjects design retention of the discrimination was assessed after HPC damage was induced 1 or 30 days following training.

METHODS:

Rats were trained on only a single visual discrimination problem. A 90% correct per session criterion was used to ensure reliable performance. Rats were given 20 daily training sessions, each comprised of 10 trials. After completion of the 20th training session, rats were assigned based on equivalent performances, to one of two surgical groups. The first received bilateral HPC lesions (n = 7) while the second group underwent the corresponding sham surgery (n = 4) 24 hours following the completion of training. After surgery, rats were given a recovery period of one week before being tested on the constant discrimination problem. Once all rats re-attained the 90% criterion, a second visual discrimination problem was introduced to test for possible anterograde learning deficits caused by HPC damage.

A third and fourth group of rats received the same set of procedures except that the training-surgery interval was longer. These groups underwent HPC damage surgery (n = 5) or sham surgery (n = 6) 30 days following the last training session.

RESULTS:

24-hr training-surgery interval:

Both Sham and lesion groups were able to learn the visual discrimination problem prior to surgery and all rats were able to reach the 90% correct criterion set for this problem. Both groups achieved a mean score of 100% correct on the final pre-operative training session. Following surgery rats with HPC damage performed as well as Sham rats and both groups performed at better than 90% correct. ANOVA showed that when HPC damage was induced 24 hours after training there was no effect of surgical

condition ($F(1,9) = 0.76, p=0.407$) on ability to retain a single elemental visual discrimination (figure 3.1).

Training on a second visual discrimination problem after retention testing of the first problem posed no difficulty for either Sham or HPC damaged rats (figure 3.2). An ANOVA between groups on the number of trials required to reach criterion revealed that there was no significant effect of lesion, $F(1,9) = 0.06, p=0.814$ on the post-operative rate of learning of a novel visual discrimination problem.

30-day training-surgery interval:

Pre-operative performance was assessed in both the Sham and HPC damage groups that were to have surgery performed 30 days after the final training session. Both groups achieved a mean score of 100% correct during the final training session.

Rats tested following a training-surgery interval of 30 days showed accurate retention (90% correct or better) of the solution to a single elemental visual discrimination problem, regardless of surgical condition (figure 3.3). The performance of the two groups did not significantly differ from each other, $F(1,9) = 2.51, p= 0.148$.

After ensuring that all rats were achieving the criterion of 90% correct each session a second problem was introduced. Rats in both Sham and HPC lesion groups were able to acquire this novel problem (figure 3.2). An ANOVA between surgical condition measuring trials to criterion revealed that HPC damaged rats did not learn at a significantly different rate than Sham rats, $F(1,9) = 0.14, p=0.717$.

DISCUSSION:

Retention of a single elemental discrimination problem was not affected by HPC damage. The HPC therefore does not appear to play a critical role in the storage of a single elemental discrimination. No retrograde amnesia was evident when surgery occurred at either 1 or 30 days after training. The results seen here support several theories of HPC function that predict this type of simple learning will occur without the HPC, including the cognitive map theory, configural association theory, and the standard model of memory consolidation. This finding does not discount the role of the HPC in elemental learning but does indicate that if it does participate, its contribution is not critical in all conditions.

This experiment utilized a between-subjects design but it is possible that if a within subject design were used a difference in performance at remote time periods might be evident. To investigate this, I utilized a within subject design for Experiment 2.

Chapter Four

Experiment 2: Retrograde effects of HPC damage on memory for multiple sequentially acquired elemental visual discriminations.

INTRODUCTION:

In Experiment 1, no retrograde amnesia was found using a visual discrimination problem presented alone. The second experiment was designed to determine if retention of multiple problems trained sequentially, with no temporal overlap during training, would be disrupted by HPC damage. It is possible that a within-subject design is a more realistic design for studying retrograde amnesia. Since problems are learned at all time-points by each animal, the comparison between those time points may yield differences in retention that are not seen when a between-subjects design is used. These ideas were investigated here by training rats on multiple discriminations at different times prior to HPC damage.

METHODS:

Rats were trained in three visual discriminations. The same task and context was used in all testing. Three distinct pairs of cues (see figure 2.2), problem one (A+/B-), problem two (C+/D-) and, problem three (E+/F-) made up the visual cues used in this experiment. Rats were trained first on a single visual discrimination for 20 daily sessions, each session consisting of 10 trials. The same 90% criterion was used to ensure

that all rats had learned the discrimination. Thus far, training was carried out exactly as it was in Experiment 1 for a single discrimination. At the completion of problem one (200 trials), rats were given a 10-day break before beginning problem two. Another 10-day break was introduced upon the completion of problem two. Problem three then began in the same fashion as the first two problems. Within 24 hours of completion of problem three, rats underwent either sham surgery ($n = 6$) or received intra-HPC injections of NMDA ($n = 8$).

Post-operatively, rats were tested for retention of the three problems. The order of testing was counterbalanced with half of each group being tested on problem one first and the other half beginning with problem three. Each rat was tested on only one of the problems each day. On subsequent days they were tested on a different problem. The order of testing was problem one, problem two, problem three; or problem three, problem two, problem one and the same orders were repeated until all problems could be performed at the 90% criterion. Then, a novel problem was introduced to assess the anterograde effects of HPC damage. The new discrimination was trained until all rats could solve it correctly at a criterion of 90% or better per session.

RESULTS:

In the pre-operative condition all rats were able to acquire all three of the discrimination problems. An ANOVA on the number of trials to reach the 90% criterion on each of the three problems revealed a trend for a faster rate of learning on each subsequent problem. There was a main effect of problem, $F(2,39) = 22.70$, $p < 0.001$, but a *post hoc* LSD test showed no difference between problems one and two ($p = 0.536$). The

third problem however was acquired in significantly fewer trials than either problem one ($p<0.001$) or problem two ($p<0.001$). Performance was maintained at or above the 90% criterion for numerous sessions before the subsequent problem was introduced or before surgery. Figure 4.1 shows the score achieved by rats in both groups during the final pre-operative training session. All rats achieved a score of 9 or 10 out of 10 trials correct during the final pre-operative session for all three problems.

Post-operatively, neither the Sham lesion nor surgery group showed any change in performance. These rats were still able to perform at or above the 90% criterion on all three problems. HPC damage resulted in a profound impairment on all three discrimination problems compared to the Sham group on the remote, $F(1,12)=48.20$, $p<0.001$, intermediate, $F(1,12)=36.00$, $p<0.001$, and recent, $F(1,12)=54.12$, $p<0.001$, problems. During the first post-operative testing session HPC damaged rats performed near chance (50%) on all three problems. An ANOVA revealed that the deficit was equally severe at all three time points, $F(2,21)=0.38$, $p=0.691$, with no evidence of a temporal gradient. The performance of Sham and HPC damage groups over the course of the first post-operative test session are contrasted in figure 4.2.

There was no difference among the three problems in the rate of reacquisition (figure 4.3) by the HPC damaged group, $F(2,21)=1.26$, $p=0.305$. Also, the rate of new learning was assessed in both groups. Rats with HPC damage were able to learn a new elemental visual discrimination problem at a normal rate. The rate of acquisition based on the number of trials required to reach the 90% level was not significantly different between groups, $F(1,12)=0.40$, $p=0.540$, with a trend for HPC rats to acquire the novel problem faster than Sham rats (figure 4.4). There was no significant difference,

$F(2,25)=1.32$, $p=0.284$, between the rate of learning of the third problem prior to surgery and post-surgical acquisition of the novel discrimination. Both Sham and HPC damaged groups acquired the novel discrimination at a rate comparable to the acquisition of the third pre-operative discrimination. Sham rats required 40 trials and HPC damaged rats required 29 trials to acquire the novel discrimination compared to the 20 trials that were initially needed to learn the third discrimination.

DISCUSSION:

Rats trained sequentially on three visual discriminations prior to HPC damage displayed retrograde amnesia for all three. Despite the obvious retrograde amnesia, the same rats were then able to relearn and retain the three discriminations and were also able to learn a novel discrimination. Because there was no temporal overlap of the three discriminations I am able to eliminate one explanation of the deficits seen here. The results of the first two experiments indicate that the HPC makes an essential contribution to elemental learning and memory when multiple discriminations are learned but not when a single discrimination is acquired.

The standard model of memory consolidation is completely incompatible with the result I have obtained in the first two experiments. Here, retrograde amnesia cannot be predicted as a function of memory type because the same type of memory can be vulnerable and invulnerable to retrograde amnesia in different circumstances (i.e. a single versus multiple discriminations). In addition, the amount of time that elapses between learning and HPC damage does not accurately predict whether or not a memory will be vulnerable to retrograde amnesia. However, the number of learning episodes that the rat

is exposed to may offer a reliable predictor of retrograde amnesia for elemental information.

It is uncertain at this point whether the number of discriminations is critical because of the similarity between the cues used in the different discriminations or if the deficit is related to the overlapping contextual elements. These opposing explanations are the subject of Experiment 3.

Chapter Five

Experiment 3: Retention of sequential visual and auditory elemental discriminations after HPC damage.

INTRODUCTION:

At this point I have excluded three possible hypotheses about retrograde amnesia for visual information after HPC damage. 1. Retrograde amnesia occurs for more than just rapidly learned episodic information, 2. Retrograde amnesia does not just affect very recent information, and 3. Retrograde amnesia does not encompass all types of information.

Learning or exposure to multiple discrimination problems in the same training context, including place, apparatus, and motivation/reinforcer, could be a critical factor in producing retrograde amnesia following HPC damage. Conversely the deficit could be related to an inability of rats with HPC damage to handle interference among overlapping elements of the cues. The present experiment was designed to distinguish between these two hypotheses. Here I trained rats on two elemental discrimination problems. Training took place in the same task and context except that the second problem was an auditory discrimination. Varying the modality of the discrimination eliminates the feature overlap in the discriminanda that might have occurred when both problems were visual discriminations. If training on an auditory problem fails to make a previously acquired

visual discrimination vulnerable to HPC damage then I have found support for the overlapping features hypothesis. If however, rats are impaired after HPC damage when the discriminations are in different sensory modalities the task contest hypothesis is supported.

METHODS:

Rats were trained on two sequential discrimination problems. One group received two visual discriminations but the second group was trained on a visual discrimination followed by an auditory discrimination. In either case, training on each problem consisted of 200 trials (10 trials per session) as in the previous two experiments. Between problems, a 10-day break was given. Surgery occurred 24 hours after the last training session for problem two.

The auditory discrimination was carried out in the same apparatus as all previous visual discriminations. The pool was modified by mounting a small speaker in the outer corner of each arm of the pool (see figure 2.1). Instead of pictures the monitors displayed identical grey screens to maintain a roughly equivalent level of ambient light in the testing room. All other elements of the context were held constant. The speakers were connected to a Macintosh G4 computer running the freeware program Winamp. The auditory cues chosen were two discrete birdcalls to allow for easy sound localization. The audio files were set so that only one call would be played through each speaker at any given time. The location of the reinforced cue was randomized using the same pattern as before.

After training and surgery, rats were allowed one week to recover before testing began. The purpose of this experiment was to determine whether exposure to a second discrimination in the same or in a different modality would be associated with HPC damage induced retrograde amnesia for a previously acquired memory. For this reason all rats were post-surgically tested on problem 1 first, before being tested on their respective second discrimination problems. Problems were tested on alternating days until all rats were able to perform at 90% or above on both problems. Rats were then trained on a novel visual discrimination until reaching the 90% criterion at which point training was complete.

RESULTS:

Prior to surgery all rats were able to acquire the first visual discrimination problem with a high degree of accuracy and performed above the 90% criterion for numerous sessions before the completion of training. Rats were also able to reach the 90% criterion on the second problem regardless of whether the second problem was an auditory or visual discrimination (figure 5.1). Post-operative testing of rats that had previously been trained on two visual discriminations revealed that the rats with HPC damage ($n = 6$) were significantly impaired on both problem 1, $F(1,9) = 66.00, p < 0.001$, and problem 2, $F(1,6) = 331.36, p < 0.001$, relative to Shams ($n = 5$) that were able to maintain an unchanged performance on both of the visual discrimination problems (figure 5.2). During the first post-operative test session HPC damaged rats performed near 50% on both problems, but Sham rats were able to perform above 90% correct on the same discriminations. Rats with HPC damage were able to reacquire both of the pre-

operatively learned problems (figure 5.3) and could also acquire a novel visual discrimination problem (figure 5.4) at the same rate as Sham rats, $F(1,9) = 0.13, p=0.729$.

In the second half of the experiment I trained rats on a visual problem followed by an auditory problem. Some rats were unable to resolve the auditory discrimination and were excluded from further consideration here. Of the rats that learned the auditory discrimination, both Sham ($n = 3$) and HPC damaged ($n = 4$) rats showed impairments during the first post-operative session (figure 5.2). During the first test session both groups performed near chance. There was no initial post-operative effect of lesion on performance in the visual discrimination task, $F(1,5) = 0.07, p=0.809$). However, Sham rats displayed an abrupt improvement to 93% correct during the second testing session at which point they no longer differed from their pre-operative performance of 100% correct, $F(1,4) = 4.00, p=0.116$. Rats with HPC damage on the other hand were impaired for a longer period of time (figure 5.3) taking a significantly greater number of trials to recover to the 90% criterion, $F(1,5) = 19.06, P=0.007$. Retention performance for the second, auditory problem showed a different pattern. Sham rats showed no difficulty in retaining the auditory discrimination. HPC damaged rats however showed poor retention of the auditory discrimination. There was a significant effect of lesion on retention of the auditory discrimination, $F(1,5) = 60.65, p=0.001$. Sham rats did not drop below the 90% criterion, but rats with HPC damage performed near the level of chance. The rate of recovery of the second discrimination problem by HPC damaged rats was not significantly different, $F(1,8) = 2.79, p=0.134$, for the group trained on a second visual versus an auditory problem. Training on a novel discrimination revealed that rats with

damage to the HPC were able to acquire new memories as well as the corresponding Sham group (figure 5.4).

DISCUSSION:

The results of the current experiment must be viewed cautiously. It is possible that for some reason Sham rats were not able to retain the remote visual discrimination after being exposed to the auditory discrimination. However, the abrupt recovery of proficient performance by the Sham rats, suggests that the first day deficit exhibited by that group may not be due to loss of the discrimination but, could have been an effect of a factor like attention shift away from the pictures on the monitors toward the locations of the auditory cues. However, the impairments exhibited by the HPC damaged rats mirrored the effects seen by HPC damaged rats when no shift of attention was required. This suggests that HPC damage induced retrograde amnesia for multiple problems even when the physical properties of the cues were different in problem one than in problem two.

Based on my results, distinguishing between common features of the cues in multiple discriminations is not the critical factor that causes multiple discriminations to be vulnerable to retrograde amnesia. Instead, my results support the idea that when multiple discriminations are acquired within the same context the hippocampus will make a critical contribution to the storage of those memories and will therefore cause the elemental information to be vulnerable to retrograde amnesia. If only a single discrimination is acquired in a given context, then the HPC is not critical and the memory will not be vulnerable to retrograde amnesia. Although I have eliminated common cue

features as an explanation of these results, further experiments will be required before I can definitively identify that multiple learning episodes in the same context is what makes the HPC critical for elemental learning.

Chapter Six

Experiment 4: Retrograde and anterograde effects of HPC damage on overlapping elemental visual discrimination learning.

INTRODUCTION:

The experiments that I have carried out to this point have all utilized a design that ensured multiple discriminations did not overlap in time. However, it may be possible that retrograde amnesia for multiple elemental discriminations will only occur if there is a temporal gap between the learning events. In other words interleaved training of multiple problems may allow for a cortical memory trace to be formed that would survive HPC damage. We have seen already that at least three visual discriminations can be acquired simultaneously by rats with HPC damage. However, these same three discriminations were vulnerable to retrograde amnesia when training was conducted sequentially. Experiment 4 was designed to test retention of multiple discriminations acquired rapidly without a long temporal gap between learning episodes. To explore this possibility I used a daily repeated acquisition and a constant discrimination problem was used. I trained rats concurrently on these two visual discrimination procedures. This design also allows us to see if the learning set acquired prior to HPC damage will be lost or retained. Most contemporary theories would predict that the memory for a simple visual discrimination task such as the one used here would be unaffected by HPC damage and that the learning set would also be unaffected by HPC damage. Cognitive map theory would predict spared memory since the task is non-spatial. Configural association theory also would

predict spared memory because the task can be solved with an elemental strategy and does not require a configural solution. The standard model of memory consolidation posits that if the HPC is required for the task it will only be needed temporarily. Longer surgery/training intervals should allow for the extra-HPC memory to be solidified resulting in spared memory.

In order to address the prediction of the standard model, HPC damage or Sham surgery was performed either 1 or 30 days after training. However, I predict that the HPC plays a permanent role in memory storage and therefore hypothesize that similar results will be obtained at both time points.

METHODS:

Initially, normal rats were trained daily to discriminate between the two cues that made up the constant problem. Each rat completed 10 trials within each session. Once a criterion of 90% or better was achieved, the second component was introduced. Within each daily training session, rats were tested during five trials of the constant problem followed by training to discriminate between two new cues (novel problem) and then an additional five trials of the constant problem. The novel problem had to be learned within each session (a maximum of 30 trials) to a criterion of 9 out of 10 consecutive trials correct within the first 15 trials. Regardless of whether the criterion for the novel component was reached the cues were never seen again following the session in which they were first used. This training protocol continued until the criteria for both the novel and constant problems were maintained concurrently for three consecutive sessions by all rats.

Four groups were randomly assigned to undergo Sham surgeries (n = 7) or HPC damage (n = 10) as described earlier. Damage was induced within 24 hours of the final training session (immediate surgery) or 30 days after the final training session (delayed surgery). Then, Sham and HPC damaged rats were allowed a one-week recovery period. Following the post-operative recovery period rats were tested the same way they had been trained until able to perform at the same criteria specified above.

Any memory deficits resulting from HPC damage may be subject to between systems consolidation effects. Therefore to test whether any deficits found in this task were specific to recent and not remote learning the same training procedures described above were repeated with a second cohort. This time however, a longer training-surgery interval was used. In this case, 30 days elapsed in this case before Sham (n = 4) or HPC (n = 5) damage was induced.

RESULTS:

Twenty-four hour training-surgery interval:

The simultaneous constant and novel discrimination problems were rapidly acquired and retained with a high degree of accuracy by all rats. Many rats were able to simultaneously perform the constant component at 100% per session and demonstrate one trial learning on the novel acquisition component. All rats were able to achieve the criteria set forth above for both the constant and novel problem and all rats were able to perform at this level for at least three sessions prior to surgery. On the final training session prior to surgery all rats recalled the constant problem at a level of 100 % correct. In the same session, the two groups had a mean rate of acquisition of 9.5 and 9.7 trials

respectively to reach criterion. The minimum number of trials possible to reach criterion is 9 and the maximum allowed is 30. An analysis of variance (ANOVA) was used to ensure that there was no difference between groups prior to surgery. The performances of the two groups did not differ prior to surgery on the acquisition problem, $F(1,15)=0.48$, $p=0.50$, and neither group committed any errors on the retention problem.

Retention of constant problem:

During the first post-operative session the Sham rats performed the retention component without error. The pre- and post-operative acquisition performance of the Sham rats did not differ. In contrast, rats that had received HPC damage showed significantly worse performance on the retention problem than rats that received the sham operation, $F(1,15) = 24.20$, $p < 0.001$. While Sham rats maintained a mean score of 100% correct on the first post-operative session, HPC damaged rats were only able to score 59 % correct during the same session (figure 6.1a).

Retention of Novel problem:

In addition to the deficits seen in the retention problem, the HPC damaged rats were also impaired relative to Sham rats on the novel problem. The difference between Sham and HPC rats on the novel acquisition component was statistically significant, $F(1,15) = 49.17$, $p < 0.001$. Sham rats efficiently acquired the novel problem requiring only 9.7 trials to reach the criterion, but HPC rats performed poorly on this component requiring a mean of 26.3 trials with many of those rats reaching the ceiling of 30 trials before reaching the criterion (figure 6.1b).

Relearning

Although initially quite dramatic, the effects of HPC damage on the retention and acquisition of simple visual discriminations disappeared with more training. Daily training sessions allowed the HPC damaged rats to recover to the pre-surgical level of performance on both the acquisition and retention components. An average of 43 trials (compared to the mean of 76 trials originally required to learn this problem) was needed before the HPC damaged rats were once again able to reach the 90% criterion for the retention problem. The constant problem took significantly more trials, $F(1,18) = 11.08$, $p=0.004$, to acquire pre-operatively than it did to reacquire the same problem in the absence of the HPC. The same rats required 73 trials to once again be able to acquire novel discriminations to a level of 9 out of 10 consecutive trials correct in less than 15 trials.

30-day training-surgery interval:

A similar pattern of impairment was seen for rats that received surgery 30 days following the final training session compared to those that only had a period of 24 hours elapse prior to surgery. Both Sham and lesion groups showed excellent pre-operative performance on both the acquisition and retention components. During the final pre-operative session the HPC damage group scored 100 % correct on the constant problem and acquired the novel problem in 9.4 trials while the Sham group also scored 100 % correct and required 9.8 trials respectively (figure 6.2). No significant effect of group was identified prior to surgery for acquisition of the novel problem, $F(1,7) = 0.98$, $p=0.356$, and neither group made an error during the final pre-operative session for the retention problem.

Sham rats showed no significant changes in performance from their pre-operative level following surgery on acquisition, $F(1,6) = 1.76, p=0.233$, or retention, $F(1,6) = 3.00, p=0.134$, components. Post-operatively, rats with HPC damage were impaired relative to sham rats on both the acquisition component and the retention problem (Figure 6.2). During the first post-operative testing session Sham rats acquired the novel problem in 12.8 trials versus the 30.0 trials without acquisition taken by rats with HPC damage. During the same testing session the HPC damaged rats showed a diminished performance of 60 % correct on the retention problem compared to the 95 % correct achieved by rats that had undergone sham surgery. The effect of group on acquisition of a new problem was significant, $F(1,7) = 76.19, p < 0.001$, as was the effect of group on retention of the constant problem, $F(1,7) = 63.52, p < 0.001$.

There was no difference in the impairments that resulted from inducing HPC damage one or 30 days after the completion of training for either the acquisition problem, $F(1,13) = 1.79, P = 0.204$, or the retention problem, $F(1,13) = 0.01, P = 0.923$. That is, within the time points assessed here there was no temporal gradient associated with retrograde amnesia.

Relearning:

Rats who sustained HPC damage 30 days after the completion of training were able to perform at their pre-operative level after retraining trials. The mean number of trials required to reach the criterion for the retention problem was 32.0 and 45.8 for the acquisition problem. Rats with HPC damage showed a trend, $F(1,8) = 4.84, p=0.059$, for faster relearning of the constant discrimination than their initial rate of learning (54 trials to reach 90%) prior to HPC damage. The rate of reacquisition measured by the number

of trials needed to re-attain criteria was equivalent in the groups with HPC damage tested at the two training-surgery time points (figure 6.3). There were no significant differences for either the rate of relearning of the constant problem, $F(1,13) = 0.79, p=0.399$, or in the amount of time taken to reach the criterion for the novel problem, $F(1,13) = 2.29, p=0.154$.

DISCUSSION:

The results of the current experiment provide further support that the HPC contributes to the retention of elemental information. Here I show, that if rats are trained on concurrent constant and novel versions of an elemental discrimination prior to HPC damage they will have retrograde amnesia for both components of the task. Post-operative retraining allows HPC damaged rats to reacquire both the constant and novel components as well as Sham rats.

This result is not predicted by most current theories. The task I have used is a non-spatial and elemental discrimination and therefore the deficits seen here contradict both the cognitive map theory and configural association theory respectively. The retrograde amnesia seen here is of equal magnitude when surgery occurred 1 or 30 days after training. The standard model of memory consolidation would predict a more severe retrograde amnesia for the training interval of 1 day than 30 days. No current theory adequately explains the results of this experiment. However, the results seen in this final experiment conform to those found in experiments 2 and 3. In all conditions tested here, HPC damage after acquisition of multiple discrimination problems whether temporally overlapping or separated causes retrograde amnesia for those problems. Finally, we also

show that the learning set acquired pre-operatively is impaired following HPC damage. Novel learning is impaired initially despite an eventual ability of rats with HPC damage to relearn the task. This demonstrates that retention of elemental information as well as the learning set that allows for these discriminations to be rapidly acquired are both dependent on the HPC when it is intact.

Chapter Seven

A revised view of the function of the hippocampus in learning and memory

Rats with damage to the HPC exhibit retrograde amnesia for simple discriminations whenever they learn multiple discrimination problems in the same context. In striking contrast, preserved performance is seen after HPC damage if only a single visual discrimination had been acquired. The results of the experiments discussed here are incompatible with the current predictors of retrograde amnesia and with theories of HPC function. My results will be discussed in relation to these predictors and a new theory of HPC function will be introduced that can account for these findings.

EXPERIMENT 1:

Training on a single well-learned visual discrimination was not disrupted by HPC damage at either training-surgery interval (1 or 30 days). Rats with HPC damage were able to learn a novel discrimination as fast as Sham rats. This experiment indicates that the HPC is not always required for the storage of elemental information.

The results of experiment one also provided a control condition against the possibility that the use of excitotoxins could disrupt memory stored outside the lesion area (Jarrard, 2002). Furthermore, this result shows that NMDA induced HPC damage does not produce a deficit related to performance effects such as impaired perception,

motivation, or attention. I show here that using an identical surgical procedure, NMDA injections into the HPC do not cause retrograde amnesia for a single visual discrimination but does for multiple visual discriminations. The same type of memory is not disrupted in all cases even though the same excitotoxin was used. This provides a great deal of reassurance that NMDA can be used to damage the HPC without causing a permanent disruption of memories stored elsewhere in the brain.

EXPERIMENT 2:

The results of the second experiment along with the results of experiment 1 show that the HPC damage disrupts elemental information for multiple discriminations but not a single discrimination. Here, rats trained on three sequential discriminations prior to HPC damage had retrograde amnesia for all three. There was no temporal gradient to the memory loss despite the different training-surgery time interval for each problem (1, 30, or 60 days). Post-surgical acquisition of a novel discrimination occurred at an equivalent rate in Sham and HPC damaged rats.

It is important to note that the retrograde amnesia associated with learning multiple discrimination problems does not spare the first problem learned. Training on the first problem in experiment two was exactly the same as for the single problem in experiment one. The only difference was the addition of a second and third problem afterwards. The first problem must have been learned without the HPC or learned in parallel by the HPC and an extra-HPC learning system. It appears more likely that the two learning systems operate in parallel during acquisition of the first problem because novel learning does not disrupt the memory of problem 1 in Sham rats. Some form of

post-event processing initiated by the learning of additional problems disrupted the cortical memory trace of the first problem. Subsequent acquisition must occur without the extra-HPC memory system because these latter problems are disrupted in rats with HPC damage.

EXPERIMENT 3:

Here I have demonstrated the memory deficit that occurs when multiple discriminations are learned is not due to overlapping visual features of the cues among the different discriminations. Rats trained sequentially on a visual discrimination followed by an auditory discrimination had retrograde amnesia for both following HPC damage. This finding suggests that retrograde amnesia occurs when multiple discriminations are learned in the same context. Anterograde acquisition of a novel discrimination was intact in HPC damaged rats.

EXPERIMENT 4:

The results of the final experiment demonstrate that elemental discrimination learning is vulnerable to retrograde amnesia for both the novel and the constant components of the task. The HPC was critical for retention of the 2 concurrently learned discriminations regardless of the training-surgery interval (1 or 30 days). Post-surgical reacquisition of both the novel and the constant components was possible for rats with HPC damage. Retraining allowed performance of HPC damaged rats to proceed as well as Sham rats.

Previous studies (Alvarado & Rudy, 1992; Astur & Sutherland, 1998) have demonstrated that in certain circumstances, both rats and humans will utilize configural solutions to elemental problems. The HPC could have been recruited to store elemental information in a configural way in order to more efficiently represent multiple similar learning episodes.

ANTEROGRADE RESULTS:

In each of the four experiments outlined above I have shown that rats with HPC damage are able to acquire novel visual discriminations as well as Sham rats. Furthermore, in all cases, rats with HPC damage were able to relearn the same discriminations that they had acquired pre-operatively and can do so with the same degree of accuracy as they had been capable of prior to HPC damage. Based solely on the anterograde findings of these experiments, my results appear to agree with the predictions of a number of contemporary theories.

The cognitive mapping theory of O'Keefe and Nadel (1978) would predict that performance on a non-spatial task such as the visual discrimination paradigm used here would not be disrupted by HPC damage because it does not require the formation or storage of a cognitive map. Configural association theory (Sutherland & Rudy, 1989) also predicts the anterograde results of my experiments. Because the task has a simple elemental solution it should be possible for rats to solve the discriminations without a HPC. This is in fact what is seen in the anterograde direction. There is nothing in particular about the type of memory in question that requires a contribution from the HPC.

RETROGRADE RESULTS:

My findings of retrograde amnesia for elemental discriminations are far less supported by current theories than is the absence of anterograde amnesia. The same theories that predict preserved anterograde learning (i.e. configural association theory and cognitive mapping theory) would also predict preserved retrograde memory. However, this is not what I have shown here. The results of the first experiment demonstrated that under certain conditions (i.e. learning multiple discriminations in the same context) the HPC is critical for elemental learning and memory. Rats, trained concurrently on a constant and a novel discrimination each day, are susceptible to retrograde amnesia.

Configural association theory

Almost no contemporary theory would predict an elemental visual discrimination task is dependent on the HPC. Included in this category is the configural association theory that identifies two distinct systems one for elemental information and another for configural information. However, My results demonstrate that the configural association system, based on the HPC, also performs simple associative learning. Therefore this theory is unable to predict the results of the current experiments because under certain circumstances (when only a single discrimination is learned) simple associative learning is performed by an extra-HPC simple association system but in other cases (learning multiple discriminations) the same type of learning is dependent on the HPC.

Cognitive map theory

Since the task used in my experiments is fundamentally non-spatial, it should not require the formation of a cognitive map and therefore according to O'Keefe and Nadel

(1978) will not depend on the HPC. However, I have shown clear retrograde deficits for this task following HPC damage. Thus, the cognitive map theory fails to predict the presence or absence of retrograde amnesia in the current experiments.

Standard model of memory consolidation

Between systems consolidation theory suggests that the HPC, when used, is only required for short term processing of a memory after which the neocortex is capable of supporting that memory on its own. By this view, remote memories are less vulnerable to HPC damage than are recently acquired ones, but my results provide two lines of evidence that contradict this theory.

First, in Experiment 2, rats were trained sequentially on a different discrimination at three different time intervals. An equal impairment was found for all three problems regardless of the amount of time that elapsed between training and surgery. The between systems consolidation view would again predict a graded deficit with the most recent problem being severely impaired and the most remote problem being completely or partially spared. Instead, I see evidence of the alternative view that once a certain memory is dependent on the HPC it will always be dependent on the HPC. Training rats on two sequential visual discrimination problems further supported this result. Following HPC damage the same pattern and magnitude of memory impairments are seen compared to those induced by HPC damage following training on three visual discrimination problems.

A second line of evidence against between systems consolidation theory arises from my final experiment. Rats were trained concurrently on both a fixed and a novel daily component. Impairments were seen on both the acquisition and retention

components if HPC damage was incurred either 24 hours or 30 days following the completion of training. Furthermore the deficit was of equal magnitude regardless of the training-surgery interval. The widely accepted view that the HPC is only needed for a limited time cannot account for the identical impairments seen 30 days after training. Consolidation theory would predict a smaller or absent impairment in rats that underwent surgery 30 days post-training. Instead, the results of this experiment suggest that the HPC is actually needed permanently for at least this form of memory.

My intention is not to refute the idea of memory consolidation but to argue the type of consolidation process that occurs. There is no evidence here to say that the memory does not change and become consolidated over time but these experiments provide no evidence that the consolidation process involves the interaction between HPC and neocortical areas. An often-overlooked alternative is that a within system consolidation process is sufficient to produce a stable long-term memory. That is, the memory in this case is actually consolidated within the HPC meaning that the HPC would always be required for recall of the memory. This alternative explanation would explain the presence of a temporal gradient in some cases where only a portion of the HPC is removed. It is possible that the dorsal HPC lesions produced in some experiments are insufficient to produce the complete effects of HPC damage. The consolidation process could proceed in a dependent manor through the different subfields of the HPC thus a partial lesion would not have the same effect as a more extensive one.

The standard model of memory consolidation posits that declarative memory is dependent on the HPC and non-declarative memories are not. It is evident from these

experiments that the type of memory cannot accurately predict whether or not HPC damage will cause memory deficits. I find that memory for elemental discriminations is dependent on the HPC if multiple problems are acquired, but I have also found that this same type of memory can be formed in the anterograde direction without the HPC. This emphasizes that different results may be obtained from studies of anterograde or retrograde amnesia. Studies of anterograde amnesia do not necessarily help address the specific contribution of the HPC. Furthermore, the same type of memory can be differentially affected as a consequence of the training history of the rat (i.e. learning one vs. two discriminations). Therefore, defining the role of the HPC by the type of memory it stores may be accurate in some circumstances but not all. Instead of age and type of memory I propose that a more accurate predictor of retrograde amnesia following HPC damage is the number of memories acquired in a given context. When multiple problems are learned in the same context, the HPC makes an important contribution to the storage of the memory and HPC damage will result in amnesia.

Reconsolidation theory

Reconsolidation theory (Misanin, Miller, & Lewis, 1968 and more recently described by Nader, Schafe, & Ledoux, 2000) also fails to predict the results of the experiments described here. This theory predicts that a memory initially dependent on the HPC will become consolidated to a neocortical storage site and thus, become HPC independent. Future reactivation of that memory will cause it to revert to a labile, HPC dependent state. Then, over time the memory is reconsolidated to the neocortex. It is, according to this theory, only when the memory is in a labile HPC dependent state that it will be vulnerable to retrograde amnesia.

Like the standard model, reconsolidation would predict retrograde amnesia at short training-surgery intervals but not at longer ones. However, I have shown here that the length of this interval does not change the magnitude of the resulting retrograde amnesia. Proponents of reconsolidation might suggest that the shortest training-surgery interval of 1-day is too long and by that time the memory has already become reconsolidated. But, this explanation cannot account for the fact that the same type of memory, reactivated the same number of times would be labile if other discriminations had been learned prior and not labile if it was the only discrimination learned.

Multiple-trace model

One of the main tenets of the multiple-trace model is that the HPC is required for only certain types of memory (i.e. episodic memory). Even without labelling this task as episodic it is possible to see why this theory fails to predict the results of my experiments. As has been the case for several theories, the multiple-trace model cannot predict retrograde amnesia based on the number of discriminations the rats learn prior to HPC damage. Instead, Moscovitch and Nadel (1997) would predict that if a certain type of memory were dependent on the HPC then it would be regardless of whether the rat learns one or two discriminations.

Two-process model

Rudy, Huff and Matus-Amat (2004) have proposed a two-process model of HPC function in declarative memory based on results of several contextual fear conditioning experiments. They have proposed that the HPC performs two separate roles. First, the HPC forms and stores a configural representation of the context and secondly, inhibits extra-HPC conditioning to the independent features of the context. The two-process

model is however, insufficient to predict spared memory after learning a single discrimination versus retrograde amnesia for multiple discriminations.

HIPPOCAMPUS DEPENDENT DECONSOLIDATION THEORY:

My results demonstrate that an interaction between HPC and extra-HPC memory systems exists but the current description of this interaction is incomplete. Instead I propose a theory of HPC dependent memory *deconsolidation*. The current dominant theory suggests a process of consolidation occurs between systems that allows for a temporary memory in the HPC to support formation of a long-term memory in the neocortex. I find that the opposite interaction must occur. Initially, the memory of problem 1 (P1) is processed and stored by two separate systems. The HPC system and a cortical system both having access to and independently store the memory for a single discrimination. Unlike the standard model of between systems consolidation, the interaction does not consist of strengthening of the memory in the neocortex by the HPC. Instead, the interaction is engaged when multiple learning events occur in a contextually identical environment (C1). Learning problem two (P2), following or concurrent with learning P1 causes the activity of the HPC to inhibit the use of the cortical memory system. The inhibition causes the suppression or even erasure of information that had been previously acquired by an extra-HPC memory system. Any subsequent recall of that information is not dependent on the memory trace stored with in the HPC. This inhibitory process also suppresses the acquisition of further memories by the extra-HPC system. Learning and memory of any additional problems still occurs as a result of the intact HPC system. Damage to the HPC after learning multiple problems will result in

retrograde amnesia for all problems but if the damage occurs after learning a single problem there is no memory deficit.

The HPC has been described as a fast learning system in comparison to the relatively slow rate of learning that occurs in the neocortex (O'Reilly & Norman, 2002). A faster rate of learning has an outcome of increasing the amount of interference between memories. A system that acquires information rapidly must be well suited to disperse interference. Many theorists believe that the HPC, especially the dentate gyrus, is organized to perform this type of action. Both the HPC and the cortical memory system encode P1 that occurs in C1. New learning (P2) in C1 causes interference between the memories P1 and P2 because there are no contextual cues to separate the problems from each other. Being placed in C1 would activate the memory for both P1 and P2. The HPC is able to reduce this interference so its output inhibits the use of the extra-HPC memory system. This interference results in inhibition that causes the HPC to be used as the default memory system even though the cortical system is able to perform this type of learning and memory when the inhibition from the HPC is removed.

A key role of the HPC is to differentiate multiple learning episodes that occur in the same context by storing the memories in a partitioned and more efficiently retrievable manner than can be achieved by cortical memory systems. It may be the case that one reinforcer (in this case, escape to the platform) can only produce a limited change in synaptic strength. This would result in competition between memory systems. In the absence of the HPC the competition is removed and the cortical system receives more synaptic weight change. Therefore, the cortical system appears to learn at the same rate as the HPC but this is probably only the case when the HPC is removed.

FUTURE DIRECTIONS:

The experiments described here provide strong support for a conceptual view of the HPC that places it in a central role for a wide variety of learning and memory processes. Current theoretical work is insufficient to explain the entire spectrum of memory impairments that can be seen following HPC damage. The theory of HPC dependent deconsolidation presented here allows for an account of deficits in a diverse array of memory tasks.

Future work will be needed to clarify if the deficit is purely dependent on multiple training episodes in the same context or if some of the impairment can be attributed to interference from overlapping visual cues. Training in the same context may only account for part of the deficits. Overlapping visual features may also play a role in the deficits seen in my first and third experiments. In order to confirm that the deficits are purely related to the identical training context rats should be trained on two discriminations in separate contexts.

Additional experiments will also be required to determine if the results of my experiment can be applied to similar learning paradigms that take place in a different task. It is possible that other tasks that are not impaired if trained after HPC damage may be impaired in the retrograde direction. These tasks may also only be affected if multiple contextually similar events are learned. Other memory tasks that are not vulnerable to anterograde amnesia should be tested in a similar paradigm to determine if my results are specific to discrimination learning or if the HPC plays a much wider role. Although I have seen evidence that the deconsolidation effect is apparent across sensory modalities

and not just applicable to the visual domain, I have not yet tested whether other tasks are susceptible to such effects.

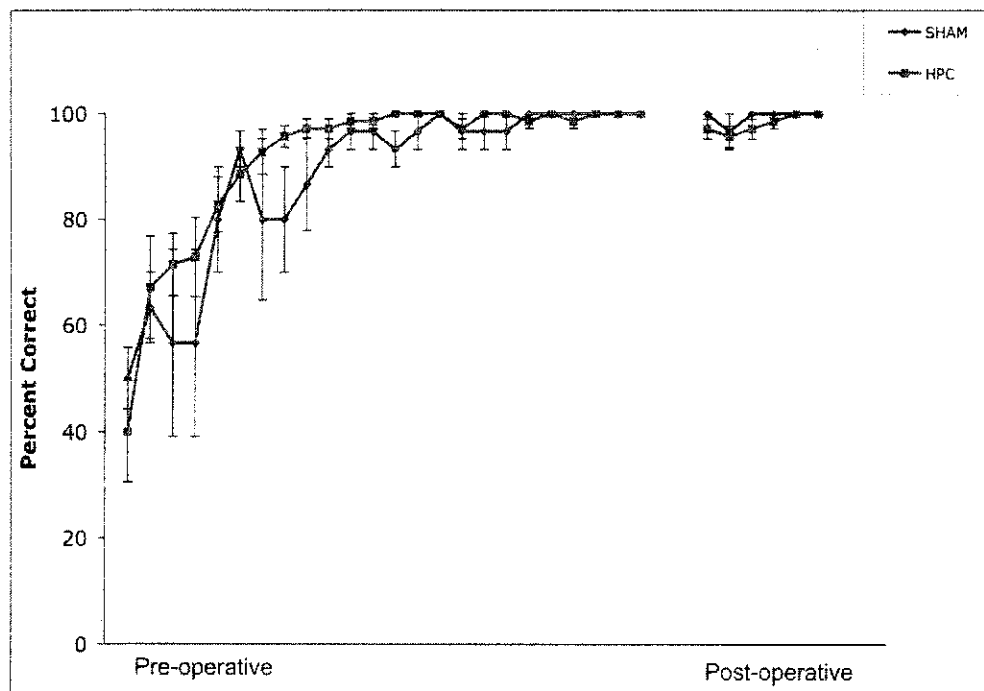
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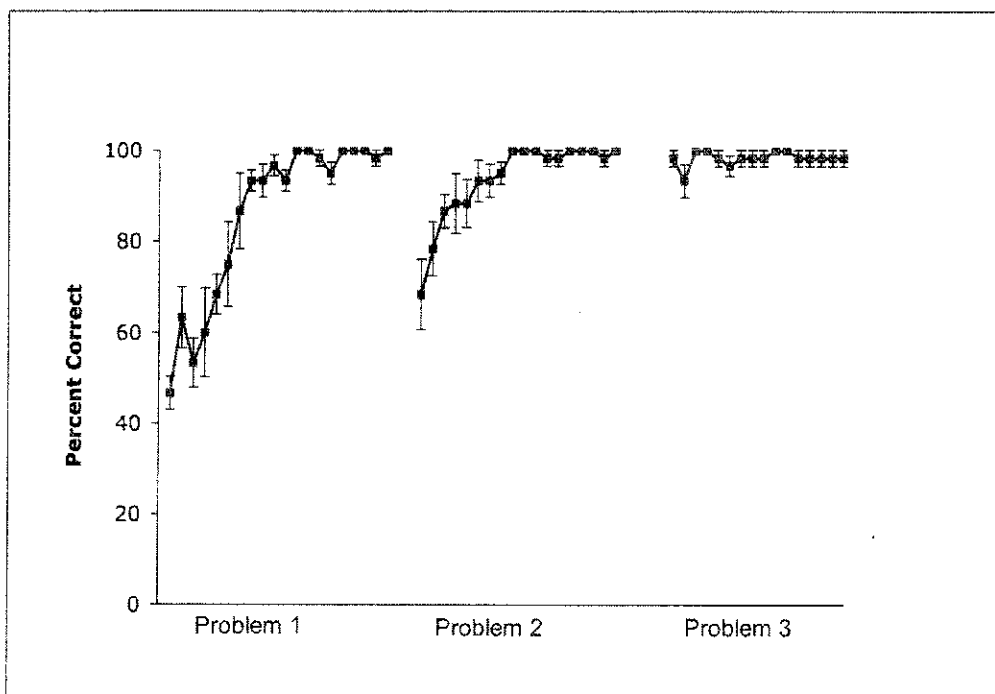
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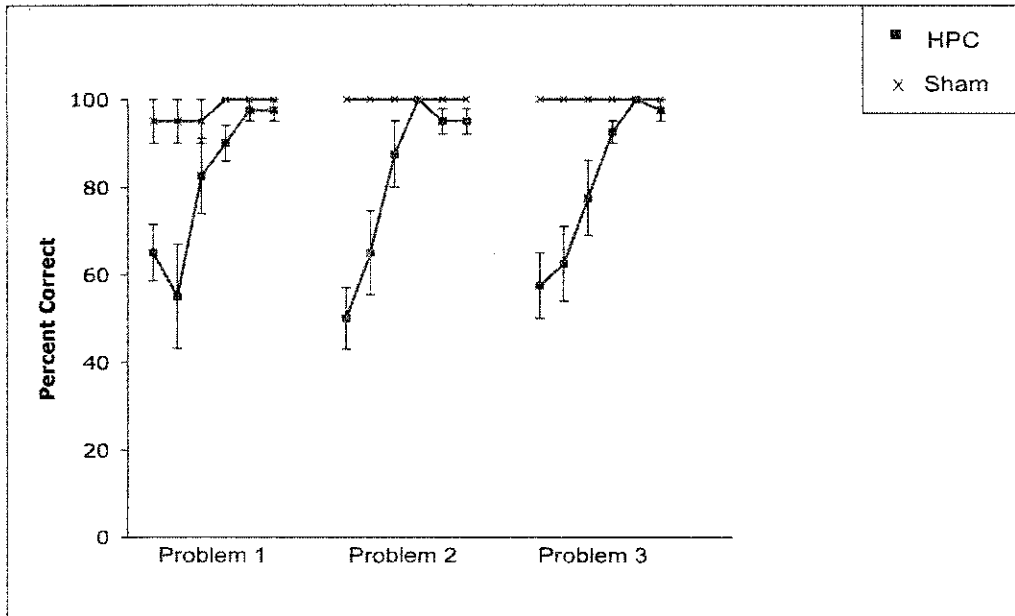
Appendix 1



A



B



C

The above figures show percent correct over training sessions. Each data point represents the mean score per session (10 trials per session) \pm STD error. Panel A shows the pre-operative acquisition and post-operative retention of a single visual discrimination. Panel B shows the pre-operative acquisition of three sequential discriminations. Panel C shows the initial retention and relearning of three discrimination problems.

Figures

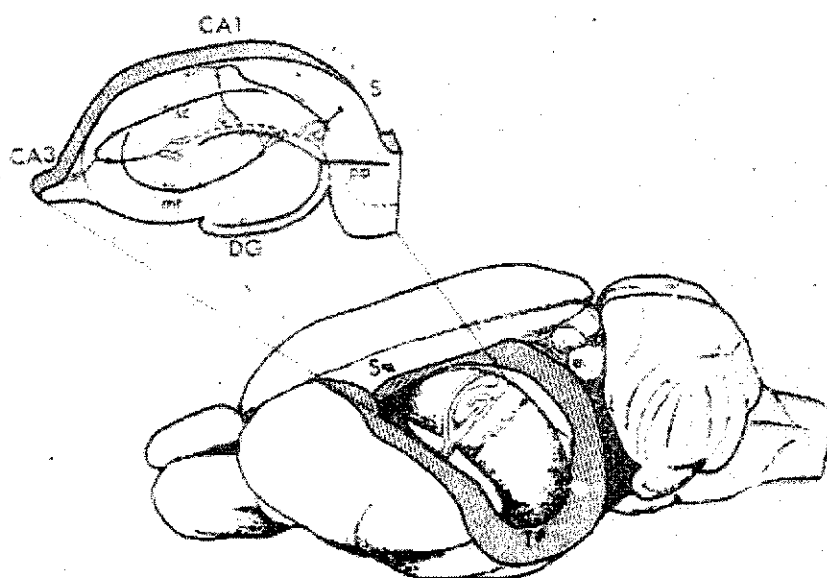


Figure 1.1. The image above shows the location of the hippocampus (yellow) within the rat brain. The cut out image is a slice through the hippocampus and shows the principal subfields and pathways. Figure adapted from: <http://www.mit.edu/people/jelevin/research.html>.

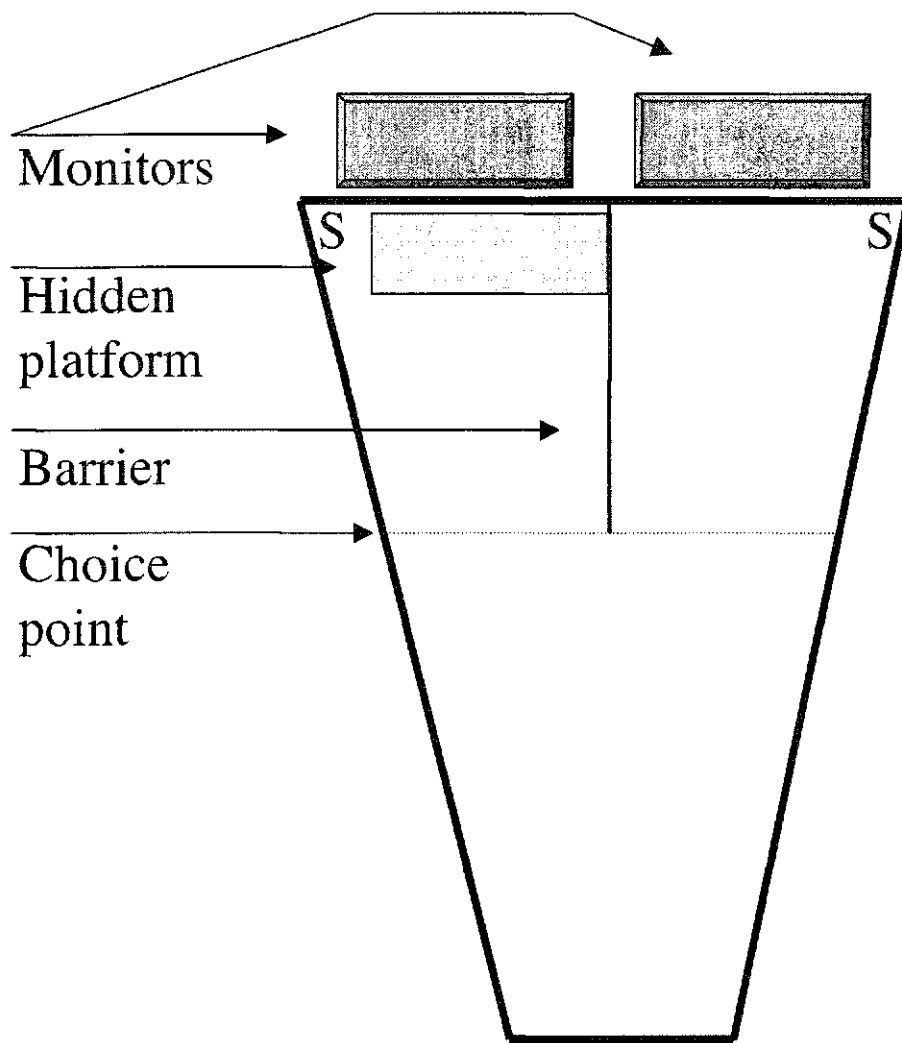


Figure 2.1. The apparatus used in the following experiments is seen above. The visual water task consists of a trapezoidal pool and two computer monitors separated by an opaque barrier. *S* indicates the location of the speakers that were only present during experiment four.

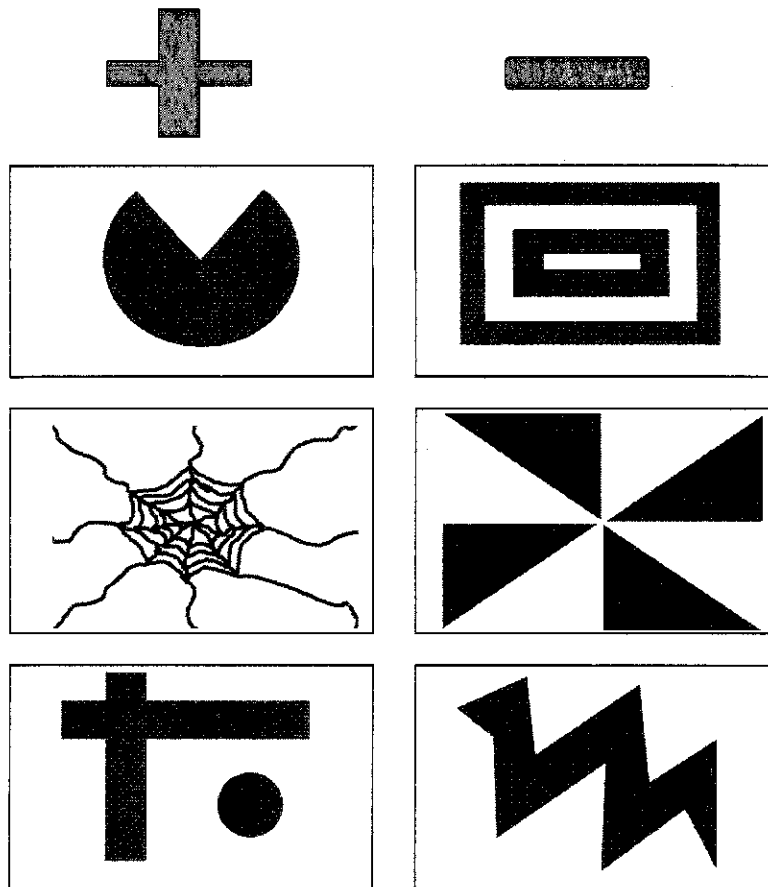


Figure 2.2. A subset of the cues used in the visual water task is shown above. The left column contains samples of reinforced cues; the images in the right column are representative non-reinforced cues.

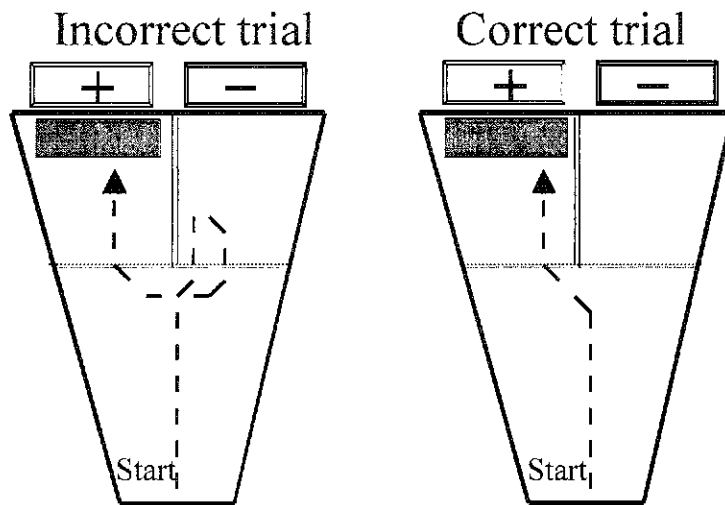


Figure 2.3. Correct and incorrect swim paths are shown in this figure. During an incorrect trial the rat approaches the non-reinforced cue, but then swims to the reinforced cue and is removed from the pool. During a correct trial the rat swims directly to the reinforced cue to terminate the trial.

	<i>AP</i>	<i>ML</i>	<i>DV</i>
Site 1	- 3.1	± 1.5	- 3.6
Site 2	- 4.1	± 3.0	- 4.0
Site 3	- 5.0	± 3.0	- 4.0
Site 4	- 5.3	± 5.2	- 7.3
Site 5	- 6.0	± 5.0	- 7.3

Table 2.1. The coordinates used for injection of NMDA into the hippocampus are shown above. All numbers are expressed relative to bregma.

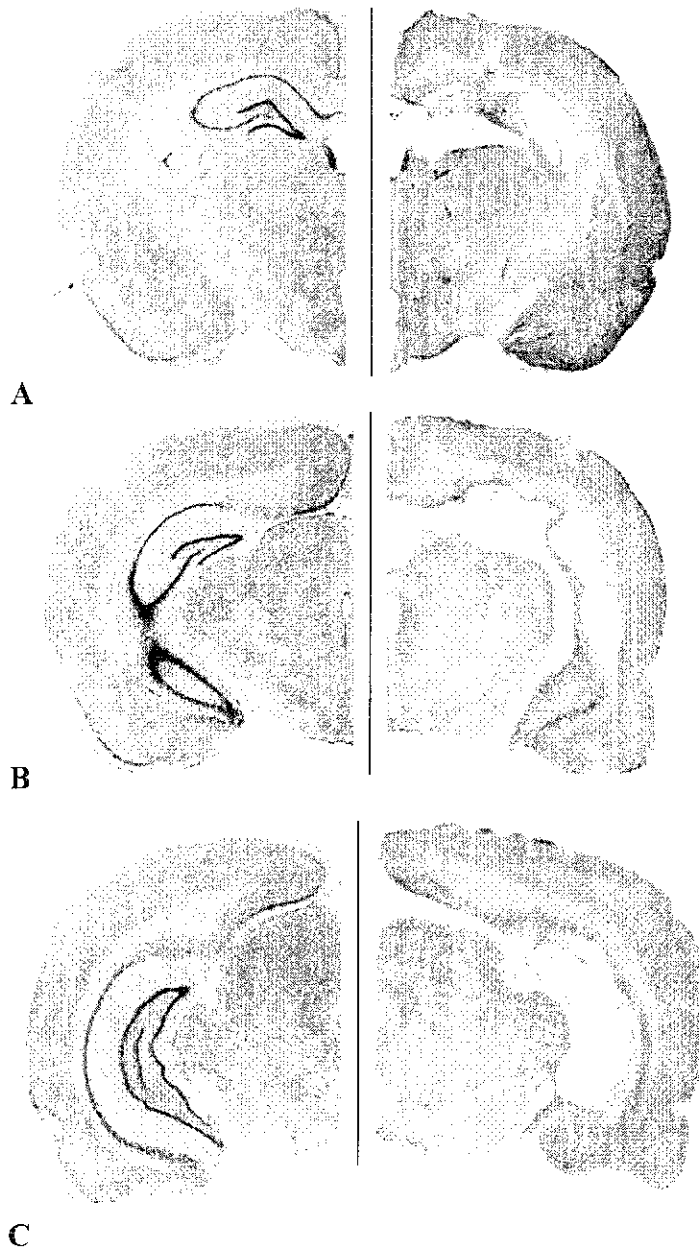


Figure 2.4. Cresyl violet sections from (a) anterior, (b) medial, and (c) posterior sections of the hippocampal region are shown above. The left hemisphere is taken from a Sham rat; the right hemisphere is from a rat with NMDA induced hippocampus damage.

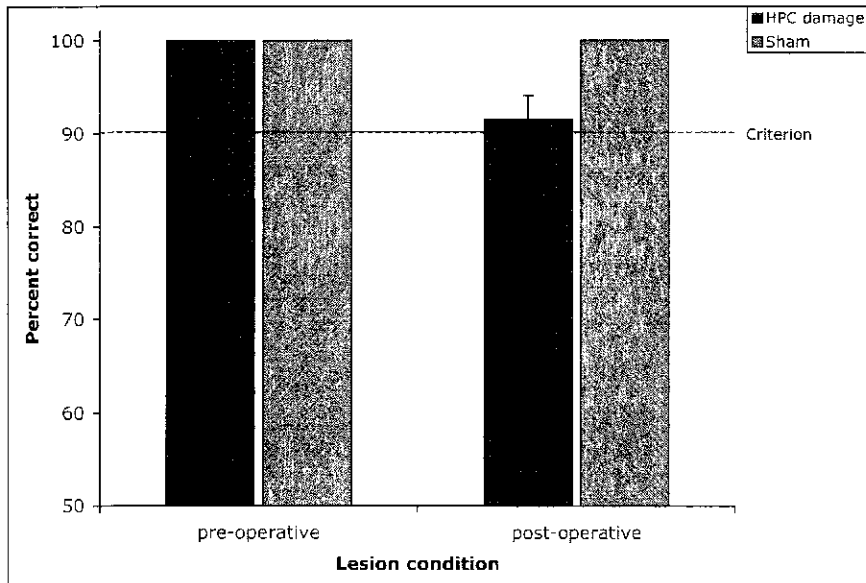


Figure 3.1. The final pre- and first post-operative session is shown above for rats trained on a single elemental discrimination. Hippocampal damage after learning a single discrimination did not result in retrograde amnesia at the 1-day training-surgery interval. Bars represent mean values \pm STD error of the mean.

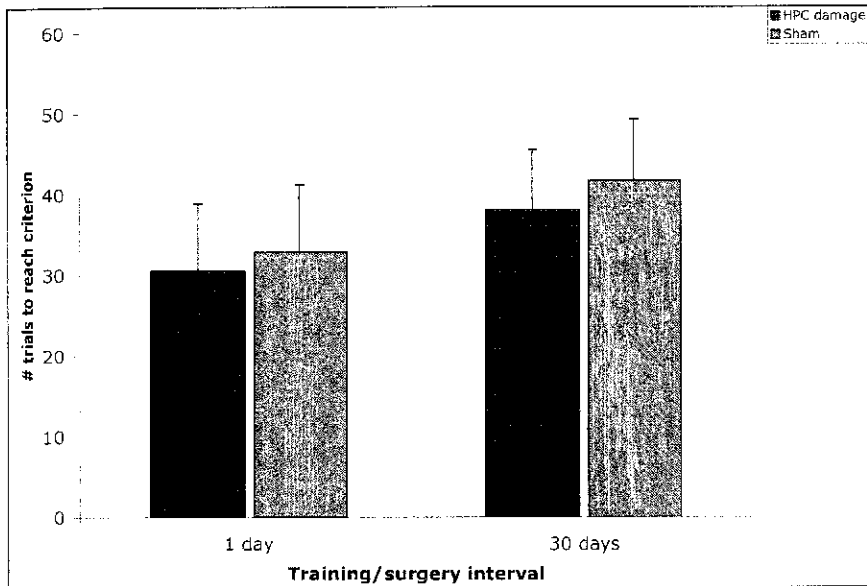


Figure 3.2. The rate of post-surgical acquisition of a novel visual discrimination problem is shown above. The number of trials to learn a new problem is equivalent regardless of surgical condition (hippocampus damage or Sham surgery) or the interval between initial pre-operative training and surgery (1 day versus 30 days). Bars represent mean values \pm STD error of the mean.

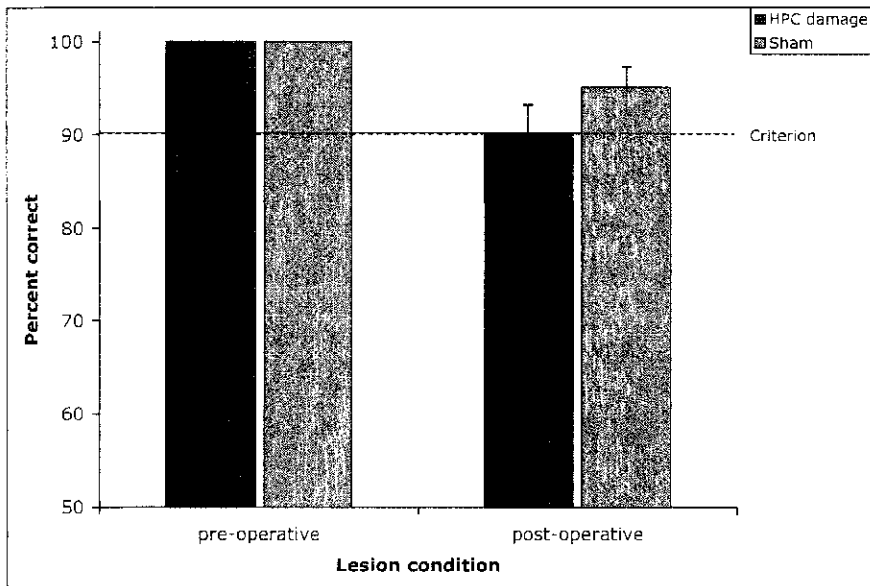


Figure 3.3. The final pre- and first post-operative session is shown above for rats trained on a single elemental discrimination. Hippocampal damage after learning a single discrimination did not result in retrograde amnesia at the 30-day training-surgery interval. Bars represent mean values \pm STD error of the mean.

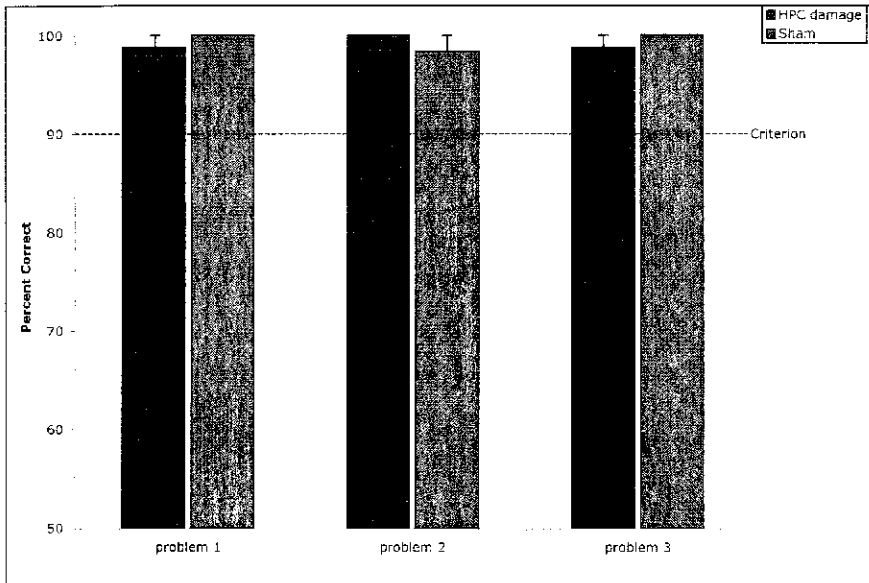


Figure 4.1. Pre-operative level of performance achieved on the final training session for each of three sequentially trained visual discrimination problems. Bas represent the mean group score \pm Std error of the mean.

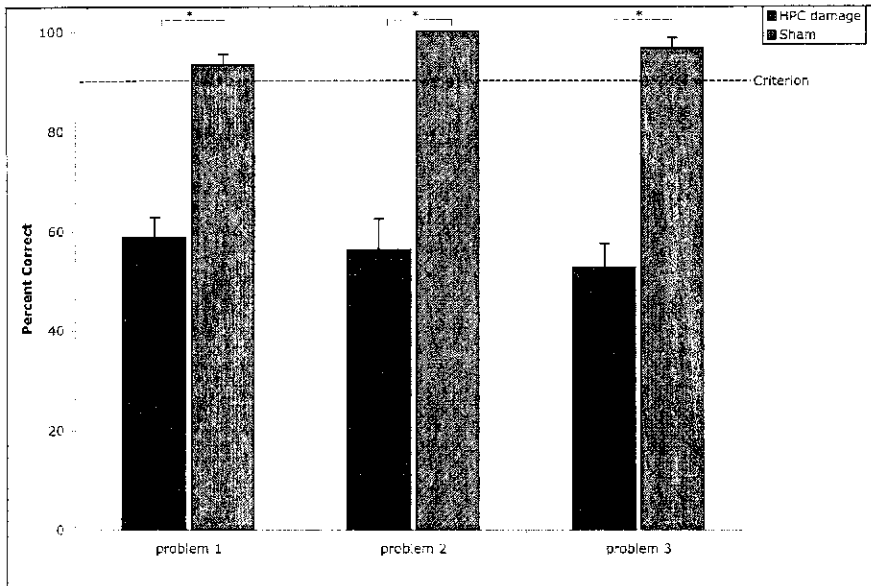


Figure 4.2. Initial retention of the three sequentially learned visual discrimination problems during the first post-operative testing session. Hippocampus damaged rats have retrograde amnesia for all three problems. Bars represent the mean score \pm Std. error of the mean.

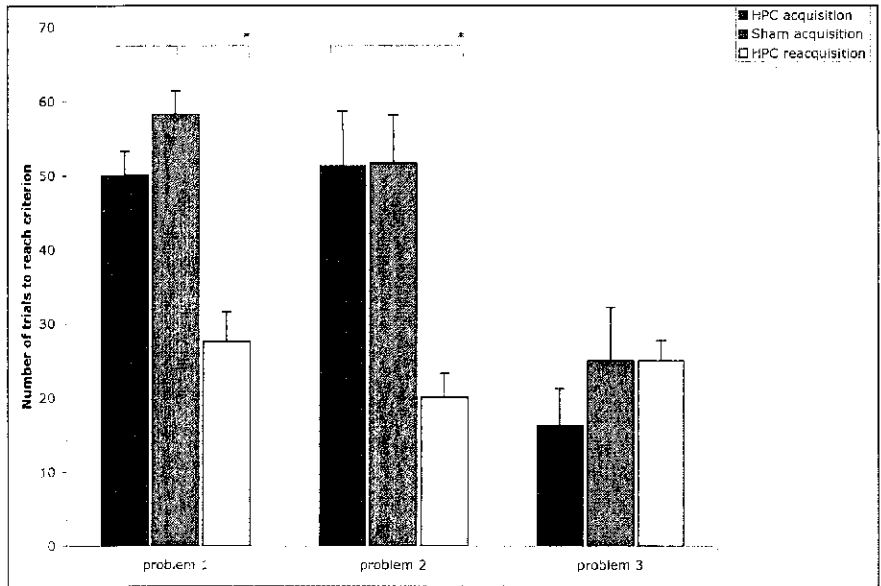


Figure 4.3. Rate of acquisition of three sequential visual discrimination problems pre-operatively and rate of reacquisition of the same problems post-operatively is shown. Bars represent mean number of trials required to reach a criterion of 90% correct \pm Std error of the mean.

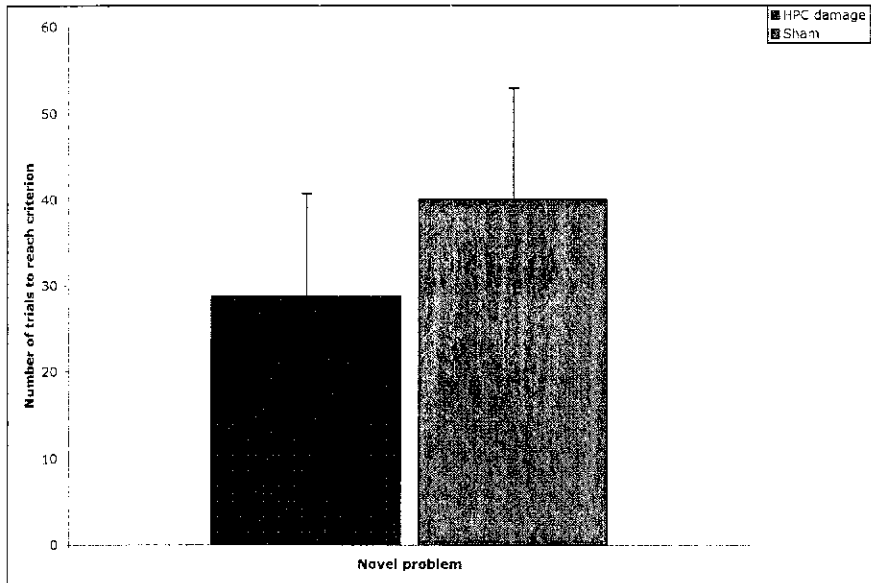
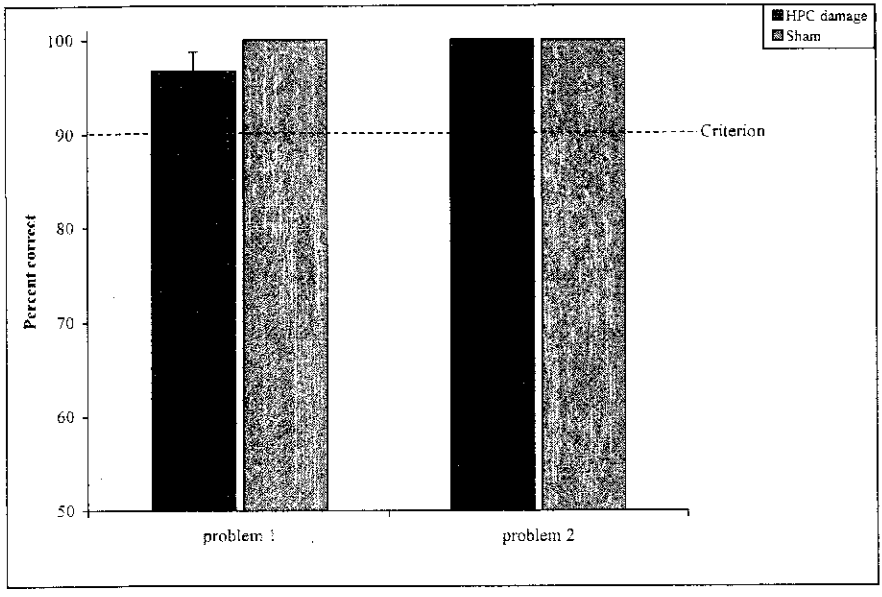
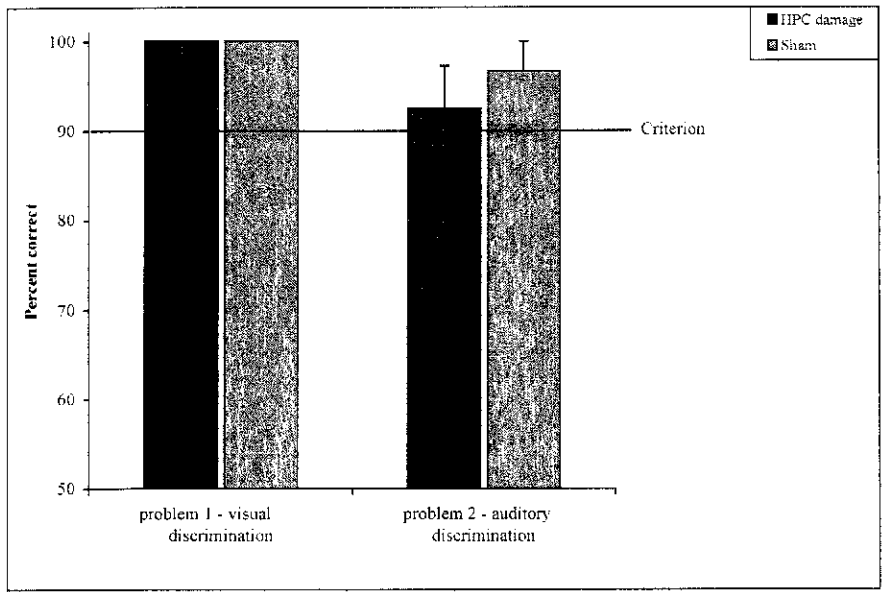


Figure 4.4. The number of trials required to reach the 90% criterion on a novel visual discrimination acquired following surgery is shown above. Bars represent mean \pm Std. error of the mean

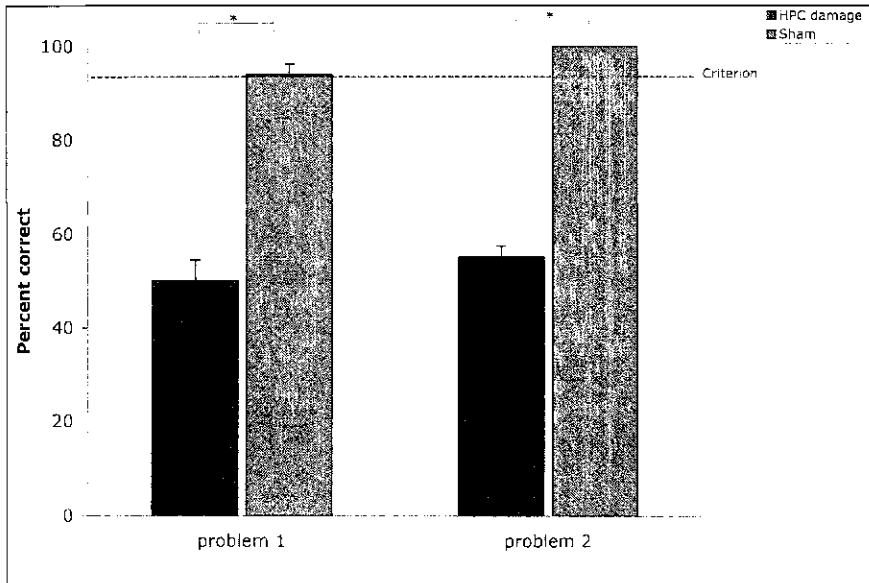


A

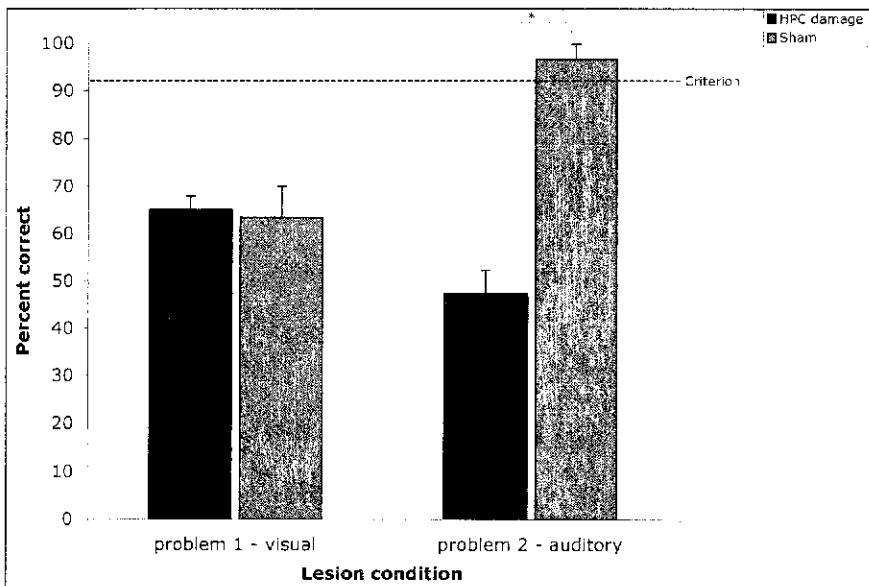


B

Figure 5.1. Shown above is the final pre-operative training session of each problem for rats trained on two sequential visual discriminations (a) or on a visual discrimination followed by an auditory discrimination (b). Bars represent the mean score \pm Std. error of the mean.



A



B

Figure 5.2. Shown above is the first post-operative training session of each problem for rats trained on two sequential visual discriminations (a) or on a visual discrimination followed by an auditory discrimination (b). Bars represent the mean score \pm Std. error of the mean.

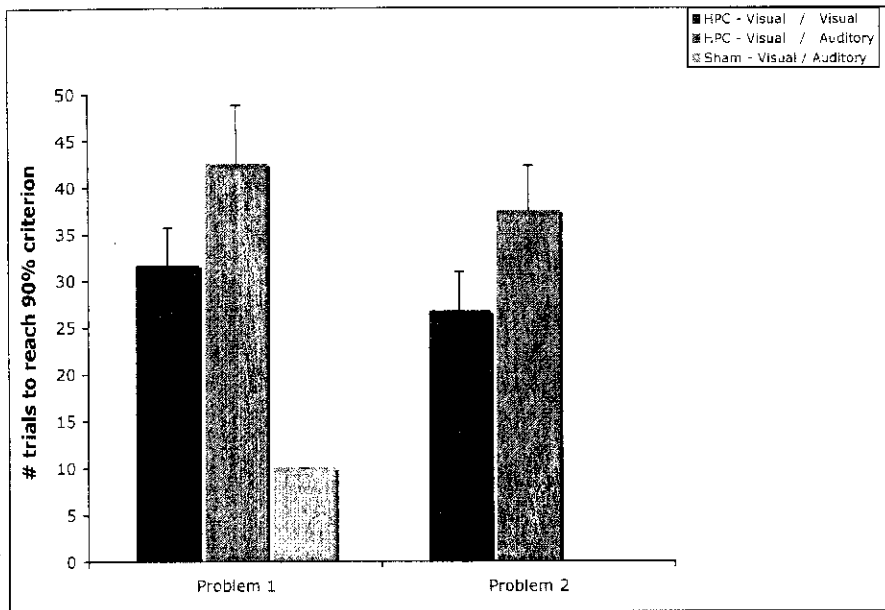
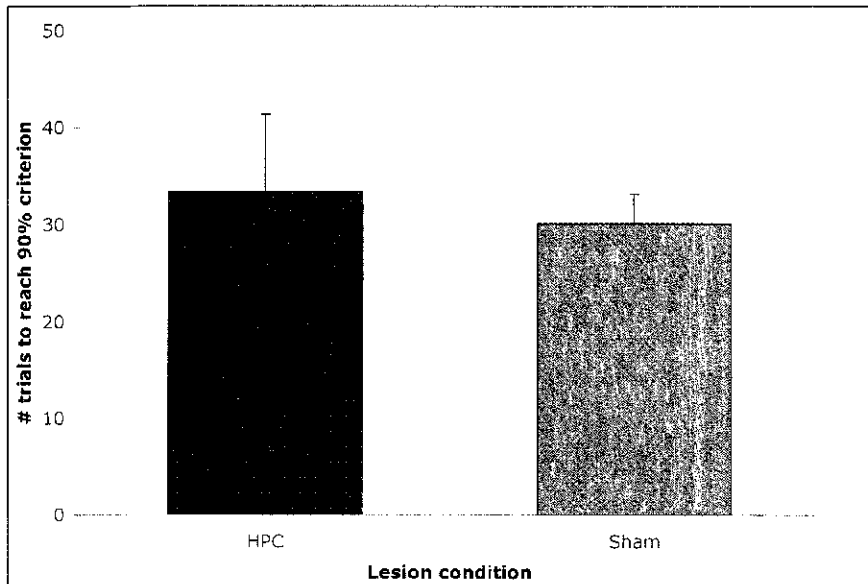
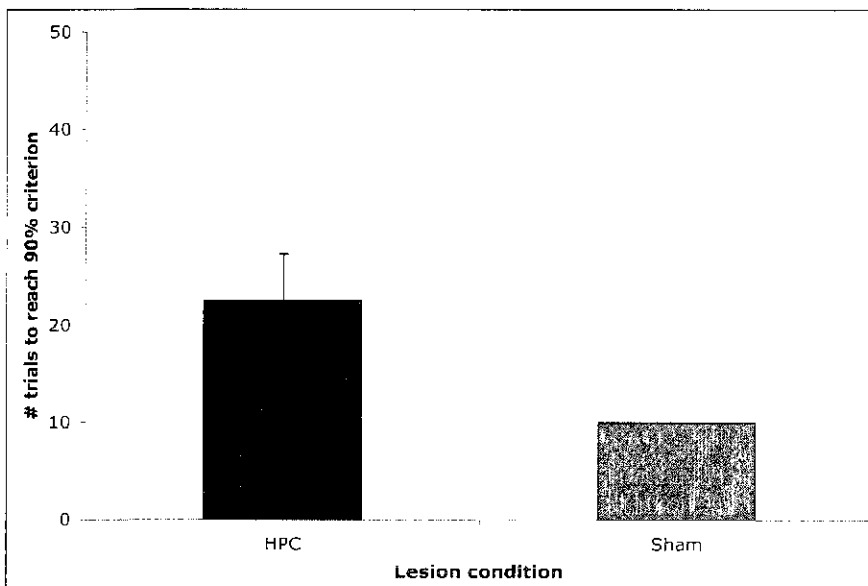


Figure 5.3. The rates of relearning of both problems are shown above. Bars represent the mean number of trials \pm Std. error of the mean.

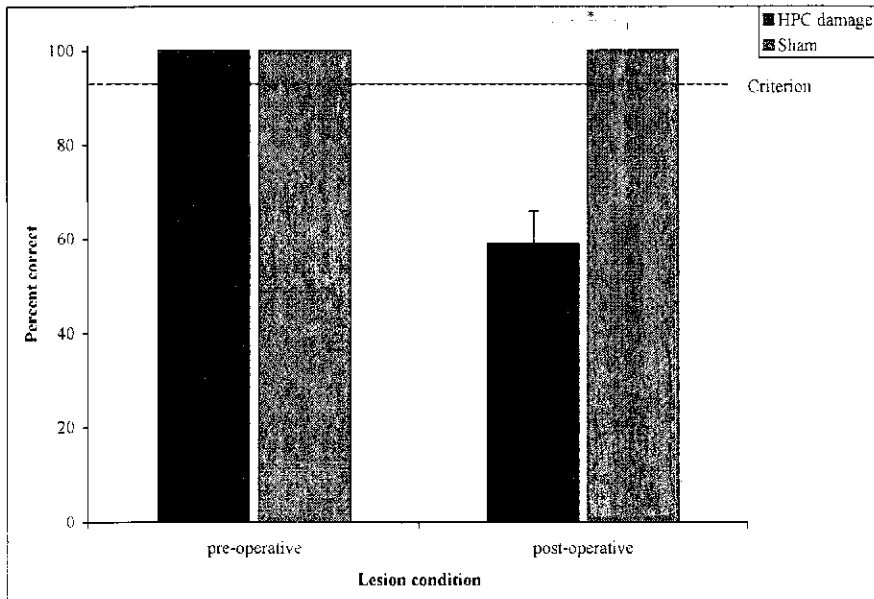


A

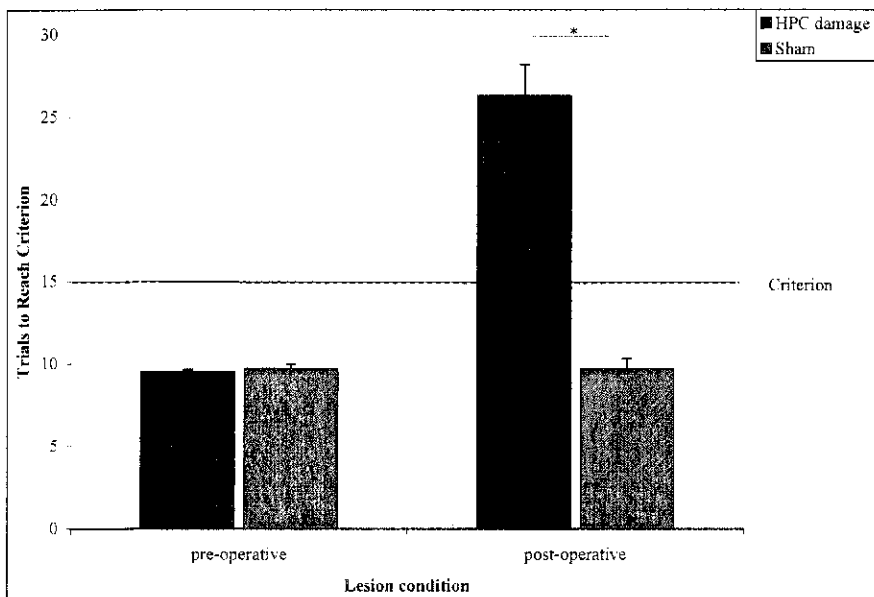


B

Figure 5.4. The number of trials required to reach the 90% criterion on a novel visual discrimination is shown above for rats initially trained on two sequential visual discriminations (a) or on a visual discrimination followed by an auditory discrimination (b). Bars represent the mean number of trial \pm Std. error of the mean.

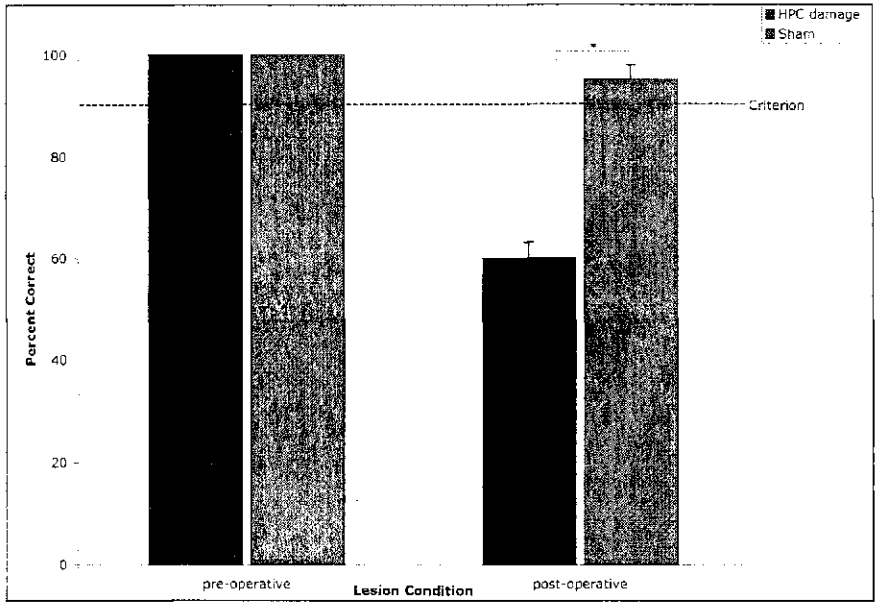


A

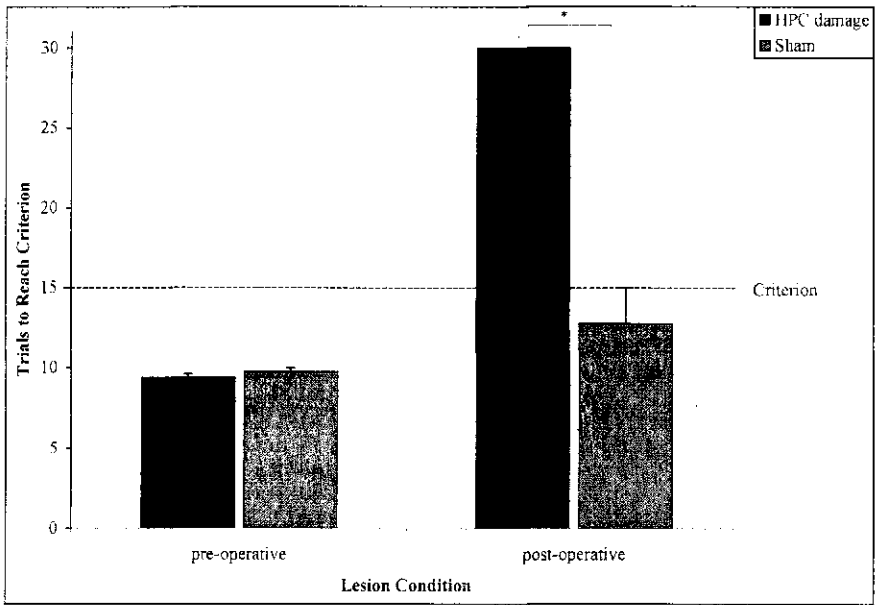


B

Figure 6.1. The figure above shows the final pre-operative training session and the first post-operative testing session for rats trained concurrently on retention (a) and acquisition (b) components. Rats with hippocampal damage exhibit retrograde amnesia for elemental visual discriminations when a 1-day training-surgery interval was used. Bars represent mean values \pm STD error of the mean.



A



B

Figure 6.2. The figure above shows the final pre-operative training session and the first post-operative testing session for rats trained concurrently on retention (a) and acquisition (b) components. Rats with hippocampal damage exhibit retrograde amnesia for elemental visual discriminations when a 30-day training-surgery interval was used. Bars represent mean values \pm STD error of the mean.

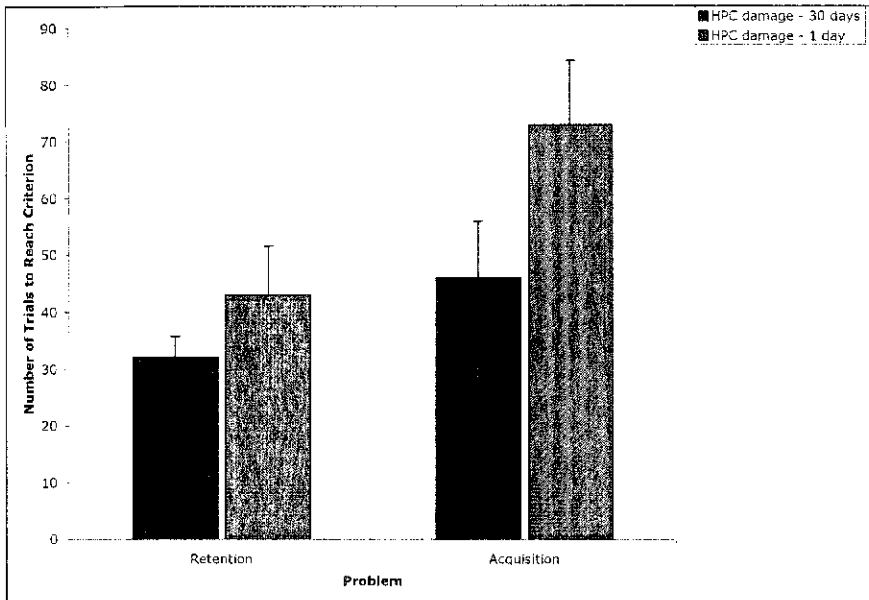


Figure 6.3. The rate of post-operative relearning is shown above. Rats with hippocampal damage were able to relearn both the acquisition and retention components of the task. Bars represent mean values \pm STD error of the mean.