HIPPOCAMPAL STRIATAL INTERACTIONS IN A PICTURE MEMORY TASK

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Abstract

The organization of learning and memory in the brain is widely held to be made of functionally distinct memory systems. It is important to identify how these complex neural systems interact with one another. This thesis investigates the interaction of the systems involved to solve a simple discrimination task. Acquisition of this task can be supported by hippocampal and non-hippocampal systems. The purposes of these experiments are: (1) to identify the non-HPC system supporting picture discrimination, and (2) characterize how the systems interact. Rats received various lesions either before or after training and assessed on acquisition or retention performances. The results indicate that picture discriminations can be acquired by hippocampal or striatal systems, and that the medial prefrontal cortex is not involved in an essential way. Furthermore, the findings suggest that a competitive interaction occurs supporting the idea that the hippocampus interferes with striatal acquisition of the picture discrimination task.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANOVA	analysis of variance
Contra	contralateral lesion group
Ipsi	ipsilateral lesion group
HPC	hippocampal lesion
HPC + mPFC	combined hippocampal and medial prefrontal lesion
HPC + str	combined hippocampal and dorsal striatal lesion
mPFC	medial prefrontal cortex
MPMS	multiple parallel memory system
MWT	Morris water task
NMDA	N-methyl-D-aspartic acid
PBS	phosphate buffered saline
PFA	paraformaldehyde
str	dorsal striatal lesion

CHAPTER 1

1.1 Introduction

The study of the neurobiology of learning and memory involves the attempt to understand the nature and organization of the brain processes that record information that can subsequently influence behaviour(s). Although it is possible to link the molecular dynamics of individual neurons to learning and memory, it cannot be ignored that neurons are organized into signalling pathways and networks that communicate and interact. To understand such complex processes such as learning and memory, we must understand not only the properties and pathways of individual cells but also the network properties of functional circuits and systems in the brain. Despite the vast amount of literature on learning and memory, there are still important controversies, especially concerning the organization of learning and memory at the systems level. The evidence suggesting that there are many types of memory and many different memory systems within the brain is an important source of the complexity in the organization of learning and memory.

This thesis attempts to enhance our understanding of the nature of learning and memory and its organization in the brain. This thesis begins with a consideration of some background information about the different types of memory and the various

related memory systems. Next, a discussion of the prominent theories bearing on these issues establishes where their explanatory power is limited. Next, how brain systems interact to organize behavioural expression of memories is explored. All of the experiments in this thesis investigate the interactions among memory systems. The results from these experiments highlight the idea that the nature of these interactions is important for a complete understanding of the nature of learning and memory and its organization in the brain.

What is Memory?

There are many perspectives on *what* memory is, and *how* it is organized in the mammalian brain. In one sense, memory can be thought of as a fundamental property of the brain's various systems, and is a natural result of the brain's processing activities. Memory in the information processing perspective, can be thought of as information gathered from an organism's environment and processed through different phases; *encoding, storage, consolidation, and retrieval.* Through experience and sensory input the brain processes information which creates a memory or representation, built up by synaptic plasticity processes within specific networks. The evidence showing that memory is manifested in a variety of forms by a variety of functionally and anatomically distinct memory systems, makes this area of research difficult to understand how memory is organized. As a result, many different theories of memory have emerged, yet none of these theories can give a fully satisfactory account of the available data.

Background

Questions regarding structure-function organization in memory originate from some of the earliest case studies of patients showing amnesia after brain damage.

Although not the first case study on memory disorders, patient H.M. provides an excellent example of *where* one of the central structures for memory is located. In an attempt to ameliorate his suffering from severe epilepsy, patient H.M. underwent a procedure involving the surgical removal of much of the medial temporal lobe, including the hippocampus. Immediately after surgery and until his death, H.M. suffered from severe memory impairments. In the 1950's, Dr. Brenda Milner tested the scope and severity of H.M.'s memory deficits and came to several conclusions. She noted that after H.M.'s MTL damage he suffered from an inability to form new long-term memories for facts or events (anterograde amnesia). Although the damage produced a loss of memories from many years prior to the surgery (retrograde amnesia), his most remote memories were spared (*temporally limited* retrograde amnesia) (Scoville & Milner, 1957). Scoville and Milner hypothesized that the MTL, specifically the hippocampus, was critical for certain kinds of long-term memories. H.M.'s memory impairments suggested that the hippocampus is not a permanent storage site for memory, but rather, the hippocampus served only a time-limited role for some memories. Milner observed, however, that H.M. was successful in motor learning and memory, but not capable of learning or remembering facts or events.

Milner's observations stimulated several alternative theoretical accounts of H.M.'s symptoms. Despite H.M.'s impairments, he had intact working memory. This was the first clear observation of the dissociation of working or short-term memory from long-term memory after a circumscribed lesion. MTL damage also spares some types of learning and memory for motor skills and acquired cue-response behaviours, sometimes referred to as "non-declarative" or "procedural memory" (Cohen & Squire, 1980; Squire, 1992). It was concluded that t other memory systems exist outside the MTL.

1.2 Different Memory Systems Mediate Distinct Forms of Memory

Scoville and Milner were among the first to provide evidence that there are multiple memory systems in the brain, and that these systems mediate different types of learning and memory mechanisms. The common observation of spared learning abilities after hippocampal damage, led to different dual memory theories, which propose that certain types of memory are hippocampal dependent and others are dependent on systems outside the hippocampus (Bachevalier & Mishkin, 1984; Cohen & Squire, 1980; Hirsh, 1974; O'Keefe & Nadel, 1978; Olton, Becker, & Handelmann, 1979; Squire, 1992; Sutherland & Rudy, 1989). Milner's findings stimulated the hypothesis that the hippocampus is the primary memory structure for facts and events, whereas non-hippocampal structures mediate habitual memories (Gaffan, 1974; Hirsh, 1974; Tulving, 1972). These findings were among the first leading to the theory that there are multiple memory systems in the brain that mediate multiple and distinct forms of memory.

Dissociation Experiments Show Functionally Distinct Systems

In an attempt to identify the functions of different memory systems, researchers have attempted to demonstrate double or triple dissociations. Dissociation studies are useful because they can highlight the independent functions of two memory systems. Double dissociations can occur when damage to one brain area impaired learning and memory for one task but not another, while damage to a second brain area impaired learning for the second but not the first task. Converging evidence from dissociation and lesion experiments suggests that there are many memory systems in the brain such as the hippocampus, dorsal striatum, amygdala and the medial prefrontal cortex (mPFC) among others that process information supporting dissociable forms of memory (Hirsh, 1974; McDonald & White, 1993; Milner, Corkin, & Teuber, 1968; O'Keefe & Nadel, 1978; Packard, Hirsh, & White, 1989; Sutherland & McDonald, 1990).

The Hippocampal System

The hippocampus is one of the most intensely studied brain structures and research on the hippocampus has contributed much of the fundamental information to the field of neuroscience. Yet, the nature of hippocampal contribution to behaviour is still a subject of vigorous debate. No theory of hippocampal function fully accounts for all of the current data. Two theories have been especially influential in research on hippocampal function in the past 25 years, namely the declarative memory theory and the cognitive map. The first claims that the hippocampus is involved selectively in the formation of memories for facts and events that can be recalled consciously; this theory is the declarative memory theory (Squire, 1992, 1994).

The second major theory arises from the observations during recording of single cell activity in hippocampus in freely moving rats. O'Keefe and Dostrovsky (1971) showed that single cells fired bursts of action potentials in discrete parts of an environment. A large percentage of neurons in hippocampus displays this property and was thenceforth termed place cells. O'Keefe and Nadel (1978), in part based on these results, suggested that the hippocampus forms a *cognitive map*, which is essential for spatial navigation and storage of spatial information. The cognitive map includes information about the relationships among stimuli (sometimes called *stimulus-stimulus associations)* with respect to a spatial framework (Hirsh, 1974, 1980; O'Keefe & Conway, 1978; Sutherland & Rudy, 1989; Suzuki, Augerinos, & Black, 1980). Thus, the hippocampal system was postulated to mediate *cognitive mapping* or *spatial* learning of an animal's environment.

An example of a task that provides further evidence that the hippocampal system mediates memory for spatial navigation is the Morris water task (MWT) (Morris, 1981). The original or standard version of the water task was developed to study spatial

navigation and memory but now the task is used to test other theoretical issues applying a variety of protocols. The original version of the MWT requires rats to swim from random starting points from the edge of a circular pool of opaque water to the location or *place* of a hidden submerged platform in order to escape from the water. The standard water task is designed so that a rat uses local cues which are placed around the room to navigate towards the hidden platform. Thus, the solution of this task requires rats to use spatial or *place* strategies to learn the location of the platform. Rats with lesions to the hippocampal system are impaired in the place version of the MWT (Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland, Whishaw, & Kolb, 1982). These findings are consistent with O'Keefe & Nadel's (1978) idea that the hippocampal system plays an important role in spatial navigation and memory.

The Dorsal Striatal System

Another memory system includes the dorsal striatum. This system appears to support simple *stimulus-response association* forms of learning or *habit* learning, in which a behavioural *response* in conjunction with an environmental *stimulus* is *reinforced* (Divac, 1968; Prado-Alcala, Grinberg, Arditti, Garcia, Prieto, & Brust-Carmona, 1975; Mishkin, Malamut, & Bachevalier, 1984; Mishkin & Petri, 1984; Viaud & White, 1989; Packard & McGaugh, 1992; McDonald & White, 1993; White & McDonald, 2002; White, 2004). Reinforcement strengthens an association between the stimulus and response in an incremental manner. In stimulus-response learning or *cue* learning, the strengthening of the stimulus-response association increases the probability that the stimulus will elicit the same response in the future. The striatal system is thought to be an essential neural system that acquires stimulus-response associations that support cue learning in order to solve procedural-like tasks.

A different version of the water task is called the *cue task* which is mediated by the dorsal striatum. The cue task requires rats to acquire the response of swimming directly to a visible or cued platform. The cue task provides an example of stimulus-response learning, and rats with damage to the dorsal striatum are impaired in the cue task (McDonald & White, 1994). These findings are consistent with the hypothesis that a striatal system mediates S–R habit formation (Mishkin, et al., 1984; Mishkin & Petri, 1984; Petri & Mishkin, 1994). Contrasting the effects of different lesions in the place vs. cue version of the water task highlights the idea that different memory systems are functionally distinct (Bussey, Muir, Everitt, & Robbins, 1997; Kesner, Bolland, & Dakis, 1993; White & McDonald, 1993).

1.3 Multiple Memory Systems Interact

It is clear that most experiences require integration of information across complex neural networks. There are investigations revealing extensive functional interactions between memory systems (McDonald, Devan, & Hong, 2004; Murray & Wise, 2004; Voermans, et al., 2004; White & McDonald, 2002). This evidence makes the view that there are totally dissociable memory systems with distinct functions insufficient in explaining how and in what way various memory systems interact to bring about behaviours to express learning and memory. Research investigating how memory systems interact has suggested that there are at least two particular ways that memory systems interact with one another, namely, *competitive and cooperative interactions*. These types of interactions are discussed in more detail in Chapters 6 and 7. Briefly, *competitive interactions* occur when two different memory systems process information that produce or lead to different behaviours (Chang & Gold, 2003; Hirsh, 1982; White &

McDonald, 2002), whereas *cooperative interactions* occur when the behavioural output of different systems lead to similar behaviours (Chang & Gold, 2003; Devan & White, 1999; McIntyre, Marriott, & Gold, 2003; McNay & Gold, 1998; Packard & Teather, 1998; White & McDonald, 2002).

1.4 The Picture Discrimination Task

Investigating the organization of learning and memory in the brain can be daunting, and understanding the nature of interactions between any two systems can be very difficult and complex. This complexity is due in large part because any given task may engage a variety of systems, and depending on the conditions of the task, these systems may interact cooperatively, competitively, a combination of both types, or the interaction may not have been identified as of yet. As the nature and organization of learning and memory is evidently complex, experimental task protocols must be designed in such a way that limits the number of possible learning strategies that can be used in solving the task, this would limit the number of memory systems engaged. In such a task, data interpretation can be simplified in relation to the nature of interactions. The main task used in the experiments here is the picture discrimination task because it offers specific predictions about which systems are important for task acquisition.

The Picture Discrimination Task Requires Stimulus-Response Strategies

The main task used in this thesis is a two choice picture discrimination task (Driscoll, Sutherland, Prusky, & Rudy, 2004). The picture discrimination task requires rats to make a simple discrimination between two pictures by approaching the picture that is associated with a reward. Discrimination tasks are thought to be solved based on *simple* stimulus-response learning strategies or habit memory strategies (Broadbent,

Squire, & Clark, 2007; Mishkin, et al., 1984; Packard, et al., 1989). The picture discrimination task requires rats to rely on stimulus-response associations, a learning strategy that is typically mediated by dorsal striatal processes (McDonald & White, 1993; Mishkin, et al., 1984; Mishkin & Petri, 1984; Packard & McGaugh, 1992; Viaud & White, 1989; White, 2004; White & McDonald, 2002). Most of the evidence suggests that rats do not engage hippocampus dependent memory when acquiring discrimination tasks of this type (Alvarado & Rudy, 1995; Broadbent, et al., 2007; Buffalo, Stefanacci, Squire, & Zola, 1998; Divac, Rosvold, & Szwarcbart, 1967; Fernandez-Ruiz, Wang, Aigner, & Mishkin, 2001; Sutherland, McDonald, Hill, & Rudy, 1989; Teng, Stefanacci, Squire, & Zola, 2000; Whishaw & Tomie, 1991; Zola-Morgan & Squire, 1984).

Hippocampal and Non-hippocampal Learning

Interestingly, there are a few reports showing that the picture discrimination task can be supported by both hippocampal and non-hippocampal systems. A picture in this context is a simple black and white image, design or picture that is displayed on a computer monitor. If rats are trained on such a visual discrimination before receiving hippocampal lesions, then they show retrograde amnesia during retention testing, but can rapidly and readily relearn (Driscoll, Sutherland, Prusky, & Rudy, 2004; Epp, et al., 2008; Sara, 1981; Sutherland, et al., 2001). These results show that the hippocampus may normally participate in picture discrimination learning. Another intriguing result is that relearning of the picture discrimination is just as rapid as prior to hippocampal damage suggesting that there is another system that can learn (Driscoll, Howard, Prusky, Rudy, & Sutherland, 2005; Epp, et al., 2008). This task appears to be a stimulus-response learning task, a task that should not critically involve the

hippocampus, yet rats show retrograde amnesia for picture memory after hippocampal damage and relearning occurs normally. Therefore, the hippocampal system is involved in picture discrimination memory but it is not required for normal acquisition rates. These results raise several questions that the present experiments are designed to resolve.

1.5 Which System(s) is the Non-hippocampal Memory System(s) for the Picture Discrimination Task?

This thesis examines two regions that may be critical to the non-hippocampal system, one is the dorsal striatum and the other is the mPFC. The rationale for selecting these regions is briefly discussed.

The Dorsal Striatum

The dorsal striatum is a likely structure to participate in simple discrimination learning because the picture discrimination task requires rats to rely on stimulus-response learning strategies, which is typically mediated by dorsal striatal processes (McDonald & White, 1993; Mishkin, et al., 1984; Mishkin & Petri, 1984; Packard & McGaugh, 1992; Viaud & White, 1989; White, 2004; White & McDonald, 2002). As described in section 1.4, the picture discrimination task must be learned by making stimulus-response associations or habit memory. As stimulus-response learning is mediated in large part by the dorsal striatum (see section 1.2), this system was chosen as a likely candidate for the non-hippocampal system for picture discrimination problem solving.

The mPFC

The mPFC was selected as a possible non-hippocampal system mediating picture discrimination learning due to the growing volume of evidence implicating the mPFC playing an important role in learning and memory. For example, neuroimaging studies have demonstrated activation of the prefrontal cortex in various paradigms and task variations, including recognition tasks, paired association tasks, cue-retrieval tasks and stimulus discrimination/categorization tasks (Buckner & Wheeler, 2001; D'Esposito, 2000; Fletcher & Henson, 2001; Freedman, Riesenhuber, Poggio, & Miller, 2001; Fuster, 1997; Janowsky, Kritchevsky, & Squire, 1989; Milner, Corsi, & Leonard, 1991; Vertes, 2006). Other reasons for suspecting the mPFC playing a role in picture discrimination learning as the non-hippocampal system arise from studies showing that the prefrontal cortex supports retrieval of visual stimuli during certain cognitive tasks (Buckner & Wheeler, 2001; Hasegawa, Sawaguchi, & Kubota, 1998; Koriat & Levy-Sadot, 2001; Miyashita & Hayashi, 2000; Naya, Sakai, & Miyashita, 1996; Sakai & Miyashita, 1991; Shallice & Burgess, 1991; Sidtis, Volpe, Holtzman, Wilson, & Gazzaniga, 1981; Tomita, Ohbayashi, Nakahara, Hasegawa, & Miyashita, 1999; Vertes, 2006).

The cognitive demands required for making picture discriminations have been shown to be associated with prefrontal functions. For example, this simple associative visuo-motor task involves processing visual information and associating it with an appropriate motor response. This has been shown to activate prefrontal regions. Petrides also provided imaging evidence showing that prefrontal areas are important for simple stimulus-response associations (Petrides & Milner, 1982; Petrides, 1997, 2000). In addition, orbital frontal activation is observed for the encoding of visual stimuli (Frey & Petrides, 2000). Results showing prefrontal participation for visuo-motor skill learning tasks has also been identified (Doyon, Owen, Petrides, Sziklas, & Evans, 1996). One

study gives particular validation for speculating mPFC involvement for picture discrimination learning. This imaging study (Takashima, et al., 2006) suggests that there is a close, functional interaction between the hippocampus and the mPFC for picture memory recall.

These examples are primarily gathered from imaging and clinical case studies of the prefrontal regions of primates (Fuster, 2001). The argument remains whether or not the PFC of primates is functionally homologous to the mPFC of rats. There is strong evidence suggesting that the function of these regions can be compared to functions of the primate prefrontal cortex as described in the examples above (Baddeley, 1986; Baeg, et al., 2003; Batuev, Kursina, & Shutov, 1990; Freedman, Insel, & Smith, 2000; Fuster, 2001; Goldman-Rakic, 1987; 1995; Heidbreder & Groenewegen, 2003; Hok, Save, Lenck-Santini, & Poucet, 2005; Laroche, Davis, & Jay, 2000; Orlov, Kurzina, & Shutov, 1988; Poucet, et al., 2004; Repovs & Baddeley, 2006; Seamans, Floresco, & Phillips, 1995; Vertes, 2006). Accordingly, Vertes (2006) recently stated that "the mPFC of rats appears functionally homologous to a fairly widespread region of the prefrontal/frontal cortex of primates sub-serving motor, emotional, and cognitive elements of behaviour; that is, the dorsal mPFC appears homologous to the supplementary/pre-motor area, the infralimbic cortex of rats may be functionally homologous to the orbitomedial prefrontal cortex and the prelimbic cortex (and ventral anterior cingulate cortex) homologous to the dorsolateral prefrontal cortex of primates, and that the infra/prelimbic cortex complex of rats, like the lateral/dorsolateral cortex of primates, exerts significant control over emotional and cognitive aspects of behavior."

1.6 Issues arising from Picture Discrimination Studies that this Thesis Addresses

The results from simple discrimination studies leave several questions unanswered that the experiments in this thesis address. Specifically, why do picture discriminations depend on the hippocampus in normal rats, but in the absence of the hippocampus pictures can be learned at a normal rate? The purpose of Experiment 1 is to determine if dorsal striatum or mPFC are part of the non-hippocampal system that can learn and recall the discrimination. Experiment 2 answers whether or not rats retain picture memory or if they show retrograde amnesia when the hippocampal system is removed after training? The results show that rats show retrograde amnesia after damage to the hippocampal system. Experiment 2 also addresses whether rats would be able to relearn if the non-hippocampal system is damaged when the hippocampal system is intact. Essentially these questions are related to a more fundamental one that Experiment 3 evaluates: **What is the nature of the interaction between the hippocampus and the non-hippocampal memory system on the visual discrimination task?** These questions are the focus of this thesis.

CHAPTER 2

General Experimental Design

This thesis is designed to answer questions about the interactions between memory systems underlying picture memory. First, as described in section 1.6, an essential component of the non-hippocampal system for picture discrimination must be identified. We decided to ask first whether the dorsal striatum or the mPFC is involved in an essential way in non-hippocampal learning and memory for this task. To this end, a set of three experiments was conducted using the picture discrimination task. The first two experiments investigated the identity of the non-hippocampal system. The third experiment examined the interaction between the hippocampal and the non-hippocampal system.

2.1 Experiment 1

Experiment 1 tested rats for anterograde amnesia for picture memory. Rats first received a lesion to one of the regions in question and then were trained in the picture memory task. In order to determine if either the dorsal striatum or mPFC were essential parts of the non-hippocampal system, a combined lesion of the hippocampus and the dorsal striatum or the mPFC was given to rats, 3 control groups were also included, creating 5 groups: Sham, hippocampus (HPC), striatum (str), or combined hippocampus

+ striatum (HPC + str) or hippocampus + mPFC (HPC + mPFC) lesions. All rats were then trained on a set of picture discriminations.

2.2 Experiment 2

In the second experiment, rats were tested for retrograde amnesia, that is, all groups were trained in the picture discrimination task before surgery and after damage were tested for retrograde amnesia after recovery (7 days). Subsequently rats were trained on a new picture discrimination problem. The lesions were restricted to the hippocampus, mPFC, dorsal striatum, as well as combined lesions of hippocampus + striatum, and hippocampus + mPFC, (six groups in total including sham surgeries).

2.3 Experiment 3

After identifying the dorsal striatum as an essential region in the non-hippocampal system as for picture memory, Experiment 3 investigated the nature of the interaction between the hippocampal system and the non-hippocampal system (critically involving the dorsal striatum). A useful way of testing functional connectivity and interactions between lateralized systems is to use a cross-lesion approach. Experiment 3 consisted of 2 parts, 3A and 3B. In part 3A, after rats had been trained on a picture discrimination problem, a unilateral lesion of the hippocampus was made and a unilateral lesion of the dorsal striatum was made in the ipsilateral or contralateral hemisphere. In 3B, rats were retrained on the same problem, and then they received a second surgery that damaged the remaining hippocampal tissue.

CHAPTER 3

General Materials and Methods

3.1 Subjects

Male Long-Evans rats (University of Lethbridge colony; 300-450 g) were housed in groups of two in standard laboratory cages, kept on a 12:12 light-dark cycle (lights on at 07:00), provided with food and water *ad libitum*. Environmental conditions in the rat colony room were held at a constant temperature of 21^{o}C, at 35% humidity. All rats were between 60 and 90 days old at the time of training/surgery. Every rat was treated within the guidelines of the Canadian Council on Animal Care under a protocol approved by the University of Lethbridge Animal Welfare Committee. All rats were handled before the start of each behavioural task and each experiment.

3.2 Apparatus

The visual water task (also known as the picture discrimination task) (See Prusky et al., 2000 for the apparatus details) was used to train rats on picture discriminations (Figure 3.1). The visual water task is composed of a trapezoidal shaped metal pool of water measuring 17.5 cm in depth, with a hidden platform of 14 cm in height located at one end of the pool. The end wall of the tank was transparent. On one side of the

trapezoid was a 45.7 cm long barrier with a height of 14 cm above water level, dividing the end in half to create two arms for the rat to swim into, each is half 40.6 cm in width. On each side of the barrier, displayed through the transparent wall, were two 17 inch flat CRT computer monitors on each side of the barrier showing a black and white picture, one rewarded stimulus and one unrewarded stimulus 30.5 by 30.5 cm. Each picture stimulus displayed on the monitors had a near equal amount of luminance. The software Vista 2.6.4; (http://www.cerebralmechanics.com) used to manipulate different pictures and side location was developed by Prusky et al, (2000). A representative sample of the pictures used in these experiments can be seen in Figure 3.2.

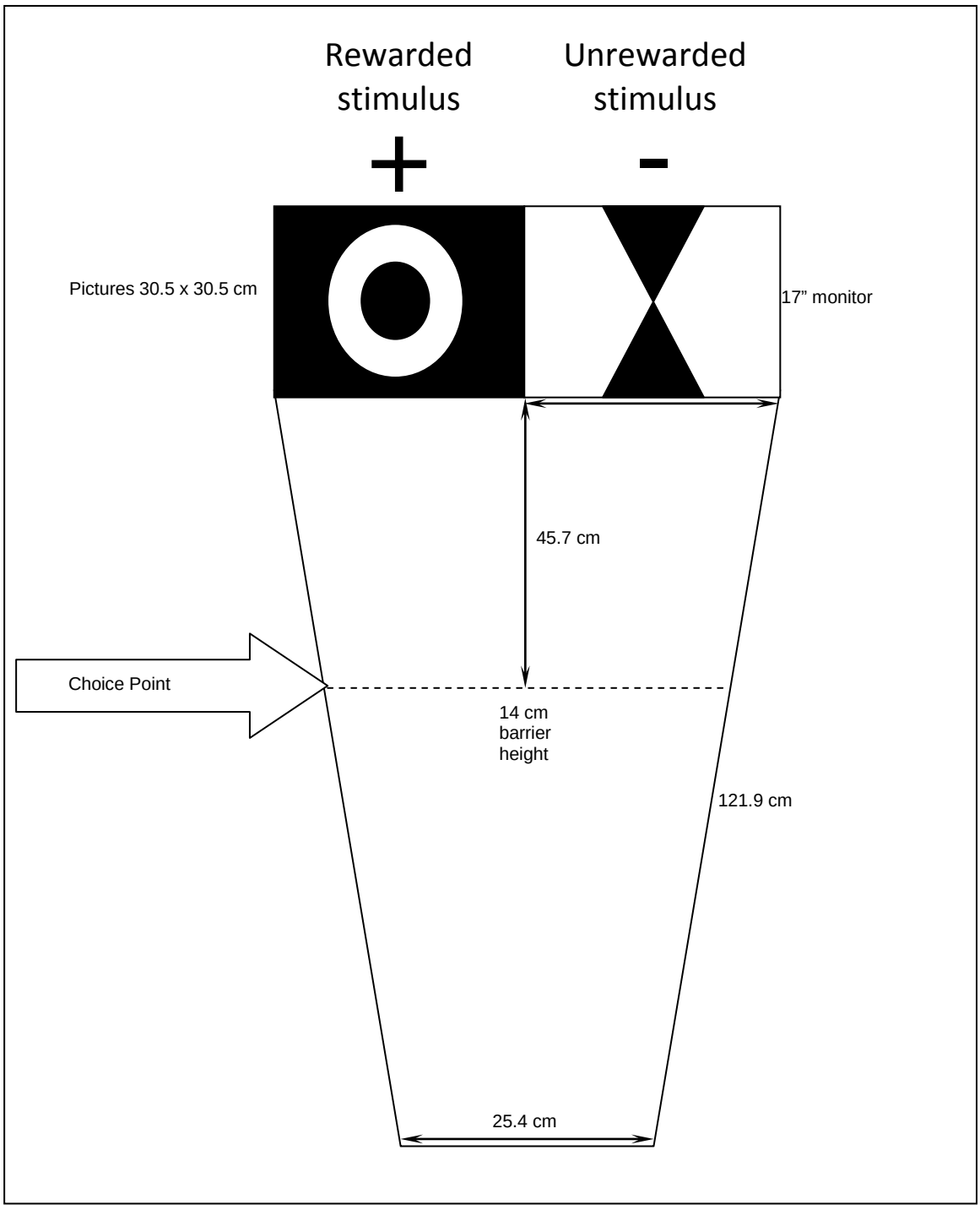

Figure 3.1. The visual water task used for the picture discriminations problems. The figure shows a top view of the trapezoidal pool. A transparent glass wall shows two flat screen computer monitors that display the pictures. A barrier is placed at the end of the pool separating the two pictures. A hidden platform is located submerged in the water corresponding to the rewarded stimuli or picture.

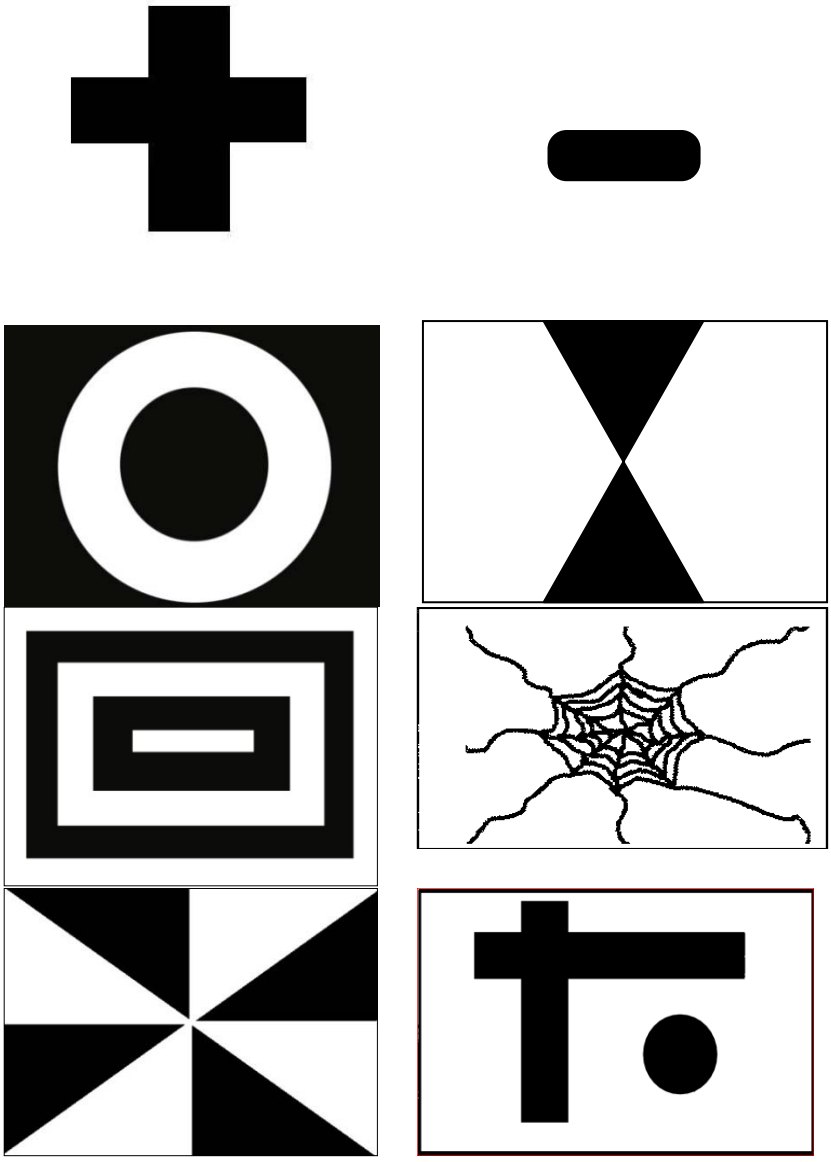

Figure 3.2. Examples of the pictures used in the visual water task are shown above. The left column contains samples of reinforced pictures; the images in the right column are representative non-reinforced pictures.

3.3 Behavioural Procedures

The picture discrimination task required rats to swim to a hidden platform that was submerged in front of the monitors that displayed the rewarded (+) picture. At the beginning of each trial, rats were released into the pool facing the wall opposite to the pictures stimuli, and allowed to swim to one of the stimuli. If the rat swam to the rewarded picture the rat reached the hidden platform to escape the water, and was returned to its holding cage. If the rat swam to the incorrect picture then, the rat was allowed to remain in the pool until it found the platform. A correct response was determined if the rat swam directly to the correct picture without entering or crossing an invisible line representing a choice point that determined an incorrect trial (See Figure 3.1). If the rat swam to the wrong picture or if its hind limbs crossed the choice point toward the incorrect picture, the trial would be judged incorrect, even if the rat had turned around before reaching the unrewarded picture. A pseudorandom pattern of the rewarded stimulus/platform side location was alternated after each trial. There was an equal amount of left-to-right variability. The dependent variable was percentage of correct choices. The inter-trial interval was approximately 3 min per rat. The pre-surgical procedure for the acquisition of a picture discrimination was to train rats at 20-30 trials per day. All rats received a single session or ten trials per day for post surgery retention/retraining of picture discriminations.

3.4 Surgery

Surgical procedures were consistent throughout each experiment although groups and extent of lesion varied slightly between experiments. Ten minutes before anaesthesia, all rats received 20 mg/kg (i.p.) diazepam (Valium) (Sabex, Boucherville, Quebec), and

were given an analgesic (buprenorphine, .05 cc; 0.3 mg/ml, i.p.; Reckitt & Colman, Richmond, VA). Rats were anaesthetized with isoflourane inhalation at 4% with 2 L/min of oxygen for 5-10 minutes, and then at 1.5-2% isofluorane for the duration of the surgery. They were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA), the skull was exposed by an incision and holes were drilled through the skull over each brain structure of interest. All infusions were done sequentially through a 30-gauge injection needle attached to a 10 μl Hamilton syringe via polyethylene tubing (PE-50). Infusion rates were ≤ 0.15 μl/min. The injection needle was left in place for an additional 3 min following each injection to facilitate diffusion. Following the lesions, the scalp incision was closed using suturing thread. If any overt signs of seizure activity were observed during surgical recovery, the rats were given additional injections of Valium. The same surgical procedures were used for the Sham rats except that no damage was done to the brain of these rats, and instead of drilled holes, bone scoring was etched across the surface of the skull. The rats were allowed to recover for a minimum of 7 days (see Table 3.1 for lesion coordinates for each structure).

Table 3.1 *Injection coordinates relative to Bregma for all lesion types*

Lesion structure	Site	Coordinates (mm)			Volume (µl)	Injection info
		AP	**ML**	**DV**		
Striatum	1	-1.6	±1.9	-5.8	0.2	30.0 mg/ml of quinolinic acid
	2	-0.5	±2.2	-6	0.2	Injection rate: 0.15 µl/min
	3	-0.8	±2.8	-4.6	0.2	Infusion time: 4.0 min
mPFC	1	4	±1.7	-4.8	0.3	10.0 mg/ml of NMDA
	2	4	±1.7	-2.8	0.3	Injection rate: 0.10 µl/min
	3	2.7	±1.7	-5.6	0.25	Infusion time: 3.0 min
	4	2.7	±1.7	-7.3	0.3	
	5	1.7	±1.7	-3.2	0.2	
Hippocampus	1	-3.1	±1.5	-3.6	0.4	7.5 mg/ml of NMDA
	2	-4.1	±3.0	-4	0.4	Injection rate: 0.15 µl/min
	3	-5	±3.0	-5.6	0.4	Infusion time: 2.5 min
	4	-5	±5.2	-7.3	0.4	
	5	-5.8	±4.4	-4.4	0.5	
	6	-5.8	±5.1	-7.5	0.5	
	7	-5.8	±5.1	-6.2	0.5	

All hippocampal lesions were damaged by 7 bilateral intracranial microinfusions of N-methyl-D-aspartic acid (NMDA) (7.5 mg/ml) dissolved in a vehicle of 0.1 mol solution phosphate buffered saline (PBS) (for Experiment 3 only, the hippocampal lesion was initially unilaterally damaged, and after subsequent testing or training, the lesion of the hippocampus was completed bilaterally). The coordinates for the hippocampal lesion were: anterior/posterior, 3.1, 4.1, 5, 5, 5.8, 5.8, and 5.8; lateral, ± 1.5, 3, 3, 5.2, 4.4, 5.1 and 5.1; and ventral 3.6, 4, 4, 7.3, 4.4, 7.5 and 6.2. A volume of 0.4 µl of solution was infused through each site. The last 3 sites in the ventral hippocampus were injected with 0.5 µl of NMDA.

The mPFC lesion group received 5 bilateral microinfusions of 0.3 µl and 0.25 µl NMDA (10 mg/ml), coordinates: anterior/posterior, 4, 4, 2.7, 2.7, and 1.7; lateral, ± 0.7; and ventral - 4.8, - 2.8, - 5.6, - 3.5, and - 3.2.

The striatal lesions consisted of 3 bilateral microinfusions of quinolinic acid (30mg/ml dissolved in PBS) (unilateral lesions only for Experiment 3) 0.2 µl per site: anterior/posterior, 1.6, 0.5, and 0.8; lateral, ± 1.9, 2.2 and 2.8; and ventral - 5.8, - 6.0, and - 4.6.

Histology

Upon completion of behavioural testing, all rats were sacrificed by receiving an overdose of sodium pentobarbital (0.7 cc; 320 mg/ml, i.p., approximately 100 mg/kg), and were perfused intracardially with 200 ml of 0.1 M PBS followed by 200 ml of 4% paraformadehyde. Their brains were excised and stored in a 4% paraformadehyde solution (PFA) for 48 hours and then allowed to be saturated in a 30% sucrose PBS solution and then sectioned via a frozen cryostat microtome protocol. Coronal slices were taken at a 40 µm thickness, and every fifth section was mounted on gelatine-

coated slides (1% gel and 0.2 % sodium azide), and stained with cresyl violet. The stained sections were examined through a light microscope (Leica, Germany) to examine the extent of the lesions (See Figure's 3.3, 3.4 and 3.5 for cresyl violet stained sections of each type of lesion).

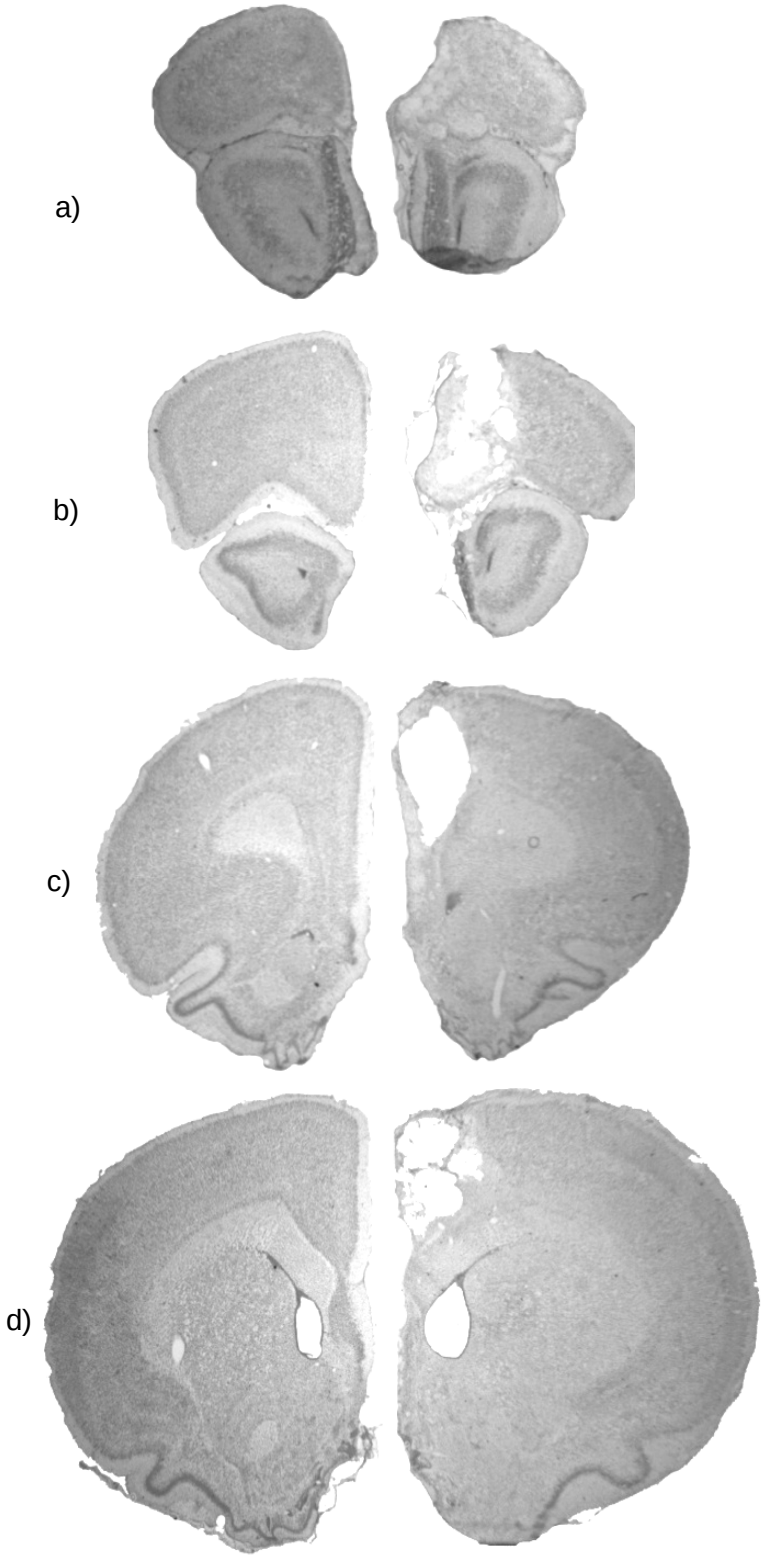

Figure 3.3. Cresyl violet sections from (a) anterior, (b) and (c) medial sections, and (d) posterior regions of the mPFC. The left hemisphere has been sectioned from a normal rat; the right hemisphere is a representative sample from a mPFC lesioned rat.

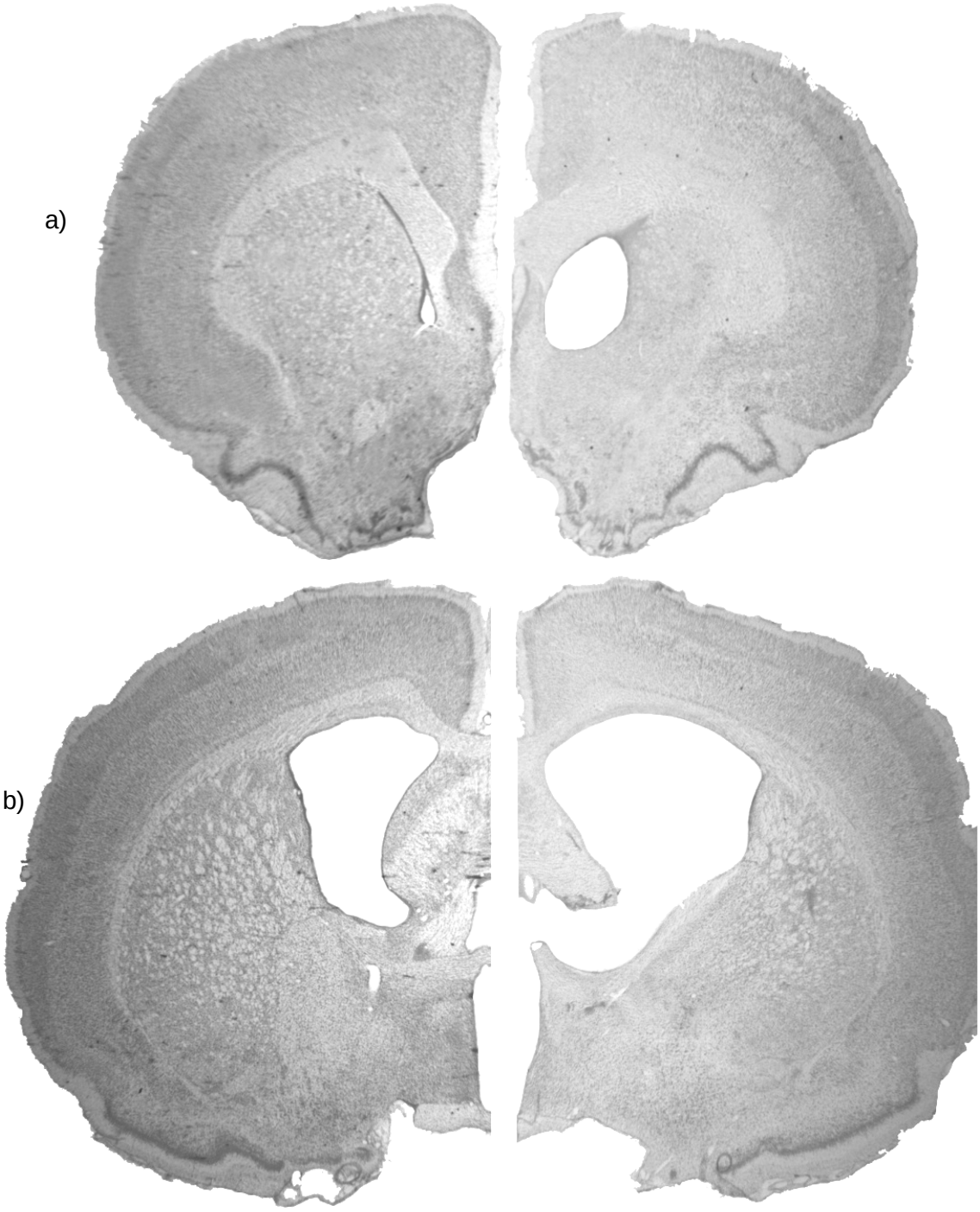

Figure 3.4. Cresyl violet sections from (a) anterior and (b) posterior regions of the dorsal striatum. The left hemisphere has been sectioned from a normal rat; the right hemisphere is a representative sample from a striatal lesioned rat.

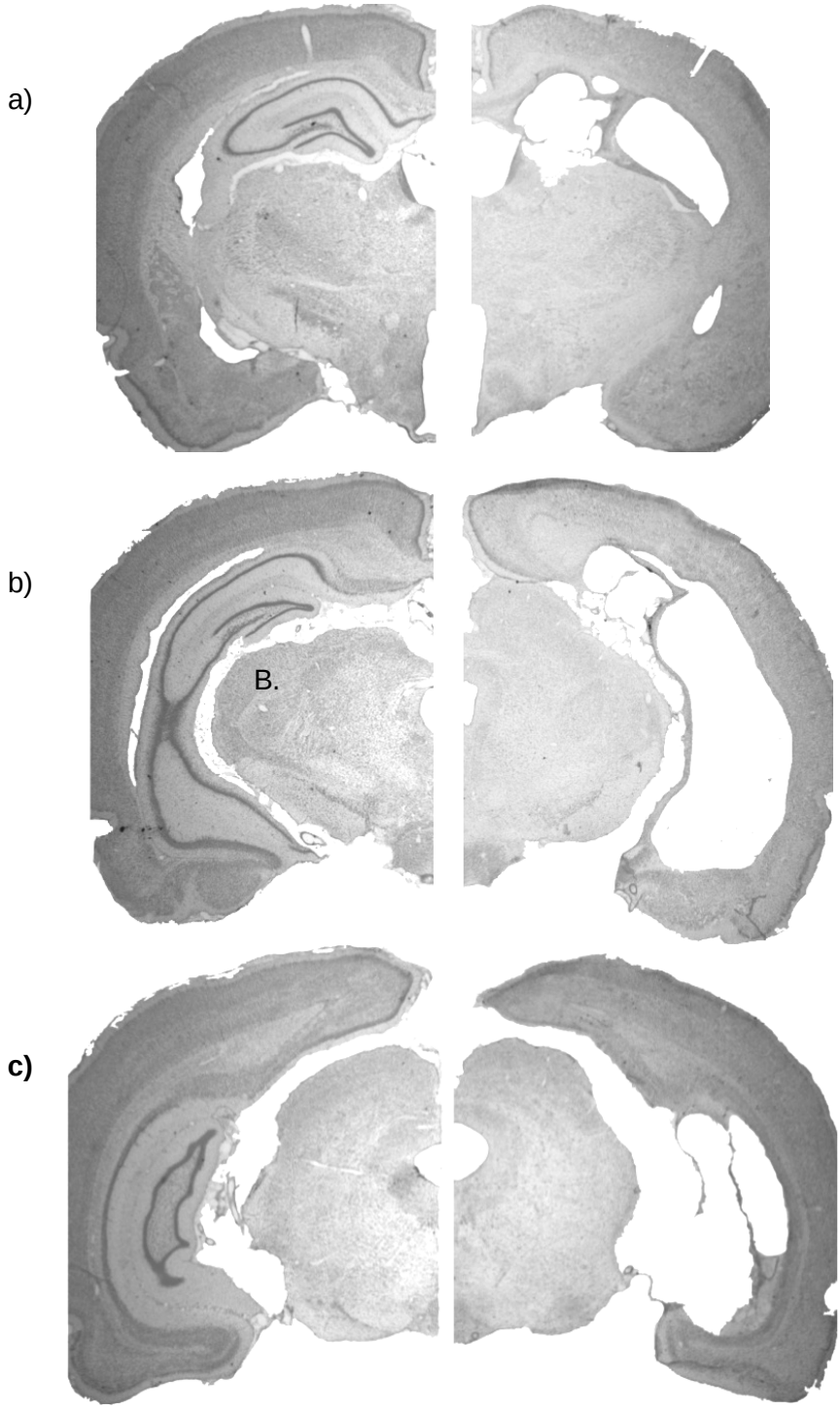

Figure 3.5. Cresyl violet sections from (a) anterior, (b) medial and (c) posterior regions of the hippocampus. The left hemisphere has been sectioned from a normal rat; the right hemisphere is a representative sample from a hippocampus lesioned rat.

3.5 Statistical Analyses

All data for the visual water task (each session was analyzed), MWT (each trial was analyzed), and the contextual fear retention was analyzed by a repeated measures analysis of variance (ANOVA) between groups, as well as the mean and standard error of each group per session/trial. Results for the MWT and contextual fear can be found in Appendices 1 and 2 respectively. An alpha level of 0.05 was used as a critical factor for significance in all instances.

CHAPTER 4

Experiment 1

Does Combined Damage of the Hippocampus and Striatum Produce Anterograde Amnesia for Picture Memories?

4.1 Introduction

A challenge in understanding the organization of learning and memory, as discussed above, is due in large part to the fact that different types of memory are mediated by different memory systems, and these memory systems may interact in different and often complex ways. Not only do different memory systems interact, but they often appear to "overlap" in function. That is, in some learning paradigms two memory systems both appear to be involved in learning and memory for a particular task. This makes the view that different memory systems are functionally distinct somewhat unclear and the lines cannot be drawn to unambiguously separate the function of one system from another. The position offered here is that multiple memory systems interact dynamically and the challenge lies in understanding the complex nature and organization of these interactions. This thesis attempts to delineate the complex

nature of learning and memory by employing a simple task that should not activate numerous memory systems in an attempt to simplify interactions.

The picture discrimination task is particularly useful because it is very simple, therefore, learning the task should not require many different memory systems to solve simple picture discriminations. An interaction between memory systems has previously been shown utilizing this task (Driscoll, et al., 2005). In this study Driscoll showed that if the hippocampus is damaged after rats learn to solve picture discriminations, rats' choice behaviour fall to chance for the familiar pictures. Significantly after hippocampal damage rats readily learn or relearn the same picture discriminations. This data suggests that there must be a non-hippocampal system that can learn, store and retrieve memories for picture discriminations in the absence of the hippocampus. **Therefore, the purpose of Experiment 1 is to identify the non-hippocampal system in picture discrimination problem solving.**

To test whether the striatum or the mPFC are essential parts of the non-hippocampal system, lesions were made prior to acquisition of the picture discrimination task. One prediction is that if either the striatum or mPFC is critical for the non-hippocampal system, then rats sustaining that lesion type, combined with a hippocampal lesion, should not be able to learn the picture discrimination or solve the problem. If the striatum is part of the non-hippocampal system then the HPC + str group will not learn, or if the mPFC is critical then the HPC + mPFC group will not learn. If both the striatum and mPFC are part of the non-hippocampal system, then neither of the combined groups will acquire the picture discrimination.

4.2 Methods

Lesions

Rats were randomly assigned to one of five lesion conditions: Sham (*n* = 6), HPC (*n* = 6), str (*n* = 6), HPC + mPFC (*n* = 6), and HPC + str (*n* = 5).

Behavioural tasks

Picture discrimination task. After a seven-day recovery period following surgery, all groups were trained on the picture discrimination for 3 sessions per day with 10 trials per session (total of 20-30 trials per day per rat). Animals were trained every day consecutively until 20 training sessions were reached (see Chapter 3 for details).

4.3 Results

Histology

Animals were included in the HPC lesion group for the statistical analysis if they received extensive damage to all of the CA fields and dentate gyrus. Three animals were excluded from the statistical analysis because the surgical procedure resulted in damage to areas outside the target regions: 1 rat from the HPC and 2 rats from the HPC + str groups had some additional damage to the subiculum. More than 85 % of the hippocampal formation was damaged. Striatal lesions included extensive cell loss and some gliosis both in medial and lateral regions of the anterior striatum, and primarily medial striatal damage in posterior regions. Damage to the mPFC consisted of extensive cell loss in the ventromedial orbital cortex, medial orbital cortex, prelimbic, and infralimbic cortex, cingulate cortex in areas 1 and 2, and secondary motor cortex in the

most posterior regions of the lesion. There was some minor sparing to the anterior cingulate.

Behavioural Results

Picture discrimination task. The picture memory learning curves for all groups after training on the visual discrimination task is illustrated in Figure 4.1. The only group that failed to reach the predetermined criterion level of 90% correct for at least 2 consecutive sessions was the HPC + str group. All other groups easily learned the discrimination without difficulty within 70-80 trials. The HPC + str lesion group did not acquire the picture discrimination (see Figures 4.1 and 4.2). A repeated measures ANOVA on performance across all training sessions revealed a main effect of session ($F(4, 25) = 39.55$, $p < 0.001$), as well as a main effect of lesion ($F(4, 25) = 7323.55$, $p < 0.001$) with a session by lesion interaction ($F(4, 25) = 4.44$, $p < 0.001$). *Post hoc* tests showed that the Sham group was significant from all groups ($p = 0.013$), the HPC and str groups did not differ significantly from one another ($p = 0.34$), the combined HPC + mPFC and HPC + str groups was significant from all other groups. A one-way ANOVA for the number of trials each group required to reach criterion revealed a main effect of group ($F(4, 25) = 21.09$, $p < 0.001$). A *Post hoc* analysis showed that the HPC and str groups did not differ significantly in the number of trials to reach criterion, when compared to the Sham group, ranging from 90 to 110 trials to reach the criterion level of performance. The combined HPC + mPFC group took 122 trials to reach criterion, which is significantly more trials to reach the criterion than the Sham group ($p = 0.02$). However, it is not significantly more trials than the HPC or str groups ($p = 0.45$ and 0.27 respectively) to reach criterion. *Post hoc* analysis of the combined HPC + str group showed that even after 200 trials they still were significantly different from all other groups ($p < 0.001$). An ANOVA comparing each group percent correct for the last

session (Figure 4.2) revealed a main effect of lesion ($F(4, 25) = 53.57$, $p < 0.001$). And *Post hoc* analyses showed that only the combined HPC + str group made significantly more errors than all other groups ($p < 0.001$).

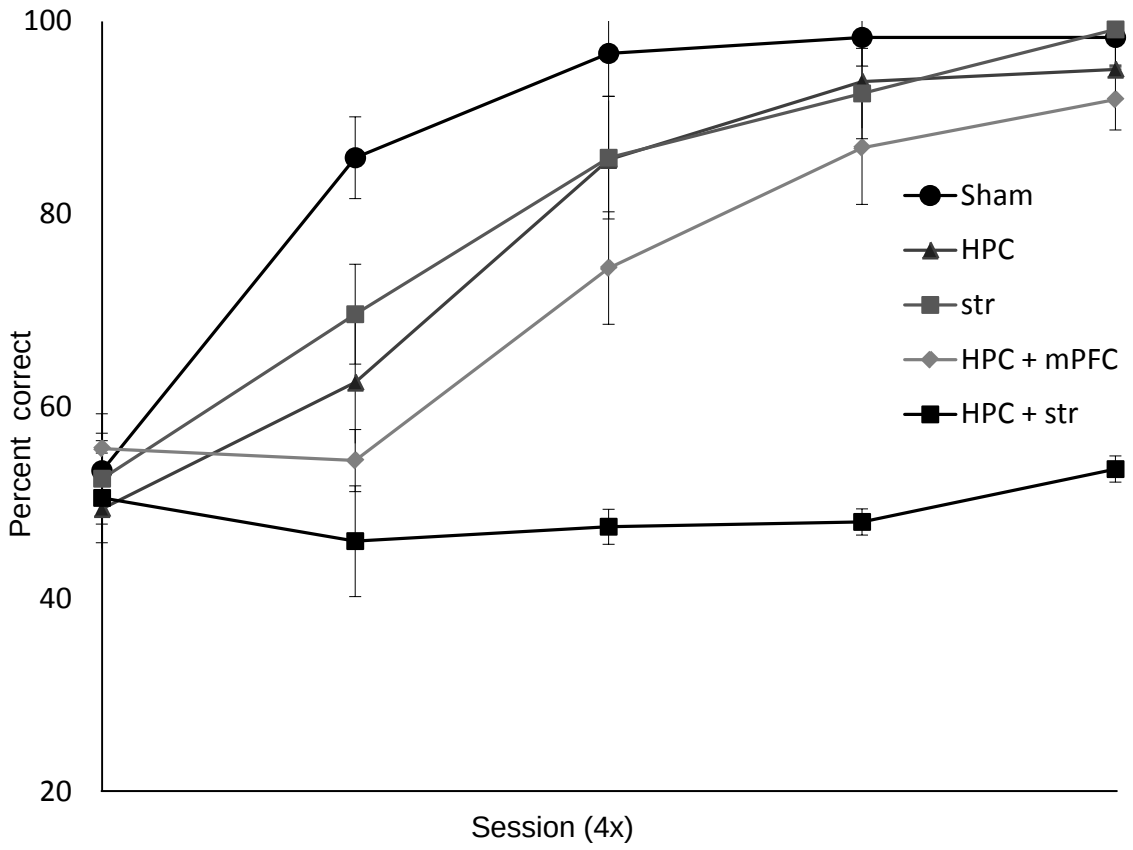

Figure 4.1. Postoperative picture discrimination task performances for Experiment 1 showing the acquisition curves for all 20 trials. Each data point represents the average percent correct for 4 sessions for a particular group. All groups were able to learn the discrimination except the HPC + str group by 200 training trials per group.

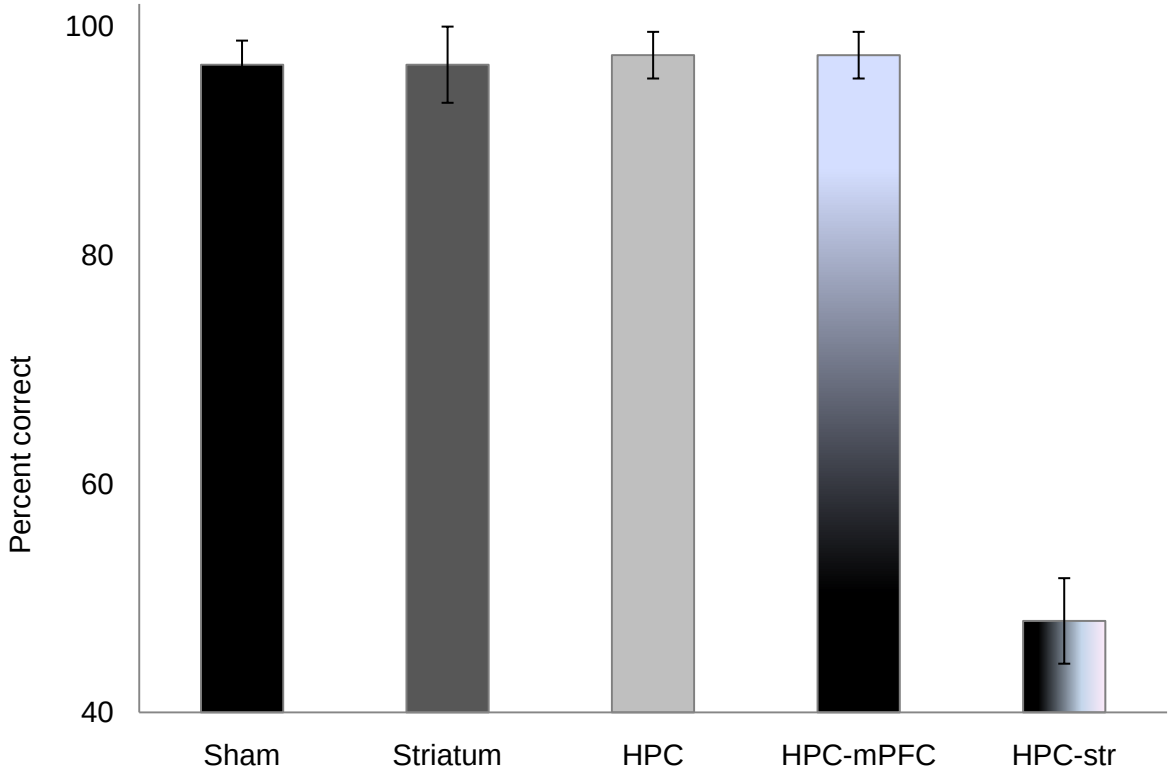

Figure 4.2. Postoperative performance for the picture discrimination task for Experiment 1 after completion of training.

4.4 Discussion

The learning curves for the picture memory problems for all but one group were very similar; all groups achieved over the 90% correct criterion level with the exception of the HPC + str group. The combined HPC + str lesion group did not learn the picture discrimination, even after 200 trials of training their performance remained at chance levels. A question posed in Experiment 1 is: does the striatum form a critical part of the non-hippocampal system? The results show that it is an essential component of the non-hippocampal system for picture discrimination learning. Neither striatal nor hippocampal damage alone produced any deficits for picture discriminations. This indicates that if one of these two structures is intact then picture discrimination learning is intact. If the striatum is damaged prior to training, and the hippocampus alone is intact during the acquisition then a system involving the hippocampus is sufficient to learn the discrimination. Similarly, if the hippocampus alone is damaged, then the striatal system can learn the pictures. If both regions are damaged, rats cannot learn the problem. Thus, the hippocampus and the striatum are conjointly necessary for resolving the picture discriminations. There is little evidence that the mPFC plays an essential role in picture memory. These conclusions point to a new hypothesis and novel prediction.

The present results suggest that in a normal rat the hippocampal and striatal systems may be conjointly necessary for retaining picture discriminations. This hypothesis makes the prediction that if rats are trained while they are intact, and if either the hippocampus or the striatum is then damaged, the remaining system should be able to support the solution to the picture discrimination problem. This hypothesis will be tested in Experiment 2.

CHAPTER 5

Experiment 2

A Combined Hippocampo-Striatal Lesion Produces Retrograde Amnesia for Picture Memories

5.1 Introduction

In Experiment 1, rats with combined hippocampal and striatal damage could not learn to resolve a picture discrimination. Rats with damage to either the hippocampus or striatum alone readily resolved the same picture discrimination. It was concluded that there are two memory systems involved in picture discrimination: 1) the hippocampus is essential to one learning and memory system and 2) the striatum is essential to the other. Either system alone can learn picture discriminations. Experiment 2 extends the findings of Experiment 1 to the retrograde direction. Here we test the idea that the two memory systems form independent records from the same experiences and that either system can support picture discrimination. On this view if normal rats learn a picture discrimination, both systems should acquire a memory that supports performance and, if either system is damaged after learning, then retrieval from the remaining intact system

should support accurate performance. In order to test this prediction, the same methods used in Experiment 1 were carried out for Experiment 2, with the exception that all rats were trained preoperatively. Additionally, if there is not a third system that can support picture discriminations then, after recovering from surgery, the combined HPC + str group should show no evidence for retention of the memory of the picture discrimination nor should they be able to relearn the discrimination. Another possibility is that one of the two systems may overshadow the other during initial learning. That is, one system may interfere with the other's ability to acquire an independent memory to support the discrimination. Driscoll et al., (2005; see also Epp et al., 2008) found that hippocampal damage alone caused retrograde amnesia for picture discrimination. Thus, an alternative hypothesis is that if both systems are intact, the hippocampal system dominates. This view would predict that hippocampal damage would be associated with severe retrograde amnesia. It has also been posited that since this type of task is a habit type discrimination, the striatal system dominates over all other systems such that if the striatal system is damaged after training then the animal will show retrograde amnesia but hippocampal damage will not produce retrograde amnesia (Broadbent, et al., 2007).

5.2 Methods

Lesions

Within 72 hours of completion of the picture discrimination task, a different combination of bilateral neurotoxic lesions was given to rats, creating six different groups of animals in all. Sham lesions (n = 6), hippocampal (n = 4), striatal (n = 6), mPFC (n = 6), a combined HPC + mPFC lesion (n = 7), and a combined HPC + str lesion (n = 6).

Behavioural Tasks

Preoperative picture discrimination training task. All rats were first trained in the visual water task to solve a picture discrimination problem. They were trained for three sessions per day until 20 sessions were completed (200 trials per rat in total).

Postoperative picture discrimination task. After a seven-day recovery period, all groups were tested for retention of the discrimination and were retrained on the picture discrimination for 1 session per day at 10 trials per session (10 trials per day per rat). Animals were trained every day consecutively until all rats could again solve the picture discrimination; excluding the combined HPC + str group who showed no learning (6 training sessions in all were given to the rats). All rats were also trained on an additional set of pictures in order to determine if they could solve a different set of simple picture discrimination problems (3 sessions per day until 20 sessions were completed).

5.3 Results

Histology

The extent of brain damage to each region was very similar to Experiment 1. Animals that were included for the damage to the hippocampal formation included complete lesions to the CA fields and dentate gyrus, and some subiculum; at least 90% of the hippocampal was damaged. All striatal lesions were dorsal lesions that included both medial and lateral cell loss from gliosis. Damage in the anterior striatum was restricted primarily to medial as well as in the posterior regions. Damage to the mPFC comprised of lesions to the ventral orbital cortex, medial orbital cortex, prelimbic, and

infralimbic cortex, cingulate cortex in areas 1 and 2, and secondary motor cortex. There was some minor anterior cingulate sparing.

Behavioural Results

The picture discrimination task. A repeated measures ANOVA across all 6 retention sessions revealed a main effect of session ($F(5, 36) = 29.44$, $p < 0.001$), a main effect of group ($F(5, 36) = 46.54$, $p < 0.001$) and session by group interaction ($F(5, 36) = 29.94$, $p = 0.028$). *Post hoc* analyses showed that the sham and mPFC groups were significantly better than the other groups (p's < 0.001), the three groups with hippocampal damage were significantly worse than the groups without hippocampal damage (p's < 0.001), and finally the str group performed significantly better than the HPC lesion group but significantly worse than the Sham group (p's < 0.03) (see Figure 5.1).

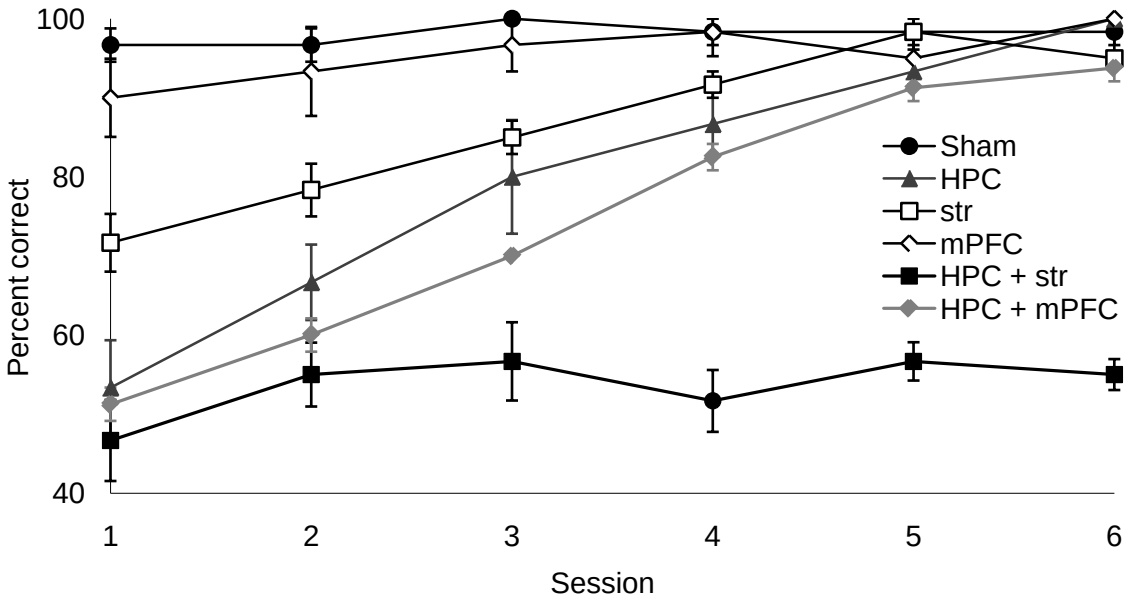

Figure 5.1. Postoperative picture discrimination retention and retraining for Experiment 2. The Sham and mPFC groups were unimpaired, the str group showed a modest, transient decrease in performance, and the HPC, and HPC + mPFC groups fell to chance levels of performance, but were able to relearn. The HPC + str group showed no retention or relearning, and remained at chance. Each point represents the average performance over 1 session.

A one-way ANOVA on the percent correct performance levels revealed that there was a main effect of lesion within the first retention session ($F(5, 36) = 20.96$, $p < 0.001$). *Post hoc* tests of the retention session revealed three different levels of performance among the groups. The first level of performance, including Sham and mPFC damaged rats showed perfect retention and significantly better than all other groups (both $p < 0.001$). The second level of performance shown by the HPC, HPC + mPFC and HPC + str groups demonstrated chance level performance and significantly worse than all other groups (all 3 groups $p < 0.001$). And finally, the str group showed a modest and transient drop in performance to 71.7% in the first session (Figure 5.1) but quickly relearned the problem to criterion level. *Post hoc* tests on the first retention session showed that the str group performed significantly different from all other groups ($p < 0.03$).

An ANOVA on the number of trials to the 90% criterion revealed a main effect of lesion ($F(5, 33) = 45.50$, $p < 0.001$). The sham and mPFC groups needed no additional training trials to achieve criterion. Post hoc analyses showed that the mPFC group did not learn significantly faster than the str group ($p = 1.000$) requiring 10 trials to reach criterion and both groups differed significantly from the groups with hippocampal damage ($p < 0.001$). The groups that received a hippocampal lesion whether alone or in combination did not differ from each other in their rates of relearning, each requiring 50-60 trials to reach criterion.

In comparing the preoperative and postoperative learning curves a repeated measures analysis of variance revealed that there was a main effect of session ($F(5, 24) = 270.94$, $p < 0.001$), a main effect of group ($F(5, 24) = 1269.69$, $p < 0.001$) as well as a main effect of session by group interaction ($F = (5, 24) = 14.87$, $p < 0.001$). These results show that the postoperative relearning occurs at a more rapid pace than initial

preoperative acquisition of the picture discrimination. A comparison of the pre and postoperative acquisition curve of the HPC group was performed using an ANOVA. The rates of learning the picture discrimination did not differ significantly between pre- and post-lesion intervals ($F(4, 2) = 3.57$, $p = 0.19$).

Picture discrimination retraining. Upon further retraining, all groups that had shown a deficit in performance relearned the discrimination without difficulty (six sessions to relearn) and their performance did not differ from sham animals. A repeated measures ANOVA showed that there was a main effect of session ($F(5, 35) = 23.37$, $p < 0.001$), and a group by session interaction ($F(5, 35) = 2.45$, $p < 0.001$). Post hoc analyses, however, showed that the combined HPC + str group was statistically significant from all other groups. This is because this was the only group that did not acquire the task (see Figure 5.2).

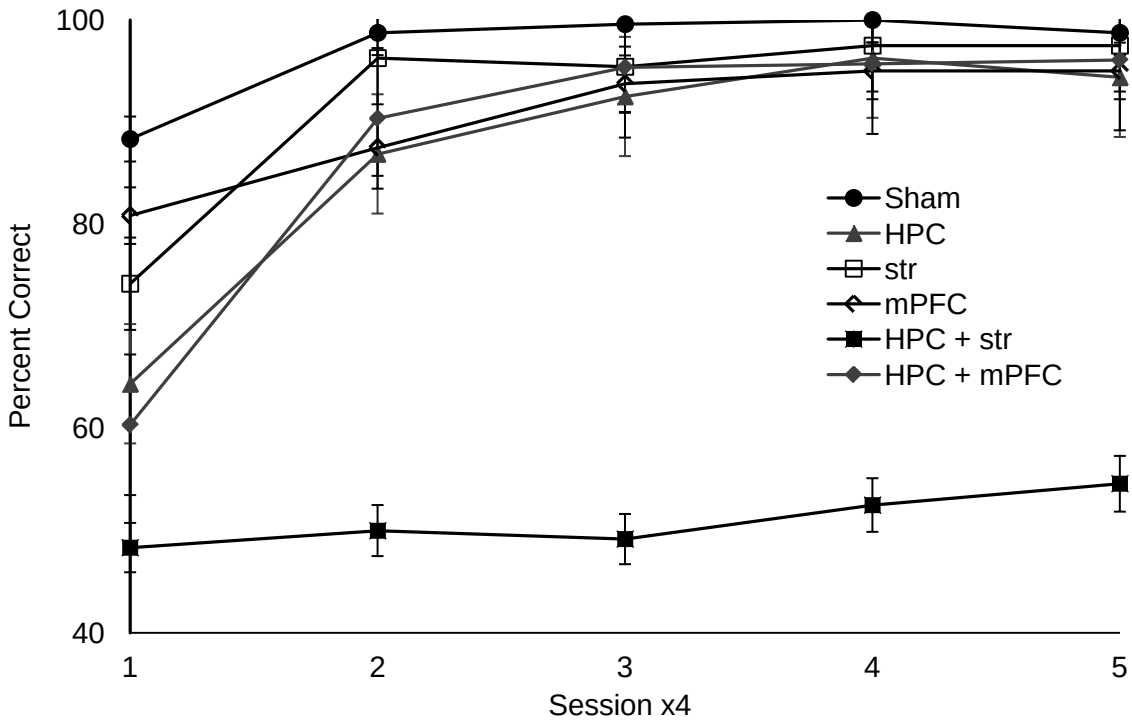

Figure 5.2. Acquisition curves for a new picture discrimination for Experiment 2. Each point represents the average for a set of 4 sessions.

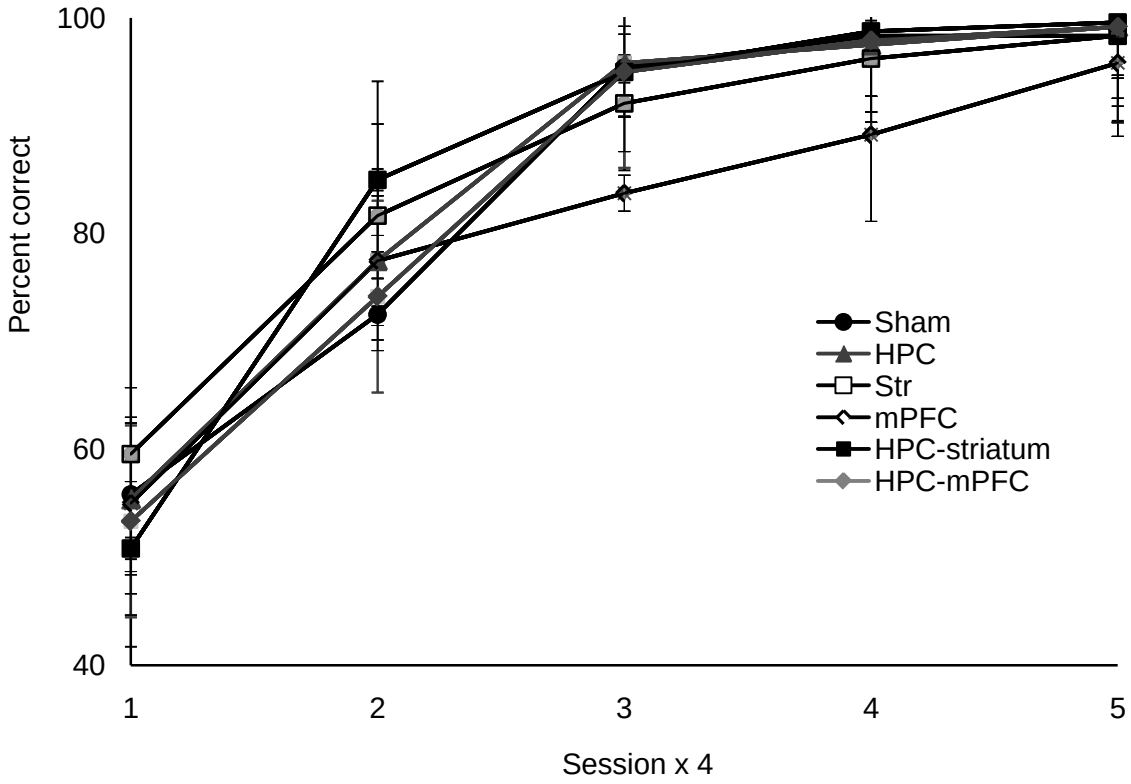

Figure 5.3. Experiment 2 preoperative acqusition picture discrimination performances. The figure shows performance by groups. This figure shows that all groups learned the discrimination at a similar rate. Each point represents the average performance over four sessions.

5.4 Discussion

The results of the present experiment show one strong similarity and some differences compared to those of Experiment 1. Rats with combined damage to the hippocampus and striatum did not retain, nor could they relearn, a simple picture discrimination. This confirms the conclusion from Experiment 1 that picture discriminations cannot be resolved unless at least one of the systems containing the hippocampus or striatum is intact. The two systems are conjointly necessary for picture discrimination learning and memory. It is likely that the outcome of processing of information in visual cortical regions directly or indirectly flows to both the hippocampus and striatum, and at some point, it can enter into associations with reinforcing events and/or responses. The fact that if either structure is intact, picture discriminations can be readily learned suggested a simple *hypothesis* (noted in the introduction of this experiment) that in the intact rat, both structures normally do participate in picture discrimination learning. It was *predicted* that if one of these structures is damaged after learning, then good discrimination performance should be supported by associations formed in the other system. The effect of striatal damage alone is consistent with that prediction. These rats showed a modest decrease in accuracy that did not last longer than 10 trials. Thus, retention performance was likely to be supported by associations formed in the system that includes the hippocampus. In striking contradiction to the prediction are the results with hippocampal damage alone. Performance by these rats dropped to a chance level and there was a protracted period of relearning. In fact, relearning took approximately the same number of trials as original learning. The outcome with hippocampal damage is consistent with the idea that a system that includes the striatum did not acquire associations that could support discrimination performance independently of the hippocampus. In Experiment 1 the mPFC does not

appear to make an important contribution to learning or retaining picture discriminations, nor does it seem to play a role in retrieving picture memories. The two experiments simply demonstrate that, if the hippocampus is present at the time of learning, then it is essential for picture memories, the hippocampus dominates over the striatum. If the hippocampus is damaged before the time of learning, then a striatal system is essential for learning picture memories. If both systems are damaged, then the rat does not learn or remember picture discriminations.

The present results are consistent with the idea that in a normal rat the hippocampal and striatal systems could independently store associations that are sufficient to support picture discriminations. This hypothesis makes the prediction that if rats are trained while they are intact, and if either the hippocampus or the striatum is then damaged, the remaining system should be able to support the solution to the picture discrimination problem. This prediction will be tested in Experiment 3.

CHAPTER 6

Experiment 3

An investigation of a hippocampal-striatal interaction using a disconnection method.

Experiment 3 addresses how the hippocampal and striatal systems might interact with one another. Experiment 3 also investigates weather the interaction is a cooperative or competitive.

6.1 Introduction

The lesion results from Experiment 1 showed that when either the hippocampus or the striatum was damaged for the picture discrimination task, the acquisition rates were similar for both groups. Experiment 1, therefore, supports the view that both the hippocampal and striatal systems contribute to the same behavioural output, in other words, for picture discriminations they interact cooperatively. However, a problem arises with the results of Experiment 2 which contradicts the simple interpretation of the results in Experiment 1. Experiment 2 shows that when the hippocampus is intact during acquisition, then it is essential for retention of the picture memories, but if the hippocampus is removed, leaving the dorsal striatum intact during acquisition then the dorsal striatum is essential for retention of the picture discriminations. When both the hippocampus and striatum are intact, however, there is little evidence that the striatum is

critical for picture learning and memory. This observation calls into question the idea that the two structures form part of a cooperatively interacting system. It is evident from Experiments 1 and 2 that from bilateral lesion studies alone we cannot determine the nature of the interaction between the hippocampus and striatum for picture discrimination. In order to probe the functional interaction between these systems alternative lesion methods, such as a cross lesion, must be applied.

Bilateral lesion studies can show similar or dissociable functions between the effects in two or more sites (i.e. the hippocampus and striatum), although bilateral damage alone does not show whether these regions are functionally interdependent. Disconnection studies or cross lesion studies, on the other hand, can identify systems that interact in concert to encode a certain type of information.

Experiment 3 used a disconnection method and was designed to investigate two main issues. First, Experiment *3A* was intended to discover if hippocampus and dorsal striatum depend upon one another for picture discrimination. To this end, all rats were trained and then received surgery. One group of rats received unilateral damage to the hippocampus and the striatum on opposite sides of the brain (Contra group). Another group received unilateral damage to these structures in only one hemisphere (Ipsi group). If the hippocampus and dorsal striatum are interdependent then retention performance by the Contra group should be significantly impaired relative to the Ipsi group. Experiment 3B further investigated the interaction between the hippocampus and the striatum (as observed in Experiment 2). All rats were retrained to criterion, and then the remaining hippocampus was removed in both the Contra and Ipsi groups. If the two systems are truly independent then the magnitude of the impairment should be equal in both the Contra and Ipsi groups. In contrast, if the hippocampus interferes with the striatum on the same side of the brain, as might be predicted from the results of

Experiment 2, only the Ipsi group should have a retention deficit despite having an identical brain injury as the Contra group during Experiment 3B.

The purpose of Experiment 3, therefore, is to elucidate the nature of the interaction(s) between the hippocampus and the striatum.

6.2 Methods

Lesions

Within 72 hours after the end of training in the picture discrimination task, unilateral neurotoxic lesions were given to rats to make three different groups of animals: a Sham group (*n* = 7), a HPC + str cross lesion group or Contra group (*n* = 8), and a unilateral, same hemisphere, HPC + str lesion group or Ipsi group (*n* = 8). For the lesion groups the side of damage was distributed between rats such that half of the groups received the hippocampal (or striatal) lesion in the left hemisphere and half in the right, etc.

Behavioural Training

All rats were trained in the visual water task to solve a picture discrimination. Rats were trained for 3 sessions per day until 20 sessions were completed (200 trials per rat in total). Animals were subsequently underwent the first surgery, and were then tested for retention. Experiment 3B: Subsequent to retention testing, rats were retrained on the problem (3 sessions per day) until they reached asymptote, 8 sessions in total. Then rats received a second surgery to complete the hippocampal lesion bilaterally after recovery rats were retested for retention. Finally, they were retrained to asymptote

taking an additional 8 trials. As a control measure, rats were trained on a new set of discrimination pictures. (See Figure 6.1 for training and lesion procedures).

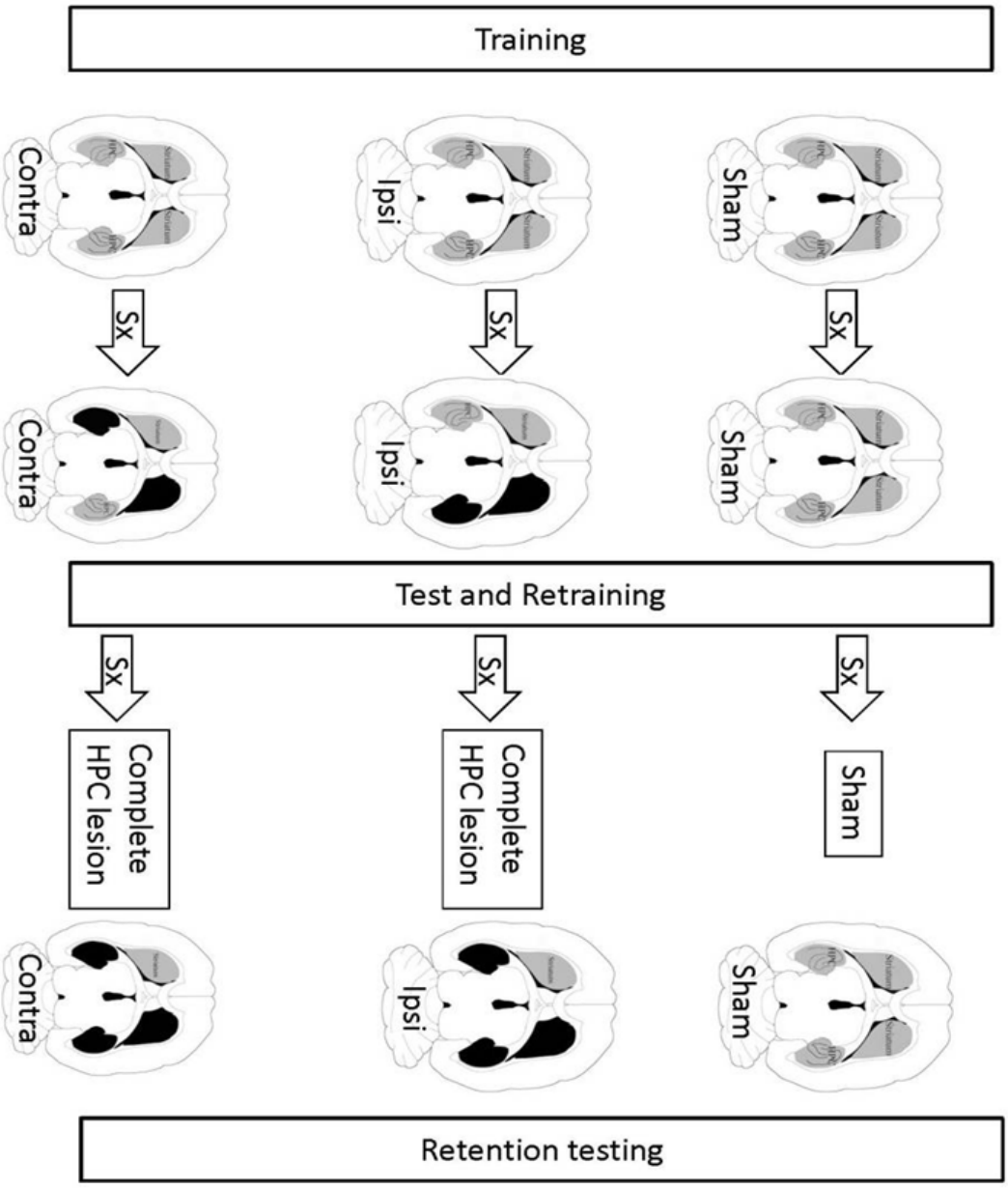

Figure 6.1. Training and lesion procedures for Experiment 3. Brain images represent horizontal slices showing intact striatal and hippocampal regions in gray and lesions in black. After training, rats received either sham or unilateral lesions to the hippocampus and dorsal striatum in either ipsilateral hemispheres or in contralateral hemispheres. The rats were then tested for retention and were re-trained to criterion levels then both lesion groups received another surgery that damaged remaining hippocampal tissue and then tested for retention once more.

6.3 Results

Histology

After the completion of behavioural testing rats were transcardially perfused with 0.1 M PBS followed by 4% PFA. Their brains were extracted, sectioned, and stained as stated above in the general methods section. The cresyl violet stained sections showed damage similar to the damage in Experiments 1 and 2 except that the striatal lesions were unilateral, while the hippocampal lesions were bilateral. The damage was limited to the hippocampal formation including almost complete damage to the CA fields and dentate gyrus, at least 85% of the hippocampal was damaged. Striatal lesions were unilateral and included both medial and lateral cell loss and more gliosis in the lateral regions of the anterior striatum, and primarily medial striatal damage in posterior regions. There were 2 rats from the Contra group and one from the Ipsi lesion group that were excluded from the data collection as the damage extended into neocortex.

Behavioural Results

Experiment 3A. Preoperative picture discrimination acquisition for Experiment 3A is shown in Figure 6.2. Preoperative performance was very similar across the groups. A one way ANOVA on the data from the first retention session showed a significant difference between groups ($F(2, 16) = 11.58$, $p = 0.001$). The sham animals showed near perfect retention while both the Contra and Ipsi groups were similarly impaired. The performance of the two lesion groups fell to 71.25% and 72.5 % respectively (Figure 6.3). *Post hoc* analysis showed that the lesion groups were not significantly different in performance from each other but that both groups were significantly different from shams animals (p's < 0.001).

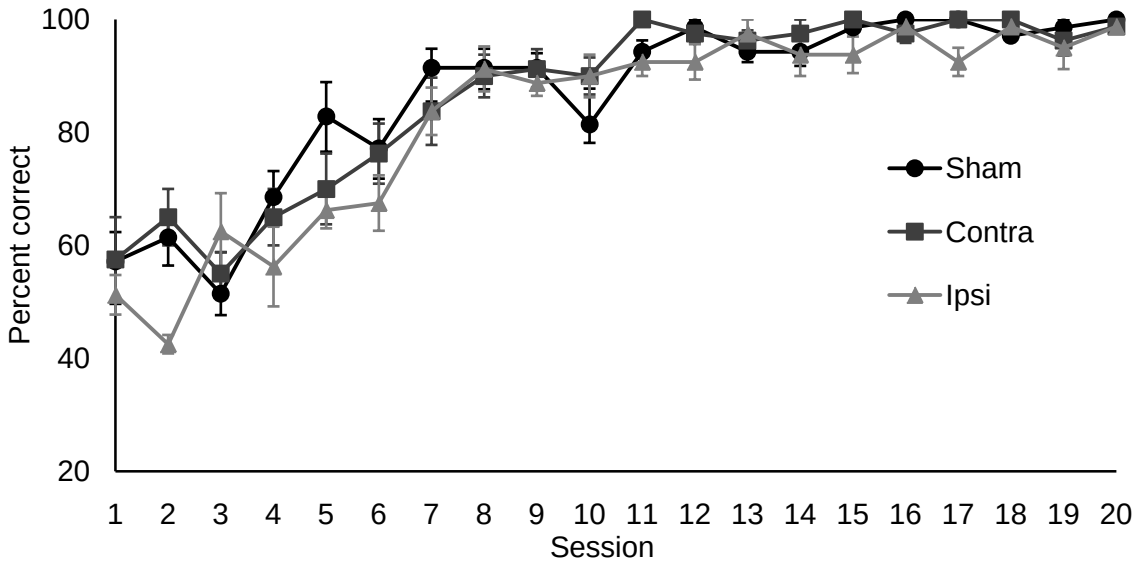

Figure 6.2. Preoperative picture discrimination training for Experiment 3A is shown above. The learning curves for all 3 groups follow normal learning rates.

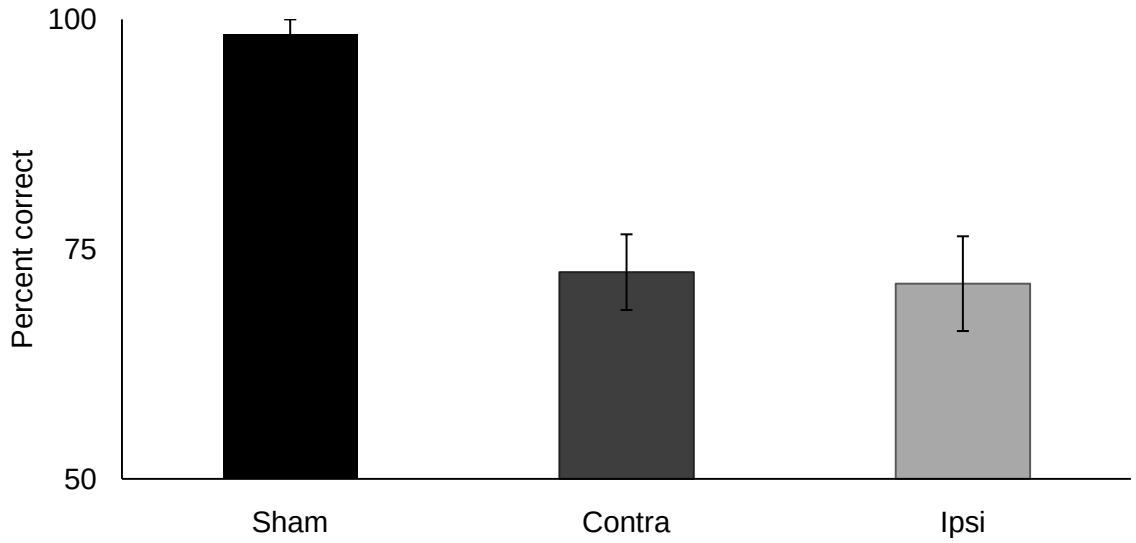

Figure 6.3. Postoperative performance for Experiment 3A is shown above. Both the Ipsi and Contra groups were impaired for the picture discrimination task.

Experiment 3B. During retraining the lesion groups readily relearned the discrimination to the 90% criterion level within 6 sessions. Their learning curves were very similar (see Figure 6.4). After recovery from the second surgery (the completed bilateral hippocampal lesion) rats were tested for retention (Shown in Figure 6.5). A one way ANOVA analysis was conducted on performance during the retention session after the second surgery and revealed a significant difference among groups ($F(2, 16) = 30.42$, $p < 0.001$). The Sham group and the Contra group showed near perfect retention of the problem and these groups did not significantly differ from each other. The Ipsi group, however, fell to chance levels at 54% correct after completion of the bilateral hippocampal lesions. Their performance was significantly poorer than either the Sham group or the Contra group ($p < 0.001$). The Ipsi group were still able to relearn the problem to the 90% criterion by 7 sessions (Figure 6.6). Training all rats on a new pair of pictures, however, took significantly longer to learn the discrimination than before the second surgery ($F(2,16) = 14.087$, $p = 0.001$) (Figure 6.7).

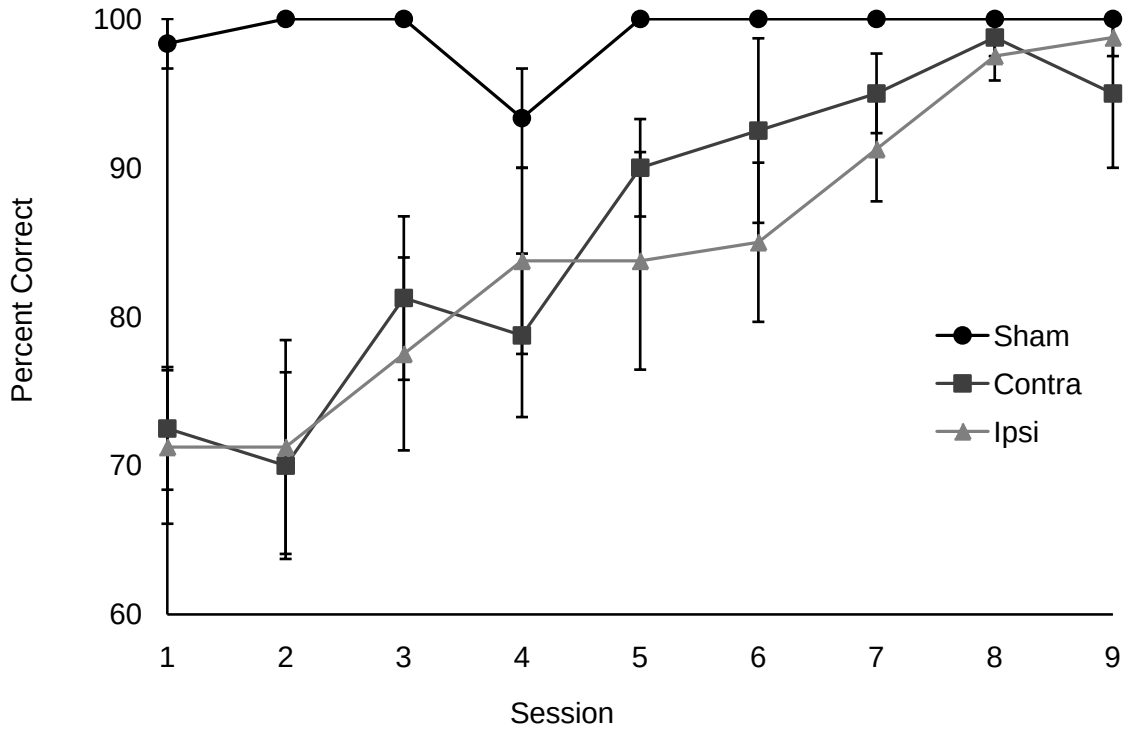

Figure 6.4. Experiment 3B retraining is shown above. Retention and relearning curves for the picture discrimination task for all groups after the first surgery. Both the Contra and Ipsi groups showed moderate retrograde amnesia but not anterograde amnesia. Rats were retrained for 7 additional sessions to relearn to the criterion level.

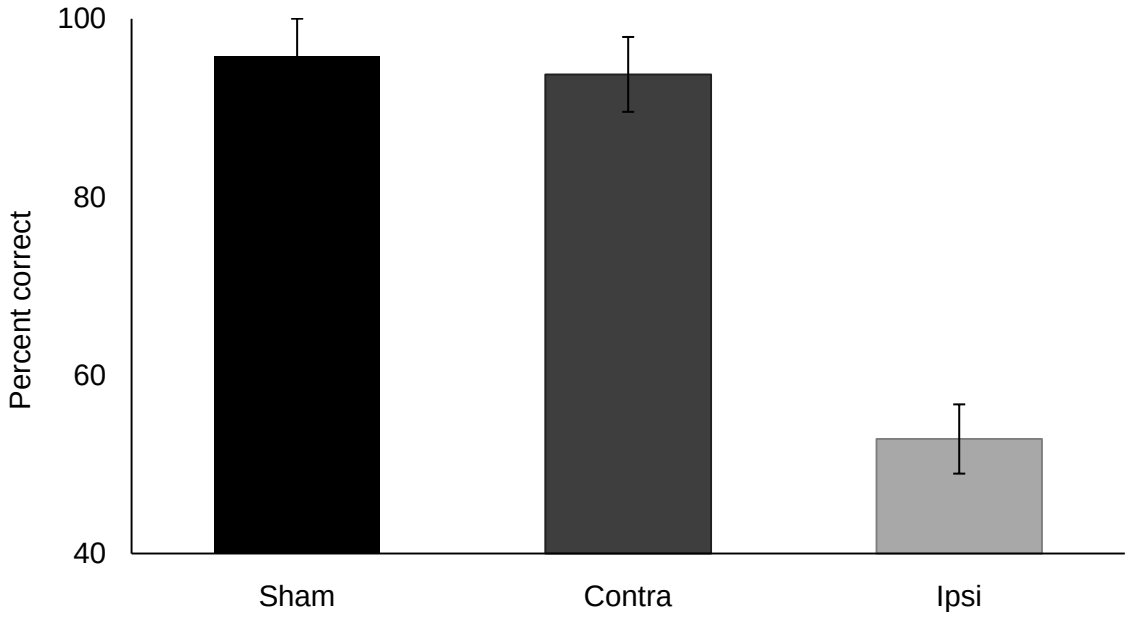

Figure 6.5. Retention performance for Experiment 3B is shown above. After completing the hippocampal lesion bilaterally, only the Ipsi group failed to recall the discrimination.

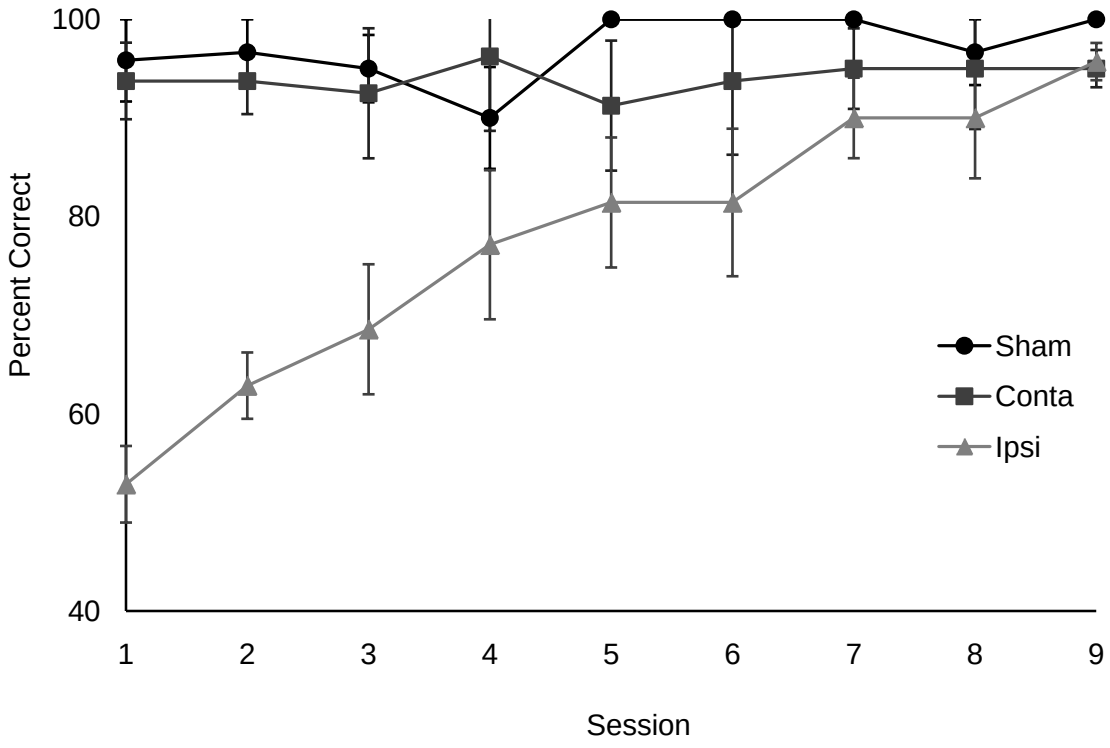

Figure 6.6. Experiment 3B retraining is shown above. Relearning curves of the picture discrimination task for all rats following the second surgery. The Ipsi lesion group showed a normal learning rate at requiring 7 sessions to relearn the problem to criterion level.

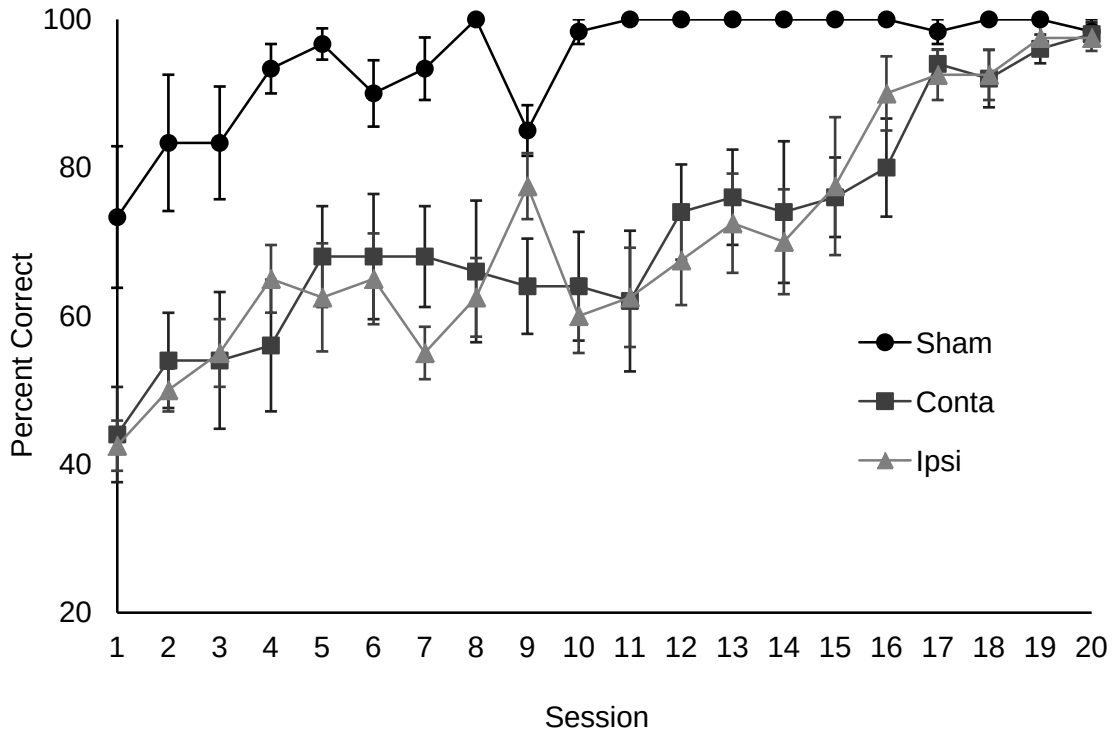

Figure 6.7. Shown above are the acquisition curves for a novel picture discrimination problem for Experiment 3B after both surgeries. Both lesion groups showed impaired learning rates.

6.4 Discussion

Experiment 3A

Experiment *3A* examined whether the hippocampus and dorsal striatum are functionally interdependent for picture discrimination by applying a disconnection method. The results indicate that the hippocampus and dorsal striatum are not functionally interdependent for the *memory* underlying picture discriminations. A note must be made here about some terminology used in describing the interaction of the hippocampus and the striatum. The term *interdependent* refers to the joint interaction of the hippocampal and striatal systems. That is, there is a *mutual* communication or interaction; both systems use the other system for normal function. Functional interdependence does not refer to one system's dependence upon the other for normal function; this is an instance of *dependence,* a one-way interaction. Thus, the result that the hippocampus and striatum are not functionally interdependent means that under normal circumstances the hippocampus and striatum do not mutually rely upon the other for normal functional output for this particular task. This conclusion is supplied by the results that showed that the Contra and Ipsi groups were equally, and only modestly impaired during retention, i.e. both groups dropped to about 72% correct. It was predicted that if the hippocampus and striatum are functionally interdependent then the Contra group should be impaired relative to the Ipsi group. The reason for this prediction is if the hippocampus and striatum are functionally interdependent, then a disconnection or Contra group will disrupt the communication between the structures. The Ipsi group, however, would have one hemisphere that is left intact so communication between the hippocampus and striatum can occur. The results present no evidence that the two systems are functionally interdependent for learning in this task. Rats with a disconnection of the hippocampus and striatum (Contra group) did not

show impaired retention performance relative to rats that did not have a disconnection or Ipsi group. Postoperative retention performance does not depend on where unilateral HPC + str lesions are made, whether the damage is confined to a single hemisphere or if the lesions are crossed. While this result reveals that the hippocampus and striatum are not functionally dependent for memory, the data do not speak as to whether there might be another form of interaction between the two systems. Indeed, the results obtained from Experiment 2 showing that the hippocampus interferes with striatal learning, indicate that there is some type of interaction. Therefore, there must be certain forms of interactions that permit the hippocampus to overshadow the striatum during learning of this task. Hippocampal overshadowing is addressed in the discussion for Experiment 3B below.

Experiment 3B

If the hippocampus interferes with learning by the ipsilateral striatum, then after the bilateral hippocampal surgery only the Ipsi group should be impaired while the Contra group should be unimpaired. This prediction, formulated based upon the interpretation of the findings of Experiment 2, was confirmed. Despite having the same damage, the Ipsi group's retention performance fell to chance performance levels while the Contra group performed at a very high level. This reveals an important aspect of the interaction during learning of this task. The hippocampus effectively blocks the ipsilateral the striatum from acquiring an independent memory to support the picture discrimination. This result is conclusive evidence that at the time of training, striatal acquisition depends critically on the integrity of the ipsilateral hippocampus only and not on the contralateral hippocampus. A hemisphere with hippocampal damage, can acquire a memory that is sufficient to support picture discrimination and we know that the dorsal striatum in that same hemisphere is involved. This result shows that for

picture discrimination memories, the hippocampus exerts a *within-hemisphere* interference or inhibition over the striatum. In view of the findings of Experiment 2 and from other studies it is suggested that the hippocampus is a system that inhibits a non-hippocampal system (Douglas, 1967; Driscoll, et al., 2005; Ferbinteanu & McDonald, 2001; Holland, 1999; Honey & Good, 1993; Kaye & Pearce, 1987; McDonald, et al., 2004; McDonald & White, 1993; 1994; 1995; Packard, et al., 1989; Rudy, Huff, & Matus-Amat, 2004; Sutherland & McDonald, 1990; White & McDonald, 2002). After the first set of lesions was made (Contra and Ipsi lesions), all rats could relearn the problem. For the Contra group, the re-acquisition of the picture discrimination must have used both the existing hippocampus in one hemisphere, and in the opposite hemisphere, the striatum was free to learn without the interference or inhibition of the hippocampus. The two different systems in opposite hemispheres acquired the picture discrimination. When the Contra group received the second surgery removing the remaining hippocampus, the remaining striatum still had a memory sufficient to guide the appropriate choice behaviour to near perfect retention because information processing was not inhibited by the hippocampus during re-acquisition. Learning picture discrimination after receiving a disconnection lesion allows both systems to acquire and retain the memory for the problem. The result that a complete hippocampal lesion did not impair performance in a simple picture discrimination task in the retrograde direction may be a unique finding to date.

The Ipsi group acquired the discrimination with the intact hippocampus, but not the intact striatum. The intact striatum was located in the same hemisphere as the intact hippocampus; therefore, during re-acquisition, encoding in the striatal system was compromised by the hippocampal system. When the second surgery removed the remaining hippocampus, the striatum was not able to participate in effective retrieval of

the picture discrimination thus, the Ipsi group performed at chance levels. In other words, the hippocampus *overshadows* or interferes with the dorsal striatum from learning picture discriminations.

The term “overshadow” coined by Kamin (Kamin, 1968), is typically used in classical conditioning in which only one of two redundant conditioned stimuli acquires control over conditioned responding. The term overshadowing used in the context of interacting systems, however, can be referred to as “inhibition” or “interference” of one system by another.

In summary, this experiment addressed how the hippocampus and dorsal striatum interact with each other to solve picture discriminations. The results indicate that the hippocampus does interact with the striatum by interfering with the striatum during the acquisition of the picture memories, such that the dorsal striatum cannot acquire a memory for picture discriminations. It is proposed that the hippocampus provides an independent representation of the solution and inhibits learning that could be supported by a system that does not depend on the hippocampus (that is, the dorsal striatum). This interaction appears to be mediated via a direct or indirect interaction from the hippocampal formation to the ipsilateral dorsal striatum (Swanson & Kohler, 1986).

CHAPTER 7

General Discussion

This general discussion reviews the conclusions of each experiment and considers their implications for mainstream theories of memory.

7.1 Experiment 1: An Anterograde Analysis

Simple Picture Discrimination

Experiment 1 assessed the effects of hippocampal, striatal, and mPFC lesions in rats that were postoperatively trained in a picture discrimination task. The results revealed a simple outcome. Only the rats with a combined HPC + str lesion did not learn the picture discriminations. All other groups were able to learn the problem within 80 trials, whereas the combined HPC + str lesion group did not perform better than chance levels even after training 200 trials. This outcome is consistent with the idea that combined damage to the hippocampus and dorsal striatum causes severe anterograde amnesia. It is important to note that both the HPC and str groups displayed similar learning rates, because it indicates that both systems are equally efficient at learning the task. Thus, neither hippocampus nor dorsal striatum alone are essential for picture discrimination learning and both structures are conjointly necessary for these problems. Taken together, the picture discrimination task results suggest 2 hypotheses:

1) There are 2 systems that can mediate picture discrimination. One involves the hippocampus and the other includes the striatum.

2) Both the hippocampal and the striatal systems acquire the discrimination at the same rate

These hypotheses contradict theories claiming that certain types of learning and memory are limited to particular independent memory systems are an oversimplification. Rather, the data support the idea that learning and memory can be mediated by more than one system which can provide a dynamic interaction among different systems. The idea that learning and memory are mediated by a dynamic interaction of plastic systems offers flexibility for encoding, storage, retrieval or otherwise processing of information.

7.2 Experiment 2: A Retrograde Analysis

Picture Discrimination

Rats in Experiment 2 were preoperatively trained on the picture discrimination task for 20 sessions; all rats were able to learn to solve the discrimination to a similar level (see Figure 5.3). During retention testing after surgery, all three groups that received hippocampal damage performed at chance levels, while the other three groups without hippocampal damage showed good retention. Although they failed to remember the pictures after surgery, the HPC and HPC + mPFC groups readily relearned the pictures (6 sessions). The HPC + str group, however, not only fell to a chance level in the retention test, but unlike the HPC and combined HPC + mPFC groups, the HPC + str group was unable to relearn the discrimination. Also, a modest, transient decline in

retention performance can be seen in the str group after their surgery but they rapidly relearned the discrimination to the criterion level after 4 sessions. The results in Experiment 1 showed that picture discrimination can be acquired by the hippocampal or striatal systems, since the str group in Experiment 2 had damage to the striatal system, the spared hippocampus must have mediated the discrimination acquisition. .

These results, taken together with those of Experiment 1, show that in order to solve picture discrimination, rats must have at least the hippocampus or the dorsal striatum intact. If both the hippocampus and the striatum are intact at the time of acquisition then the hippocampus performs an essential role in supporting the discrimination. Thus the hippocampus must interfere with or overshadow learning by other networks that interact with the striatum. This contradicts the idea that the hippocampal and striatal systems process information in parallel. If, however, the hippocampus is damaged during learning then striatal networks will learn to solve the picture discrimination readily. The two systems are conjointly necessary for picture discrimination learning and memory.

Experiment 1 showed that damage to mPFC alone or in combination with the hippocampus did not prevent rats from acquiring the picture discrimination. Likewise, there did not appear to be any contribution of mPFC to retrograde amnesia for the same discrimination. It is unlikely that the mPFC significantly contributes to picture discrimination learning or memory. The combined HPC + mPFC group also did not differ from the HPC group. The mPFC, therefore, can be ruled out as part of an essential system for acquisition, storage, or retrieval of picture memories.

7.3 Experiment 3: A Systems Disconnection and Interaction Analysis

Experiment *3A* investigated the nature of the interaction between the hippocampus and the striatum in solving picture discriminations. Specifically, the experiment sought to determine if they make interdependent contributions to picture discrimination memory. Taking advantage of the almost totally ipsilateral interconnections between these structures (Swanson & Kohler, 1986) combined unilateral hippocampal and striatal damage, either as a crossed lesion or Contra group or as lesions within the same hemisphere or Ipsi group was made in rats that had learned the picture discriminations. If the hippocampal and striatal systems acquired discriminations with interdependent interactions, then rats with damage to part of that circuit (Contra group) would produce greater deficits than rats with the hippocampus and striatum left intact in one hemisphere (Ipsi group). But if the hippocampus and striatum are not functionally interdependent for picture discrimination, then the groups should show no differences in performance because both groups have equal damage to both systems. The retention test showed that performance deficits were equal for both groups. The drop in performance of the Ipsi group was no different than that of the Contra group. This result is consistent with the idea that the hippocampus and dorsal striatum are not functionally interdependent for picture discrimination.

Experiment 3B explored further the *nature* and *type* of the hippocampal-striatal interaction. All of the rats from Experiment 3A were retrained in the picture discrimination and both lesion groups received hippocampal damage bilaterally and were then re-tested. Only the Ipsi group showed a deficit. As a disconnection of the hippocampus and dorsal striatum in Experiment 3A showed that the systems are not functionally interdependent for picture discrimination acquisition, the results from Experiment 3B demonstrated some important conclusions about an interaction. The

hippocampus interferes with the ipsilateral striatum acquiring a memory that can support the picture discrimination. Relearning in the Contra group must have occurred in at least the striatal system because removing the contralateral hippocampus did not affect retention. In contrast, removal of the hippocampus in the Ipsi group produced large retention deficits suggesting that acquisition must have been mediated by the hippocampus that blocked striatal acquisition.

Caveat. Given that lesions did not damage any commissural tracts, the possibility remains that there may have been communication from one hemisphere to the next. The Contra and Ipsi groups may have showed the same performance in Experiment *3A* because the striatum of the Contra group may have received information from the hippocampus in the contralateral hemisphere. The hippocampus and the dorso-medial striatum have direct connections where information can be processed and passed to the systems where an interaction can take place (Kohler, 1984, 1985; Kohler, Shipley, Srebro, & Harkmark, 1978; Shipley & Sorensen, 1975; Sorensen & Witter, 1983; Swanson & Kohler, 1986) and indirect (Hjorth-Simonsen, 1973; Rosene & Van Hoesen, 1977; Shipley & Sorensen, 1975; Sorensen & Shipley, 1979; Swanson & Cowan, 1977). Further discussion of indirect routes is addressed in the general discussion section. The direct connection of the hippocampus and dorsal striatum have few, if any, cross connections that project from one hemisphere to another (Swanson & Kohler, 1986). If the interaction is taking place via a direct route then results are not likely to be due to any cross connections. Any information projecting via indirect routes, however, can cross hemispheres in any a number of different areas. The data from Experiment 3B shows that the interference from the hippocampus on the striatum is a within-hemisphere effect. This suggests that the interaction is taking place via the direct route. These results, however, cannot account for crossing of any interactions that may

occur during postoperative memory of the picture discrimination. It is more parsimonious to interpret that learning interactions and postoperative memory interactions (if any) is used by the direct route that contains very few cross connections. In order to determine the mechanisms for these interactions future studies using temporary lesions (by inactivation techniques) would be useful. For instance, if the hippocampus is inactivated at the time of learning and then reactivated during retention, would the hippocampus interfere with expression of the memory that was acquired by the striatum? It could be that the hippocampus only interferes with the striatum at the time of learning.

In summary, the results of Experiment 3 showed that in a normal rat, picture discrimination acquisition does not depend on a functional interdependent circuit involving the hippocampus and striatum. There is, however, a competitive interaction where the hippocampus overshadows the striatum by blocking acquisition. The hippocampal interference with striatal learning is dependent on the *ipsilateral hippocampal-striatal connections*. The results confirm that the hippocampus is interfering with or overshadowing the non-hippocampal or striatal system within the same hemisphere during the acquisition of picture discriminations.

7.4 What do the Data and/or Results from each Experiment Suggest?

In every experiment under investigation here, there is one important finding that is consistent among all results. That is that there is more than one system that can mediate learning and memory for pictures. There is a hippocampal system and a non-hippocampal system for which the dorsal striatum play a critical role. In the picture discrimination task, a non-hippocampal system includes the dorsal striatum. There is

evidence showing that the hippocampus interacts competitively with the striatal system. The hippocampus interferes with the striatal system from acquiring an independently retrievable memory to support the discrimination.

For the picture discrimination task the interaction between the hippocampal and striatal system in each experiment can be summarized as follows: Acquisition does not depend on a functional interdependent circuit of the hippocampus and striatum. However, there is a competitive interaction suggesting that the hippocampus interferes with striatal acquisition of the task.

Taken together, the evidence suggests that the hippocampus engages in an important interaction with other memory systems which may or may not be the same system for each learning task. The interaction can be cooperative or competitive depending on the task the nature of the information to be processed. Experiment 3 is especially instrumental in demonstrating the subtleties that can exist for learning and memory among multiple memory systems. The results of Experiment 3 shows that acquisition in different memory systems does not always occur in parallel and that one system can overshadow or interfere with information processing in other memory systems. The results of this thesis are important because they show that there are major differences in the way memory systems can interact depending on the nature of what is learned. They highlight the differences and commonalities that can occur between memory systems and that the nature of learning and memory and its organization in the brain is not always as strait forward as many theories suggest. Understanding the differences in of these interactions can be of vital importance in the interpretation of experimental results. Erroneous conclusions gathered from experimental results can be avoided with a correct knowledge of the subtle ways systems can interact. It is evident that a proper understanding of memory systems and

their interaction(s) during learning is critical for any investigation of learning and memory. How these results here fit into the different theories of memory are the next topic of discussion

7.5 Implications for Contemporary Theories of Learning and Memory

The Declarative Memory Theory

Perhaps the most prevalent and popular theory on memory among researchers is the *declarative memory theory* of amnesia. In the early 1980's Squire and Cohen presented the declarative memory theory as an explanation of the deficits seen in human cases of amnesia (Cohen & Squire, 1980; Squire, 1992; Squire & Butters, 1984; Squire, Stark, & Clark, 2004; Squire & Zola-Morgan, 1991). This theory contains 4 key propositions, 3 of which may be in conflict with the results of this thesis. The first proposition is that the fundamental function of the hippocampus is in memory. Second, the hippocampus is selective, that is, it only mediates the memory of *facts* and *events,* or *semantic* and *episodic* memory respectively. Together, semantic and episodic types of memory are termed "declarative memory". This type memory can only be consciously declared. Non-declarative memory, such as procedural memory, is hypothesized to be mediated by non-hippocampal systems, involving, for example, the striatum or neocortex. The third proposition identifies the hippocampus as one of a number of structures that comprise the MTL. The MTL system mediates the formation and early storage of declarative memories, although, each component may have a separate sub-function. The fourth key proposition distinguishes the hippocampus as having a time-limited role in memory. After the formation of a memory, the hippocampus participates in a consolidation process whereby the memory trace is permanently stored in the

neocortex, and the hippocampus is no longer required or involved in recall of the memory.

Problems with the declarative memory theory arise when it is applied to non-human animals, because declarative memories must be consciously declared. It may never be determined whether animals are *conscious* of their memories, however, there are many studies that show that animals can *remember facts* (e.g., that a certain food is safe to eat, or that approaching a certain picture results in a reward) *and events* (e.g., that an initially novel object or picture has been seen before). The jury is still out, however, as to the extent to which animals are *conscious or aware* during encoding and recall of an event (Cowey & Stoerig, 1995; Kao, Davis, & Gabrieli, 2005; Sole, Shettleworth, & Bennett, 2003; Weiskrantz, 1997).

In consideration of the picture discrimination task within the declarative memory theory, it should be identified whether the task is declarative or non-declarative. It may be argued that learning a simple discrimination can be considered semantic or factual (e.g., approaching picture "A" always leads to a reward), in which case this type of memory falls into the declarative category, the task therefore, must be dependent on the hippocampus. The problem with this designation is the difficulty in determining whether a rat is *conscious or aware* of its memories. If rats are not conscious or aware of the memories of the picture discrimination task then the task falls into the non-declarative category, and the task is not hippocampal dependent. Indeed, it is very possible that the task is procedural in nature, and would, according to the declarative memory theory, be a striatal dependent task.

The results of the present experiments are not easily accommodated within the main propositions of the declarative memory theory, whether the memory mediating the

task is classified as declarative or non-declarative. A subset of the results support this theory, for instance, the task is only dependent on the hippocampus in the retrograde direction, and not necessarily in the anterograde direction. This is because rats can learn the task with the dorsal striatum without the hippocampus, thus the memory mediating this task cannot be declarative according to the declarative theory. Moreover, the postulate that memories are consolidated in the neocortex is also insufficient to accommodate the data because the striatum is part of neither the MTL, nor the neocortex. The mPFC, however, was shown not to contribute significantly to this task. There is no evidence in these experiments showing that the neocortex makes a significant contribution to the task. These experiments, however, were not designed to assess consolidation nor temporal gradients in retrograde amnesia. The present findings are insufficient to adequately determine neocortical contributions and further research is required.

Recently there has been evidence that discrimination learning and memory is dependent upon the striatal system more than the hippocampal system, and therefore the task must be a procedural task (Broadbent, et al., 2007). In this study rats were trained on a pattern discrimination and given either a hippocampal lesion or a dorsal striatal lesion. Although hippocampal rats showed a significant deficit compared to controls, the str group were more impaired than the HPC group. The interpretation of this result was that rats rely on the dorsal striatum more than the hippocampus for discrimination learning. This evidence is in contrast to the idea that the hippocampus overshadows the non-hippocampal system. However, there are several reasons that the results of the Broadbent et al. study cannot be compared to the results of this thesis. First, the apparatus and procedure of their task is very different from the one used here. Instead of rats learning though negative reinforcement by associating the picture with

escape from water, Broadbent et al. (2007) used a dry land Plexiglas runway that used food pellets as reinforcement rewards. These tasks differ in the way the rat learns. These different ways of learning may activate different memory systems and may not be comparable. Next, the pictures used for discrimination are also fundamentally different. Broadbent used patterns of either vertical or horizontal stripes painted on blocks that measured 5.9 cm by 5.9 cm, whereas the pictures used here were displayed on a monitor and measured 30.5 by 30.5 cm. In addition, the pictures used in these experiments differed from one another far more than stripes that differed only in orientation. These differences in the Broadbent et al study allow for a far more difficult discrimination than the ones used here, which also may account for the difference in results. Their rats took over 600 trials for rats with striatal or hippocampal damage to learn at 60% correct, whereas the str or HPC groups took less than 80 trials to reach 60% and by 150 trials both groups learned the discrimination to over 90% correct. The difference in results may also be attributed to the fact that their lesion sizes were larger and were performed with radiofrequency probes. Radio frequency lesions destroy fibres of passage whereas the neurotoxic lesions of NMDA used here do not. In the Broadbent study, the dorsal striatal lesion was large enough to cause considerable distortion to the remaining tissue which may not have been functional. Their damage also extended into ventral regions of the nucleus accumbens as well as areas of the septal nucleus, corpus callosum and neocortex. These large lesions may explain why these rats had difficulty in learning the discrimination. For these procedural and methodological differences between these studies, the results and conclusions cannot be compared.

Cognitive Mapping Theory

The cognitive mapping theory, which was first articulated by O'Keefe and Nadel (1978), has a somewhat different taxonomic organization for the various types of

memory. While the declarative theory categorizes memory into *declarative* vs. *non-declarative* forms, mediated by hippocampal vs. non-hippocampal systems respectively, the cognitive mapping theory, on the other hand, dichotomizes learning and memory into *spatial*/locale and *non-spatial/taxon* forms mediated by hippocampal and non-hippocampal systems respectively. The cognitive mapping theory posits that the hippocampus is the locus for creating a spatial representation, or *cognitive map*, which is used for spatial navigation and storage of spatial information. This theory predicts that damage to the hippocampus should not produce memory deficits because the picture discrimination task is not a spatial task, rather the theory would claim the task to be a form of associative conditioning. Contrary to the theory the hippocampus appears to be a critical system if it is intact, that is, rats will show retrograde amnesia after hippocampal damage (Experiment 2). The cognitive mapping theory cannot fully account for the picture discrimination data.

Configural Association Theory

Sutherland and Rudy (Sutherland & Rudy, 1989) (See also Rudy & Sutherland, 1989) first outlined the configural association theory, which is able to offer a more flexible theory of hippocampal function, because it accounts for a broader range of data. Both the declarative and cognitive mapping theories fail to explain why performance in certain associative learning tasks is often impaired in combination with hippocampal damage (note that these 2 theories consider associative conditioning as non-declarative or non-spatial). Neither the declarative memory theory nor the cognitive mapping theory is able to explain how an animal can solve ambiguous associative problems or *non-linear* problems (for example, a light predicts food in one situation but it does not in another). The configural association theory, however, provides an explanation for hippocampal relevance for non-linear, nonspatial problems. The fundamental idea of the

theory is that the hippocampus serves as a *configural association system,* and it can solve associative problems by the configuring or compiling of multiple stimuli together, often called *configural associations* or *conjunctive representations*. In contrast to the hippocampal configural association system, simple problems or elemental discriminations can be solved without the hippocampus. Elemental discriminations are solved by a non-hippocampal system or a simple *association system.*

The picture discrimination problems used in this thesis are simple or elemental discriminations. Thus, according to the configural association theory, rats should not show retrograde impairments following hippocampal damage, however, the results of Experiments 2 and 3 demonstrate that rats do have retrograde impairments after hippocampal damage. These data contradict predictions the configural association theory would propose.

Multiple Parallel Memory Systems Theory

The multiple parallel memory systems (MPMS) theory (White & McDonald, 2002) approaches learning and memory from a different angle than the most popular theories of memory. The cognitive mapping and declarative memory theory places the hippocampus as the locus or primary processing structure for certain classes of memories, but the MPMS theory also posits that the information of many different forms of learning and memory are being processed simultaneously in more than one location or system of the brain, and that the processing of these multiple systems interact, compete or are otherwise coordinated in the brain. This idea contrasts sharply with the ideas of the major theories of memory (the declarative theory in particular) and the ideas provide many more insights into the interpretation of data. The major theories form their hypotheses based upon the rigid assumption that a particular brain function is localized to a particular brain structure, or in other words, that distinct brain areas are *functionally*

dissociable from one another. Contrary to the emphasis these major theories maintain on the functional dissociation of memory systems, the MPMS theory allows a relatively more flexible interpretation of structure-function relationships. For example, MPMS experiments would be designed to investigate how memory systems interact rather than how they are exclusively different from each other. The MPMS theory, however, does recognize that different parts of the brain do different things in different ways, but the theory also takes into account the fact that various brain areas must *interact* in order to realize the fluid control of all aspects of learning, memory and behaviour. The MPMS theory emphasizes that no brain structure is an isolated island, and research efforts are aimed to gain an understanding and knowledge of how and when various brain networks interact competitively and/or cooperatively within a given learning episode. It is the MPMS theory that best accommodates the results and conclusions here.

There are several key points to the original theory as proposed by White and McDonald (White & McDonald, 2002). The first point is that there are at least 3 neural systems that process and store information, and these systems function simultaneously and independently. Second, the central structures of each of the 3 systems are the hippocampus, the dorsal striatum and the amygdala. Next, environmental stimuli and information is processed and stored in these 3 systems, and each system processes the information with a specific style (processing style), and each system promotes an individual response or behavioural output depending on the stimuli. For instance, the processing style of the hippocampal system corresponds highly to the spatial elements of the stimuli, or to *stimulus-stimulus* type learning. This processing style differs from the dorsal striatal processing style which corresponds more toward a *stimulus-response* type stimuli, which promotes habitual or procedural behavioural output. Although each system functions independently, the 3 memory systems interact so that the output of the

systems converge producing either a cooperative or a competitive response to a certain environmental event. For example, if a hungry animal is presented with the choice of turning left or right (R or L) for a food reward, a cooperative interaction between systems means that both systems promote the same behavioural response (turning right). In contrast, a competitive interaction means that the systems do not promote the same behavioural response (the hippocampal system promotes R and the striatal system promotes L). But the system that is most compatible in processing the elements of the event, that is, the system that has the strongest processing style for that situation will win the competition and its behavioural output or response to be elicited (the hippocampal system processing style corresponds best in this case, the hippocampal system predicts the reward best; animal turns R).

The principles of the MPMS theory are very compatible with the results of this thesis. First, the theory's emphasis on how memory systems interact rather than how these systems are exclusively different from each other; a premise that is supported by the results that the hippocampal system interacts with the striatal or other non-hippocampal systems. Next, the MPMS theory hypothesizes there are multiple memory systems that interact in the brain**;** this is supported by Experiments 1, 2 and 3 suggesting that the hippocampal system interacts with another non-hippocampal system. Next, the theory proposes that there are three central neural substrates that processes and stores information namely the hippocampus, striatum, and amygdala. Although the results of the three experiments cannot attest to the involvement of the amygdala, the hippocampus and striatum are clearly shown to be important systems in learning memory. Finally, the MPMS theory proposes that the systems may interact either cooperatively or competitively. The results showing that the hippocampus

overshadows or inhibits non-hippocampal systems support the idea of interacting memory systems.

There are, however, at least two points in which the results of this thesis are in conflict with the MPMS theory. First, one of the primary propositions of the MPMS theory is that information from the environment is processed independently in each of the neural systems *simultaneously* and in *parallel*. The experimental results showed no evidence of *parallel* or *simultaneous processing* in the intact rat. In fact, the contrary result was observed. Experiment 2 showed that either the hippocampal or the striatal system will learn picture discriminations but not both, neither in parallel nor simultaneously. This was shown by the hippocampus interfering or blocking the striatal system from learning picture discriminations. The striatal system only showed learning in the absence of the hippocampus. There may be *parallel* processing in many learning situations but not in the picture discrimination task. In this task the acquisition in the striatal system is *conditional* on the functional integrity of the hippocampal system.

The second conflict of the MPMS theory and these results involves the way interactions are defined (White & McDonald, 2002). A cooperative interaction is defined by two or more memory systems with independent representations that promote *similar* behaviours. By contrast, competitive interactions involve representations that would promote *different* behaviours. These definitions are in conflict with the picture discrimination results showing a competitive interaction in that the hippocampal system inhibits the striatal system from acquiring a representation. The conflict stems from the result showing that both systems promote similar behaviours for this task, nevertheless, by definition, a competitive interaction involves representations of a situation that promote different behaviours (White & McDonald, 2002). The data do not fit within the definitions of a competitive interaction, therefore, this means that either the picture

discrimination task produces an interaction that is simply an exception to the current definition, or a more appropriate definition must be made in order to satisfy the data. Altering the definition to include the data here would be difficult. A reformed definition that identifies a competitive interaction as leading to both similar and different behaviours offers a little clarification between the types of interaction. There is insufficient information about the nature of the interaction to characterize and distinguish between cooperative and competitive interactions. Further research into the attributes that distinguish between cooperative and competitive interactions must be made in order for the definition to be meaningful.

7.6 Final Notes and Future Directions

There are some important implications of findings of the results of the 3 Experiments. First, the data shows some short-comings of the major contemporary theories of memory. Among the major theories, the MPMS theory can best account for the results when considering the findings from Experiments 1-3. Nevertheless, the theory must be modified if it is to accommodate the present data. First, the proposal that information is processed in each system simultaneously and in parallel was not supported by the results, instead, the information processing for the picture discrimination task occurs in the hippocampal system and processing in the striatal system is *conditional* upon the functional integrity of the hippocampus. In addition, the data do not fit the definition of a competitive interaction, in that the hippocampus and striatum interact competitively but they also produce the same behavioural outputs. Thus, either this learning situation is an exception to the rule, or the definitions must be modified to account for the data.

The results from these analyses prompt several future investigations. One fascinating experiment stems from the conclusion that by removing either the hippocampal or striatal system, the learning of picture discriminations will be mediated by the remaining system without any interaction(s) of the other system. By exploiting this idea, it can be determined how long it takes a memory to decay in either the hippocampus or striatum. Does one system maintain the memory longer than the other, does the memory remain indefinitely, or do both systems need to be intact for the memory to last a normal period of time. Prior to the discovery of this non-hippocampal system (striatal system) it has been extremely difficult to investigate the properties of only one memory system or another. The results gathered from Experiments 1-3 provide exciting opportunities for future research in this line of research.

Other future experiments can include increasing or decreasing the number of sessions or time for pre-training a rat. This may show that after many sessions or days, the picture discrimination task shifts from a hippocampal dependent task to a striatal dependent task. It may be that both systems can support the memory at the same time without one system interfering with the other from learning. Or, perhaps another third system may slowly integrate a memory of the pictures.

Another manipulation is the use of temporary lesions or temporary inactivation of either the hippocampal or striatal systems. For instance if the hippocampus were to be inactivated at the time of learning pictures, the striatal system will learn without hippocampal interference. If, however, during a recall session the hippocampus is no longer inactivated, would the striatal system be able to express the memory or would the hippocampus interfere with the striatum from showing retention as well as learning? In either case the results would provide valuable information as to the nature of the interaction.

The experiments above investigated rats, future directions should include primates and other species to better our understanding of the interactions between memory systems. Studies that include primates must necessarily involve less invasive techniques to probe system interactions, for example, methods of imaging such as magnetic resonance imaging, electroencephalography, etc, paired with novel behavioural tasks can be an important source of information ready to be tapped. Not only can some imaging techniques be applied *in vivo,* but it also allows for direct cross-species comparisons. There are other imaging techniques such as tract tracing and immediate gene activation have recently shown an increase in popularity among learning and memory researchers. This thesis only used large, permanent, bilateral and crossed lesions as a means to study interactions, although this method proved effective, other manipulations such as receptor specific drugs, sectioning of hemispheres or large tracts, or more selective lesions types (both permanent and temporary), etc, can all be valuable in this line of research.

7.7 Conclusion

The results of thesis offer important implications for any investigation in the field of learning and memory. Researchers must consider the importance of the *nature* of the interactions among memory systems. It must be taken into account which of the system(s) are interacting, and whether the interaction(s) are competitive or cooperative. This approach is not always the most parsimonious at first glance but the evidence shown in this thesis supports the idea that understanding the nature of interactions between multiple memory systems holds greater explanatory power than other theories of memory. It is too simplistic to hypothesize that a specific system is only involved for specific kinds of information. The view that the nature and organization of learning and

memory can be separated into categories with distinct boundaries is tempting to adopt because it is straight forward, easy to understand and it offers a simple way to explain data. However, this categorical view simply does not account for all of the data. A close study of the details of how learning and memory occurs must take into account the interactions and/or overlapping of memory systems and their functions. Another problem with the categorical view is that the ability to identify the differences and similarities between two different facts can be distorted. If too much emphasis is placed on boundaries, then the dynamic way that systems interact can be overlooked and this can lead to erroneous conclusions and/or theories.

In the past four decades, there have been enormous achievements in our understanding of memory and the challenges ahead in the study of learning and memory will hold formidable accomplishments. The relatively new approach of studying how memory systems interact will aid the progress to be made in the unravelling of the mysteries in the study and characterization of learning and memory. If the focus of research is pointed toward the study of the nature of memory systems and their interactions, our understanding of learning and memory would increase at a more rapid pace. Understanding the nature of interactions among systems is critical for a complete understanding of how experience changes behaviour.

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APPENDIX 1

Morris Water Task

Experiment 1 included two other behavioural measures apart from the picture discrimination task; the standard version of the MWT, as well as contextual fear conditioning. Details of the context fear conditioning task are discussed in Appendix 2.

Experiment 1

Introduction

As discussed in section 1.2 the MWT measures spatial memory, but it is also useful for many other applications for various cognitive functions. Because Experiment 1 includes 5 different groups with varying levels of brain damage, it can be expected that there may be various cognitive deficits among groups. One reason for using this task is that the behavioural effects of these lesions are well documented, thus if the lesions in this experiment are similar to the ones in the literature, the effects in this experiment should be similar to those in the literature. If the behavioural effects are similar, then there can be more confidence in the generality of the results on the picture discrimination task. In other words, are the systems that are involved in the picture discrimination task the same as the systems that mediate MWT learning? It was hypothesized that acquisition of the MWT would be impaired by lesions of the

hippocampus and mPFC, but striatal damaged rats would show acquisition rates comparable to sham animals. To this end all rats were trained to find a hidden platform for a single location for the MWT over a period of five days.

Methods

Subjects

Rats used in the MWT were the same ones that were used in Experiment 1 for the picture discrimination task. See section 3.0 for details.

Apparatus

The place-learning single location version of the MWT (Morris, 1981) was conducted in a circular pool, 137 cm in diameter and 46 cm high, and filled with water (23 ^{0}C ± 3 ^{0}C) to a depth of approximately 30 cm. The water was made opaque by adding instant skim milk powder. A movable Plexiglas platform (10 cm x 10 cm x 28 cm) was hidden approximately 2-4 cm below the surface of the water. The rats could not see the platform, but several extramaze cues (e.g., posters, shelves, a computer, ventilation duct, etc.) were visible or audible from within the pool, and the rats could learn the location of the platform relative to these distal cues. Swim paths and latencies were recorded using a VP118 Super Tracker with HVSWater software (HVS Image Ltd., Hampton, UK) and these raw data were stored on computer (IBM compatible, 486 DX) for later analysis.

Surgery

See the surgery section in chapter III.

Behavioural Procedures

In Experiment 1, rats were trained in the MWT which is typically used to measure "spatial reference memory" by using the protocol used here. Briefly, a hidden platform is

in a fixed location relative to the room cues and across trials and days (in this case the platform was located in the SW quadrant of the pool). In Experiment 1, after 14 days from surgery rats were trained eight trials per rat per day for five consecutive days. The rats were placed in the water facing the wall of the pool at randomly different start positions (N, E, S, and W) for each trial. A trial ended when the rat climbed onto the visible platform or 60 seconds had elapsed. If a rat had not found the platform after 60 s it was guided there by the experimenter. Rats were left on the platform for 10 s to allow for rearing or to allow the rat to identify its location, and were then removed to their home cages. On each training day, rats were given their first swim trial, and then rats were given their second trials, etc. The inter-trial interval varied based on the rats' level of experience (Day 1-approximately fifteen minute delays versus Day 5, a 3 minute delay). Both the latency to locate the platform and the distance traveled to the platform (a measure of how direct the swim path is) was used as a measure of spatial learning and memory.

Histology

See the histology section in Chapter 3 for details (See also Figure's 3.2, 3.3, and 3.4 for cresyl violet stained sections of each type of lesion).

Statistical analyses

All data for the MWT (each trial was analyzed) was analyzed by a between groups factorial ANOVA with lesion (i.e., Sham, hippocampal, striatal, mPFC, HPC + str, and HPC + mPFC), as well as the mean and standard error of each group per trial. An alpha level of 0.05 was used as a critical factor for significance in all instances.

Results

In Experiment 1, a hidden fixed location platform version of the MWT was used. The rats were trained for 8 trials per day for five days. Illustrated in Figures 4.3 and 4.4 the learning curves for each group (group escape latency and the distance to reach the platform respectively) show three distinct learning curve patterns; rapid, moderate, and poor learning rates (Figure 4.5 shows representative search patterns for individual rats from each of the three types of performances). The groups with the best learning performances were the sham and striatal rats (Figure 4.3). Another learning curve is the HPC group, who show an impaired learning and performance, but the group does improve across days. Rats with damage restricted *only* to the hippocampal formation eventually developed a search strategy by circling the perimeter of the pool several inches from the edge, presumably to increase the chance of "bumping into the platform". The combined HPC + mPFC group performed similar to the HPC group (Figures 4.3 and 4.4). The third, and most impaired learning performance curve, is the combined HPC + str group, whose escape latencies average between 40-50 seconds across days; a significant increase compared to the HPC and HPC + mPFC groups (an average of about 15 seconds longer than the HPC group). The HPC + str group did not seem to develop any type of search strategy; rather, they randomly crossed the centre of the pool and circled near the edge of the pool, rarely finding the platform (see Figure 4.5).

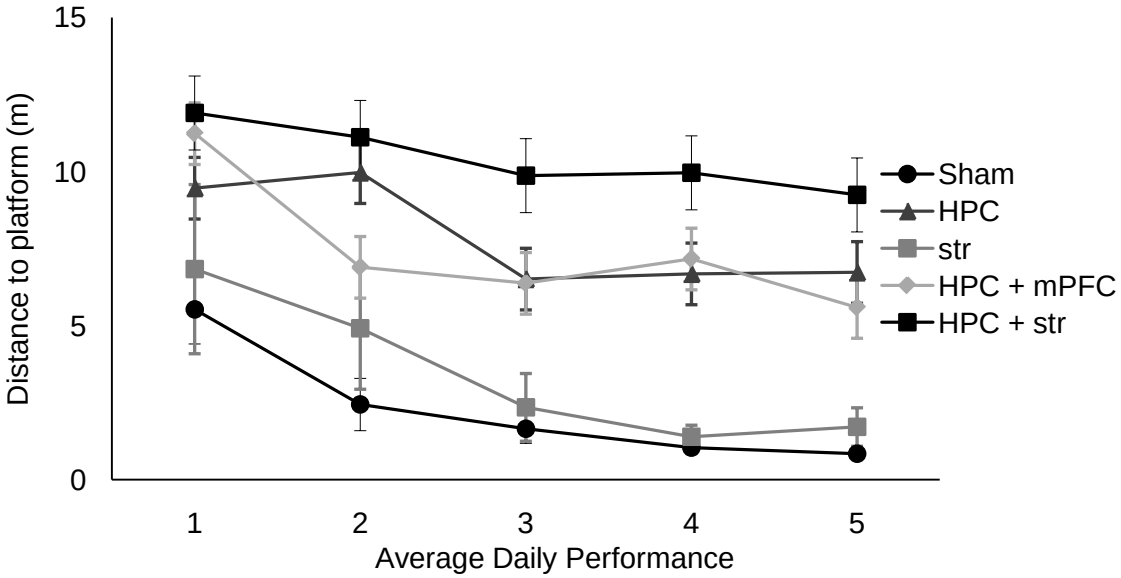

Figure 4.3. The figures show postoperative performance learning and memory with distance as a measure of performance in the MWT (hidden platform version) for Experiment 1. Each data point represents the average daily score at 8 trials per day, per group. Rats with damage to the hippocampus showed poor performance, particularly the HPC + str group.

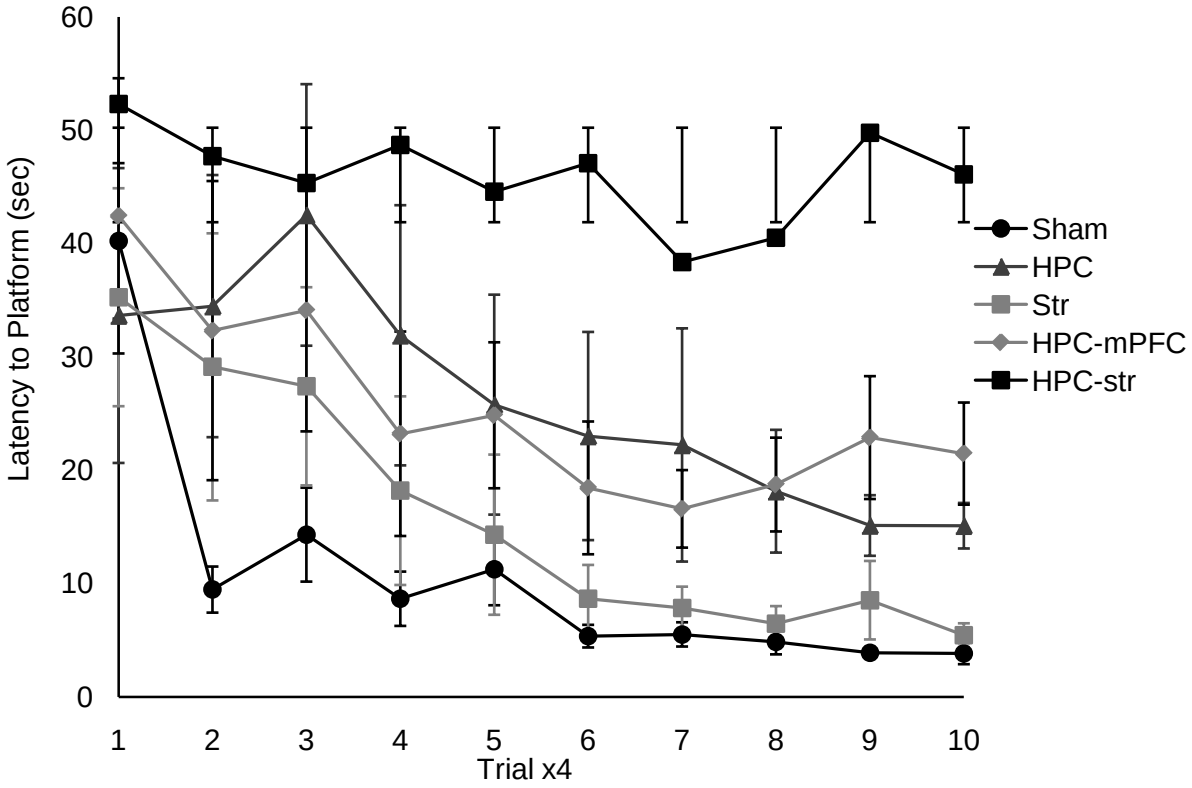

Figure 4.4. The figures show postoperative learning and memory with latency (seconds) to reach the platform as a measure of performance in the MWT (hidden platform version) for Experiment 1. Each data point represents the average daily score at 8 trials per day, per group. Rats with damage to the hippocampus showed poor performance, particularly the combined HPC + str group.

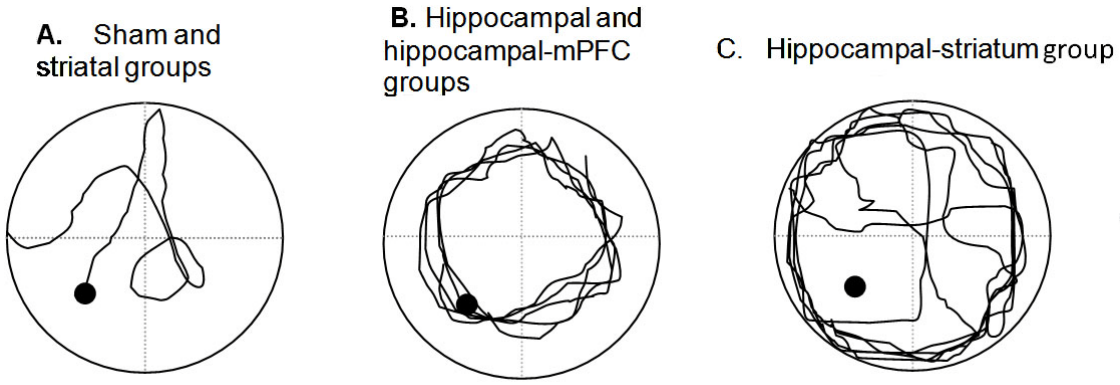

*Figure 4.*5. MWT post-training probe tests (no platform) for Experiment 1. The platform is removed after post-training probe tests. A. Sham and str groups typically swam within the quadrant where the platform was located. B. Groups with hippocampal damage showed swimming patterns distributed among all quadrants displaying a strategy of swimming a few cm away from the edge of the pool (referred to as thigmotaxis). C. The HPC + str group showed a distributed swimming pattern around all quadrants and does not develop the same search strategy, rats mostly stayed near the edge of the pool.

A repeated measures analysis of variance for escape latencies of the five groups across trials, showed a significant main effect of trial ($F(4, 25) = 5.05$, $p < 0.001$), a significant main effect of group ($F(39, 25) = 5.89$, $p = 0.03$), and a significant interaction between groups and trials ($F(156, 25) = 1.26$, $p = 0.026$). Post hoc tests revealed significant differences between the Sham group and the hippocampal ($p = 0.006$), combined HPC + mPFC ($p = 0.007$), and combined HPC + str ($p < 0.001$) groups, but not the str group ($p = 0.261$). The HPC group showed a significant main effect to the combined-HPC + str group ($p < 0.001$), but not the combined HPC + mPFC group ($p = 0.935$), and the difference for the str group nears the significance level ($p = 0.066$). The hippocampal and combined HPC + mPFC groups showed similar learning curves (Figure 4.4). The combined HPC + mPFC group shows a difference that approached significance relative to the str group ($p = 0.077$). Lastly, the combined HPC + str group differs significantly from the sham and str group ($p < 0.001$) (both groups have the same p value) and the hippocampal and combined HPC + mPFC group ($p < 0.001$); (again, both have the same p value). Post hoc analyses for the escape distances for the five groups reflect similar main effects and significance compared to escape latencies.

Discussion

Rats that received a hippocampal lesion alone performed worse than sham rats. Hippocampal lesioned rats, however, did significantly decrease the distance and latency over trials, although their performance did not improve to the level of the sham rat's performance. Morris and colleagues (Morris, Davis, & Butcher, 1990) also showed that overtraining leads to shorter latencies and eventually hippocampus damaged rats were no longer different from control rats. Although it is unclear which systems are

responsible for this over-trained learning, it can be speculated that rats may be using an approach or cued strategy to locate the platform. If rats are using cue strategies then the striatum is probably an important structure in over-training learning. Indeed, McDonald and White (1994) showed that dorsal striatal lesions impair rats in the cue task. An alternative is that there are other place systems left intact following a hippocampal lesion.

The most striking observation here is that the combined HPC + str group could not learn simple picture discriminations nor could they navigate in the MWT. In both tasks, rats performed poorly. It is plausible that the poor performance is due to: 1) deficits in place navigation from hippocampal lesions and 2) deficits in cued navigation from the striatal lesions. Whatever the reason, the results of these two tasks make it clear that there is something important about the interaction or complementary functional specialization between the hippocampus and the striatum.

APPENDIX 2

Contextual Fear Conditioning

Experiments 1 and 2 included an anterograde contextual fear conditioning task and a retrograde context fear conditioning analysis respectively. This appendix discusses these behavioural measures for Experiment 1 and 2.

Experiment 1

Introduction

In Experiment 2 all rats were also tested in the contextual fear-conditioning task in addition to the picture discrimination task (see Chapter 4) and the MWT (see Appendix 1). In terms of hippocampal function, there are differential effects of hippocampal lesions before and after being conditioned to fear the context (Kim & Fanslow, 1992; Phillips & LeDoux, 1992, 1994; Maren et al., 1997; Frankland, Josselyn, Bradwejn, Vaccarino, & Yeomans 1997). Typically, if hippocampal lesions are made before fear conditioning then rats will acquire fear of a context but not if the lesions are

made after fear conditioning (Kim & Fanslow, 1992; Phillips & LeDoux, 1992, 1994; Maren et al., 1997; Frankland, et al., 1997). In Experiment 1, rats received lesions prior to conditioning, then a 24-hour delay before the retention testing assessing memory for conditioned fear to the context. It was predicted that the HPC group would show retention but two of the lesion groups (a combined HPC + str and a HPC + mPFC lesion) may or may not show memory for fear. The main question for this task is; can rats with a combined HPC + str or HPC + mPFC lesion learn conditioned fear to the context?

Methods

Subjects

Rats used in the context fear conditioning task were the same ones that were used in Experiment 1 for the picture discrimination task. See section 3.0 for details.

Apparatus

In the Contextual fear conditioning task four identical chambers (30 × 24 × 22 cm; MED-Associates, Burlington, VT) made from aluminum (side walls) and Plexiglas (rear wall, ceiling, and vertically hinged front door) were used for the conditioning. The floor of each chamber consisted of 19 stainless steel rods (4 mm diameter) spaced 1.5 cm apart from centre to centre. Each chamber was located in a front-opened chest equipped with a ventilation fan that provided background noise (65 dB). Foot shock (1 mA; 2 sec) was delivered through the floor steel rods that were connected to a shock generator and scrambler (MED-Associates). The chambers were cleaned with clinicide before and after each rat received conditioning and retention testing. A video camera, connected to a computer, was located in front of the chests such that the behaviour of each rat during the conditioning and test sessions could be digitally recorded and analyzed using

FreezeFrame Video-Based Conditioned Fear System software (Actimetrics, Wilmette, Il). The test for fear generalization used similar chambers and contexts except for the following: Ceiling was made of stainless steel mesh with a 1.7 cm circular hole in the centre. The hinged front door was half the height of the former (24 x 11 cm) and was oriented in a horizontal manner with the hinges on top of the front wall (front wall measured 24 x 11 cm from bottom to middle of front panel) where the door swings to the top of the chamber to be latched. Each chamber consisted of 25 stainless steel rods (3.2 mm diameter) spaced 1.2 cm apart from centre to centre. There were no ventilation fans. The chambers were cleaned with an organic solvent/disinfector. Behaviour was recorded on a video camera and manually analyzed.

Surgery

See the surgery section in chapter III.

Behavioural Procedures

The Contextual fear conditioning task has three components or phases. (1) Exploration. Rats were transported four at a time in separate cages and placed in one of the four chambers and were allowed to explore for a period of 5 minutes. The exploration period is important for the rat to form a representation of the shape, odours, and other cues of the chamber, so that a cohesive, integrated memory of the box (context) can be formed and associated with the foot shock and fear conditioning occurs successfully. (2) Conditioning. Next, rats experienced two mild shocks (1 mA; 2 sec/shock) that were delivered through the metal gratings. There was one shock at the 5 min mark and then another shock 58 sec later. One minute after the second shock was delivered rats were removed and returned to their home cage. (3) Retention. After recovery from surgery in Experiment 2 (in Experiment 1, rats were conditioned after the surgery and tested for retention 24 hours later), each rat was returned to the chamber in

which it received a 5 min retention test (no shock was delivered in this retention test). The percent time freezing for each minute- block was calculated then averaged for each rat by using FreezeFrame software. Freezing was defined as the absence of movement except for breathing.

Rats were placed into chambers and were allowed to explore the context for five minutes (phase 1; exploration) before receiving foot shock through the floor bars twice for two seconds (1 mA). The foot shocks were separated by one minute. Rats remained in the chambers for one additional minute making seven minutes total (phase 2; context conditioning). Twenty-four hours later rats were again placed in the chambers and their activity was monitored for five minutes (phase 3; memory test). Freezing was used as a measure of fear of the context.

Results

During the testing for contextual fear conditioning all groups showed equivalent levels of freezing in the context; they froze more than 50% during the 5-minute period. No significant differences were observed between any of the groups (Figure 4.6). All groups showed retention by showing fear (measured by the amount of time freezing) for the context. An ANOVA revealed that there was no main effect of group ($F(4, 25) = 0.512$, $p = 0.727$).

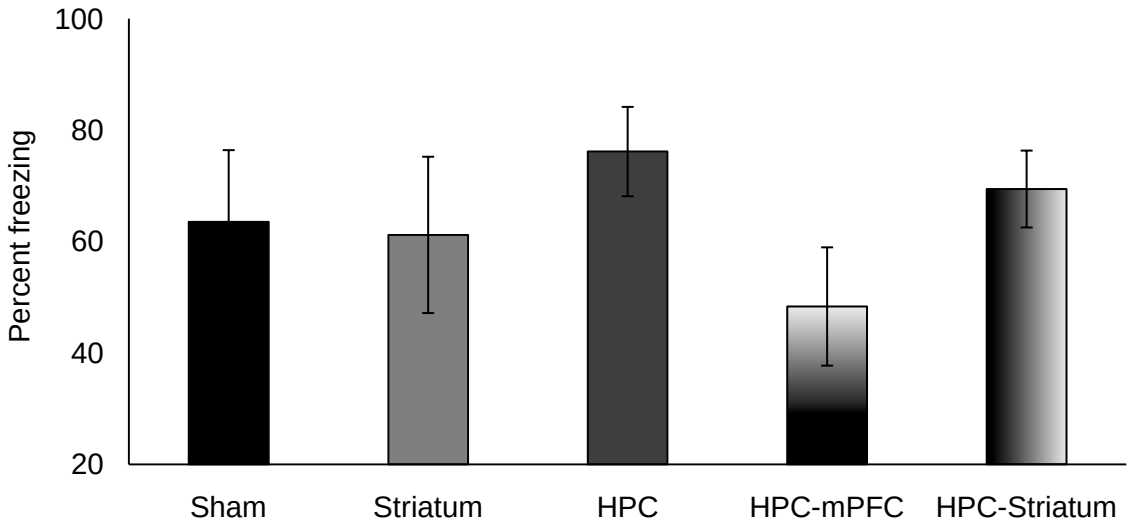

Figure 4.6. Postoperative Contextual fear conditioning for Experiment 1. Shown is the retention performance measured by percentage of freezing. The data represented above shows the average percentage of freezing over a five min period. There is no significant difference between groups.

Discussion

The 24-hour retention test of the contextual fear-conditioning task did not show any significant differences amongst groups. All groups successfully conditioned to fear the context. The results above replicate other anterograde data (Kim & Fanslow, 1992; Phillips & LeDoux, 1992, 1994; Maren et al., 1997; Frankland, et al., 1997).The result that all groups showed no learning impairments for context fear are in contrast to the results of the picture discrimination task and the MWT in that the combined HPC + str group could not acquire picture memories nor the place of a hidden platform. The reason for these differences is unknown. It is probable, however, that these different tasks require different sets of interacting memory systems. This may be due to the different motivational and/or emotional factors that are required for each task.

Experiment 2

Introduction

In Experiment 2 rats were exposed to the conditioning chambers before lesions were given so as to measure retrograde effects. According to the well-documented retrograde contextual fear data (Kim & Fanslow, 1992; Phillips & LeDoux, 1992, 1994; Maren et al., 1997; Frankland, et al., 1997). Experiment 2 predicted that groups receiving hippocampal damage after being shocked would not show fear memory of the context or in other words these groups would show retrograde amnesia. This prediction, similar to the above picture discrimination prediction, asserts that rats should show no retention of a context after receiving hippocampal damage.

Methods

Behavioural Tasks

Preoperative contextual fear conditioning. The rats received the first two phases of the contextual fear conditioning task. The exploration phase (five minutes) and the context conditioning phase, where rats received two mild foot shocks. Rats remained in the chambers for an additional minute.

Postoperative retention. After the rats had completed testing on the visual water task, they were again placed in the contextual fear chambers where their activity was monitored for five minutes. Inhibition of movement, or freezing, was used as a measure of retention of the context as described above. The rats were subsequently tested in another chamber of a different context but some cues/elements were similar. For example, the new context was located in a different testing room, had a different odour in the boxes, and there were different views of each wall, but like the original context, the new context had steel gratings and similar spatial dimensions.

Results

Behavioural Results

After recovery from surgery (7 days), rats received phase-3 of the contextual fear conditioning task; the postoperative memory test. Retention measured by percentage of freezing for context conditioning showed a main effect of group over the five minute period ($F(5, 35) = 13.67$, $p < 0.001$) (see Figure. 5.4). *Post hoc* analyses showed that sham rats froze significantly more than all other groups. The str group did show decreased freezing relative to shams. They did show, however, freezing for over 40% of the time, indicative that the striatal rats learned an association of fear with the context. Similarly, the mPFC group associated fear with the context (57% freezing) and differed from all groups except the str group. Contrary to the groups without hippocampal damage, the three groups that did receive hippocampal damage did not differ from each other, and each showed a significant decrease in freezing behaviour (between 9 and 26%), indicative of a failure to associate fear to the context (Figure 5.4). Post hoc analyses did not reveal a main effect of minute-block divisions; that is, freezing did not

change over the 5-minute period. Rats were subsequently tested in another chamber in which cues/elements were different. The freezing behaviour of all groups did not differ significantly as analyzed by ANOVA ($F(5, 35) = 0.415$, $p < 0.834$) to each other averaging 20% (Figure 5.5).

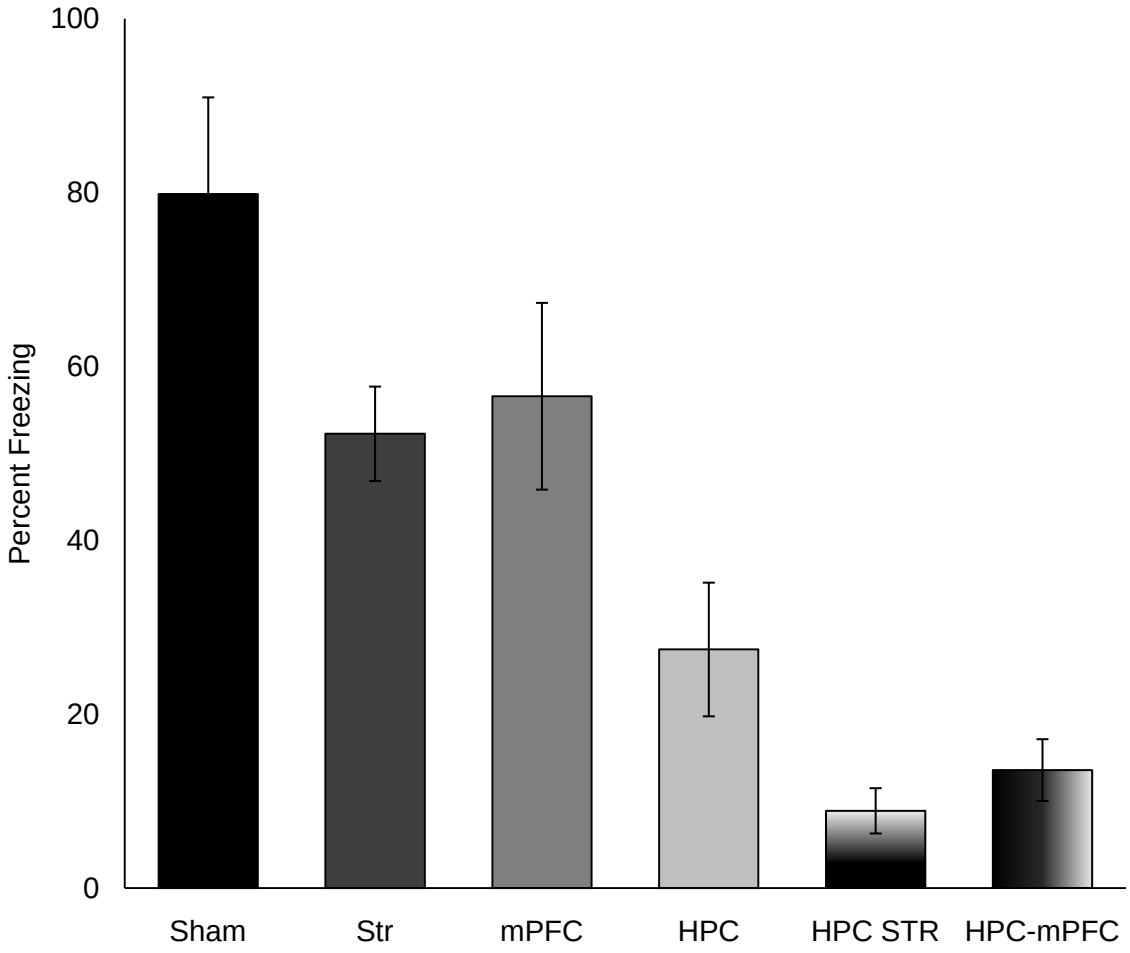

Figure 5.4. Percent freezing for contextual fear conditioning for a 5 minute period for Experiment 2. Conditioned fear of the context is lost for groups that received hippocampal damage after training. Relative to shams, the striatal and mPFC groups show only a modest decrease in freezing, but they still show memory for the context.

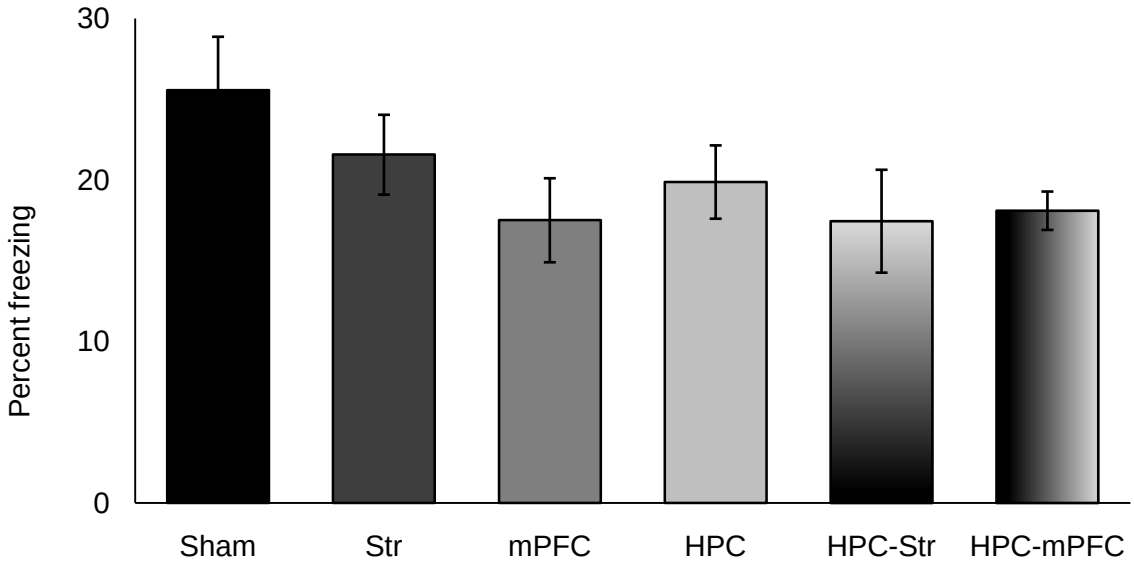

Figure 5.5. The transfer of conditioned fear to a new, different context for Experiment 2. All groups showed similar freezing levels; an average of 20%. The drop in freezing levels show that rats did not generalize fear across contexts.

Discussion

Retrograde results of the contextual fear conditioning showed that groups with hippocampal damage did not retain fear of the shock context. All other groups showed better retention of learned fear. These results are not surprising as they replicate retrograde contextual fear conditioning studies (Kim & Fanslow, 1992; Phillips & LeDoux, 1992, 1994; Maren et al., 1997; Frankland, et al., 1997). The fact that rats with hippocampal damage showed no fear of the context after their lesions supports the theory that the hippocampus is an essential structure for forming contextual memories (Kim & Fanslow, 1992; Phillips & LeDoux, 1992). In comparing the results of Experiment 1 and Experiment 2 concerning both the picture discrimination task results to the contextual fear conditioning results, one difference and one similarity can be identified. Rats with combined HPC + str damage *can* learn to associate fear to a context but they cannot learn picture discriminations. In contrast, both pre-trained picture memories and contextual memories are lost after damage to the hippocampus, regardless of additional structural damage. This is evidence that the hippocampal system interferes or overshadows non-hippocampal systems during acquisition training in the normal rats. The difference between the tasks is the non-hippocampal memory system that is used to learn in the absence of the hippocampus. For the picture discrimination task the non-hippocampal system is the striatal system whereas in the contextual fear conditioning task the non-hippocampal system is yet unknown. The non-HPC system for contextual fear conditioning is not established here, but based upon other work it almost certainly involves the amygdala (Helmstetter & Bellgowan, 1994; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990 Romanski, 1990; Maren, et al., 1997; Wilensky, Schafe, & LeDoux, 1999 1999) or neocortical circuitry (O'Reilly & Rudy, 2001; Sanders, Wiltgen, &

Fanselow, 2003; Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006 Sage, & Fanselow, .2006).

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