MULTIPLE-OBJECT MEMORY REQUIRES THE HIPPOCAMPUS

Tonia Tan-Ling Yim

B.Sc. in Psychology, University of British Columbia, 2002

A thesis

Submitted to the University of Lethbridge School of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

Department of Neuroscience

University of Lethbridge

LETHBRIDGE, ALBERTA, CANADA

Thesis Abstract

This thesis investigates the role of the hippocampus in object memory. Currently, the role of the hippocampus in object recognition is unclear, with some studies demonstrating a delay-dependent impairment after hippocampal damage, others showing no impairment. The present thesis used the novel object recognition task and its variants to investigate various types of object memory in hippocampal lesion rats. In the first study, impairments were observed in discriminating object order and associating objects with contexts, while no impairment was observed in novel object recognition. In the second study, it was found that encountering another object shortly prior to or after encountering a target object impairs the recognition of the target object. In a control procedure, encountering a novel context either shortly before or after encountering the target object did not impair object recognition. In sum, in the absence of the hippocampus, object memory becomes vulnerable to interference, rendering rats unable to discern memories of multiple objects. The present thesis concludes that the hippocampus discriminates multiple objects via pattern separation. A stimulus-response model relating the role of the hippocampus to object memory is proposed.

Acknowledgements

I owe my thanks to many people for this project. First and foremost, I am eternally grateful for my supervisor Dr. Ian Whishaw's guidance and the countless hours he had spent with me preparing this document. I thank my collaborator Dr. Hugo Lehmann for assisting with many aspects of this project. I thank all my committee members for their kind input and for caring about my well-being throughout this thesis. I thank my former supervisor Dr. Darren Hannesson for providing the direction for the first part of this project.

I thank Dr. Matt Tata and Simon Spanswick for intellectual input that facilitated the interpretion of my data. I thank everyone from the former Hannesson lab for their friendship throughout many difficult times. For technical assistance, I thank Geetha Subramaniyam, Yelena Arjannikova, and our directed studies student Vannessa Flak. I thank Maede Ejaredar and Mohammad Reza Rahavi for their support. I also thank Geetha for proofreading parts of this thesis.

I thank my friends Nhung Hong, Melinda Wang, Scott Hess, and Steve Fox for their friendship and support. I thank Martin Warszawski for being so understanding, and I thank the Warszawski family for their kindness. I thank everyone in the Whishaw lab for their kindness and assistance. I thank my friends in Hong Kong for their unconditional support, especially my best friend Judy Yung. Lastly, I thank my family for allowing me the freedom to pursue this degree.

Table of Contents

List of Tables and Figures

List of Abbreviations

AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
CA	*Cornu Ammonis*
CS	Conditioned stimulus
DG	Dentate gyrus
DMTS	Delay-matching-to-sample
DNMS	Delay-non-matching to sample
dHPC	Dorsal Hippocampus
EC	Entorhinal cortex
EEG	Electro-encephalographic
EPSP	Excitatory postsynaptic potential
ff	Fimbria fornix
HPC	Hippocampus
i.p.	intraperitoneal
LIA	Large irregular amplitude
LTP	Long term potentiation
LTD	Long term depression
mf	Mossy fibers
MRI	Magnetic Resonance Imaging
MWM	Morris water maze
NMDA	N-methyl-D-aspartate
PBS	Phosphate buffered saline
PKMζ	Protein Kinase M zeta
pp	Perforant path
RAM	Radial arm maze
sc	Schaffer collateral
s. c.	Subcutaneous
SIA	Small irregular amplitude
US	Unconditioned stimulus
vHPC	Ventral Hippocampus
ZIP	z-substrate inhibitory peptide

1. OVERVIEW

The hippocampus is important in various types of memory, in particular spatial memory. Its role in object memory, however, is unclear. While experiments with the delay-matching-to-sample (DMTS) task indicate a delay-dependent role of the hippocampus, data from the novel object recognition task indicate object memory is independent of the hippocampus.

One explanation for the inconsistent results is that DMTS and novel object recognition tasks have different task requirements. Specifically, DMTS paradigms require cognitive faculties unrelated to object memory, such as remembering task rules and associations. As well, performances in DMTS are motivated by external incentives (i.e. fear escape or desire for food reward). Given that the place cells in the hippocampus may have a role in learned associations and motivation, data from DMTS paradigms can be confounded by these factors. From this perspective, novel object recognition is better suited to test object memory.

The present thesis aims to elucidate the role of the hippocampus in object memory by using the novel object recognition task and its variations. As is known, the hippocampus is not needed for discriminating between a familiar object and a novel object. Whether the hippocampus is involved in object memory beyond this recognition is largely unknown.

This thesis asked whether the hippocampus is involved in object recognition and object association. In the first set of experiments, rats with complete and partial hippocampal damage were tested on object recognition, object recency discrimination, and object context-recency association. The object recency task tests rats' memory for the order of objects. The object context-recency task tests rats' memory of associating objects, context, and recency information. Based on various theories of the hippocampus, the present thesis hypothesized that 1) the hippocampus is not needed for object recognition, 2) the hippocampus is required for recency discrimination of objects, and 3) the hippocampus is required for forming associations between objects, context, and recency. Object discriminations of rats with complete hippocampal lesions were compared against rats with dorsal and ventral hippocampal lesions to examine whether regions of the hippocampus contribute differently to object memory.

The results from the first set of experiments suggest that while object recognition is independent of the hippocampus, object recency memory and object context-recency memory are not. Both dorsal and complete hippocampal lesion rats showed impairments in object recency and object context-recency memory, yet showing intact object recognition. Ventral hippocampal lesion rats showed intact object recognition and object recency memory, as well, they showed some indication of object context-recency association, likely due to intact recency memory. Due to small sample sizes, results from the partial hippocampal lesion rats are not definitive. The first part of this thesis concluded that impairment in object recency and object context-recency memory after hippocampal lesions may be due to interference between object memories of multiple sample objects.

The second set of experiments investigated the validity of interference as a mechanism of the observed object impairments in hippocampal lesion rats. Rats with complete hippocampal lesions were tested on the effects of object interference and context interference on novel object recognition. For the present thesis, interference is conceptually defined as an event that disrupts the memory of another event which occurred close in time. Operationally, interference is observed via rats' performance on the novel object recognition task following an encounter of another non-sample stimulus. Object interference is induced via presenting rats with another object either shortly before or after encountering a sample object. Context interference is induced via presenting rats with a novel context shortly before or after encountering a sample object. If rats retain memory of the sample object, they would show normal preference for the novel object and thus demonstrating no interference. Alternatively, if rats' preference for the novel object is disrupted, then rats' memory of the sample object is likely affected via interference. Object recognition of rats with hippocampal lesions was impaired following object- but not context- interference. The second part of the study concluded that object interference is a likely mechanism for object memory impairment in rats without the hippocampus.

Conclusion

The present thesis concludes that the hippocampus is important for discerning memories of multiple objects via reducing interference between encoded objects. Interference is the greatest between trials with significant feature-overlap, indicating that the intact hippocampus engages in pattern separation to discern memories of similar object-related events. The present thesis ends with a proposal of a stimulus-response model of object memory that requires the hippocampus.

2. AN INTRODUCTION TO THE HIPPOCAMPUS

2.1 History

The hippocampus, a prominent structure in the limbic system in the medial temporal lobe, has long been known to be essential for declarative memory (Squire, 1992). Declarative memory is defined as the memory for facts and events that can be consciously recalled or declared.

Giulio Cesare Aranzi in 1564 aptly named the structure “hippocampus” (which translates to seamonster in Greek) for its curved shape. Figure 2.11 shows the location of the hippocampus in the human brain. Historically, the hippocampus was first thought to be involved in olfaction (Kolliker, 1896 and Cajal, 1911; as cited in Green, 1964). Collectively, the hippocampus, the entorhinal and piriform cortices, and some parts of the amygdala were referred to as the rhinencephalon, meaning nose brain.

During the mid 1930’s, Klüver and Bucy (1937, as cited in Green, 1964) discovered that bilateral removal of the temporal lobes in monkeys produced bizarre emotional behaviors that included the lack of fear towards previously feared stimuli. In the same period, Papez (1937) proposed the hippocampal/ mammillothalamic circuit of the limbic system in which information passed from the hippocampus to the mammillary bodies of the hypothalamus, the anterior thalamic nucleus, the cingulate cortex, the entorhinal cortex, and back to the hippocampus. This second view of the hippocampus was that it was a supportive structure in the limbic system circuitry that provided the anatomical substrates to emotions (Papez, 1937; as cited in Anderson et al., 2004).

Interest in the hippocampus as a separate entity with mnemonic functions began in 1957 with Scoville and Milner’s clinical observation of patient H. M. After undergoing bilateral medial temporal lobe resection as a treatment for epilepsy, H. M. suffered from severe anterograde memory deficits and less severe retrograde memory deficits for events that occurred up to eleven years prior to the procedure (the terms anterograde and retrograde refers to memory post-surgery and prior to surgery, respectively).

The operation, which mainly involved the bilateral removal of H.M.'s hippocampal formations (i.e. the dentate gyrus, Ammon's horn, and the subiculum --- see section 2.2 on anatomy) rendered him unable to form new declarative memories while his intelligence and his ability to form procedural memories were preserved. Verifications from magnetic resonance imaging (MRI) indicate that most of the amygdala and entorhinal cortex were damaged bilaterally as well (Corkin et al., 1997). In the last fifty years, this initial observation has spawned an entire field of research dedicated to the role of the hippocampus in memory.

Over the years, investigations of the role of the hippocampus in memory generated many speculations. Some of the prominent theories concerning the functions of hippocampus include the formation of spatial cognitive maps (O'Keefe and Nadel, 1978), working and reference memory (Olton, 1977; Jarrard, 1978, 1986), configural associations of stimuli (Sutherland and Rudy, 1989; Rudy and Sutherland, 1995), and relational processing (Cohen and Eichenbaum, 1993; Eichenbaum 2004). While the specific function of the hippocampus is an ongoing investigation, there is a consensus that the hippocampus is important in memory in general (for a review of the debate surrounding the functions of the hippocampus, see Redish, 1999).

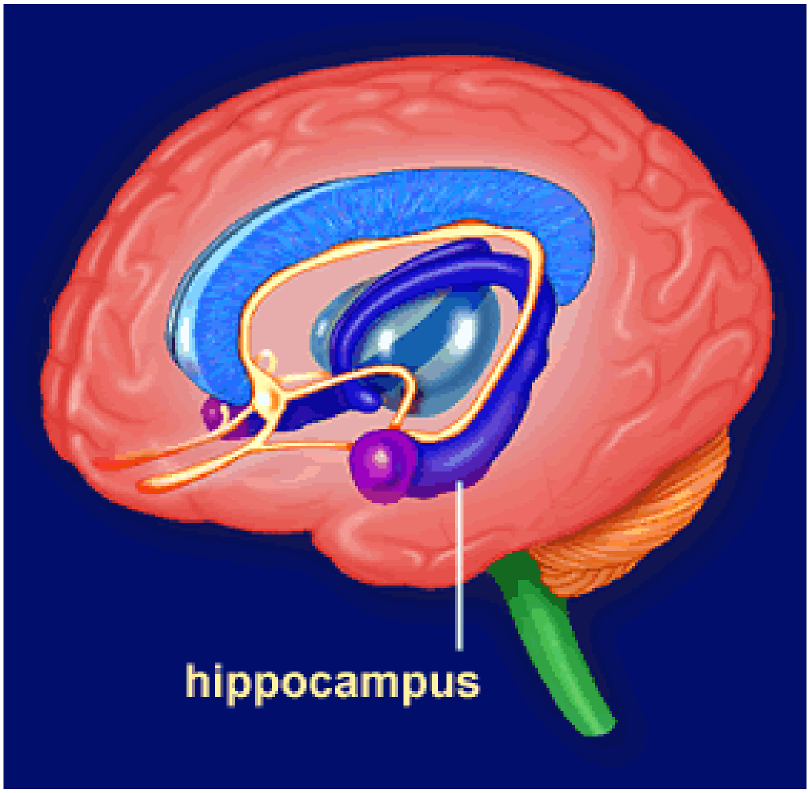


Figure 2.11. Location of the hippocampus in the human brain. The hippocampus is the curved subcortical structure illustrated in purple. Surrounding structures include the corpus callosum (in light blue), the amygdala (in purple), and the thalamus (in transparent blue under the hippocampus). (Figure from http://morphonix.com/software/education/science/brain/game/specimens/hippocampus.html [1])

---

[1] Figure reprinted with permission from Morphonix, LLC.

2.2 Anatomy

For the purpose of this thesis, our discussion on hippocampal anatomy will concentrate on the rat's hippocampus. The term hippocampal formation refers to the dentate gyrus (hereon refer to as the dentate), Ammon's horn, and the subiculum. Ammon's horn is divided into multiple subfields --- *cornu ammonis* (CA) 1 to CA4 --- with neurons from each subfield differing in cytoarchitecture and synaptic properties (Amaral and Witter, 1989; Leutgeb et al., 2004). Neurons from CA3 to CA1 are pyramidal cells; CA3 cells are closely packed and are interconnected, with a low number of neurons in the region active during firing. In contrast, CA1 cells have no intrinsic excitatory connections; they are output cells that provide the primary hippocampal output to the entorhinal cortex and the subiculum. CA4 is commonly known as the hilus and is considered a part of the dentate. Figure 2.21 illustrates the hippocampal formation.

There are three major fiber systems associated with the rat hippocampus. The first system is the angular bundle. It is a fiber bundle tract that connects the adjacent entorhinal cortex (which receives inputs from the associational, perirhinal, parahippocampal, and prefrontal cortices) to the dentate, CA1 and CA3, and the subiculum. It contains the perforant path. The second system is the fimbria-fornix pathway, a major afferent / efferent pathway that arises from the CA1 and the subiculum and connects the hippocampus with the basal forebrain, hypothalamus, and the brain stem. The third system is the dorsal and ventral commissures, which connects the two hippocampi across the left and right hemispheres via the corpus callosum (Amaral and Lavenex, 2007).

The flow of information within the hippocampus is largely unidirectional. The perforant path that contains efferent projections from the entorhinal cortex is the start of the trisynaptic circuit (Andersen et al., 1966; Swanson, 1978; Witter, 1989). First, layers II and III (and minor contributions from layers IV and V) of the entorhinal cortex send projections to the granule cells in the dentate via the perforant path. The granule cells in the dentate then send mossy fiber projections to the CA3 subfield. Finally, pyramidal cells in the CA3 subfield send projections to the CA1 subfield and to the basal forebrain via the Schaffer collateral axons. Aside from sending axons ipsilaterally from CA3 to CA1, the Schaffer collateral also sends axons to the contralateral CA1 via the Associational commissure. The pyramidal cells in CA1, which project to the subiculum, provide the major output of the hippocampus. From the subiculum, axons are sent out of the hippocampal formation and return to the entorhinal cortex (Witter et al., 2000).

Recent evidence on the topography of the hippocampal transverse connections suggests a complex three-dimensional view of hippocampal circuitry (Amaral and Lavenex, 2007). For example, it was discovered that the probability of a neuron from a given subfield innervating to the next is determined by the transverse position of the neuron itself. For instance, proximal CA3 cells (i.e. CA3 cells that are located near the dentate) are more likely to project to distal CA1 cells (i.e. CA1 cells that are located near the subiculum). It is beyond the scope of this introduction to discuss in detail the three-dimensionality of the intrinsic hippocampal network. One should note that the trisynaptic circuit view of the hippocampus presented here represents a simplistic view of the topic.

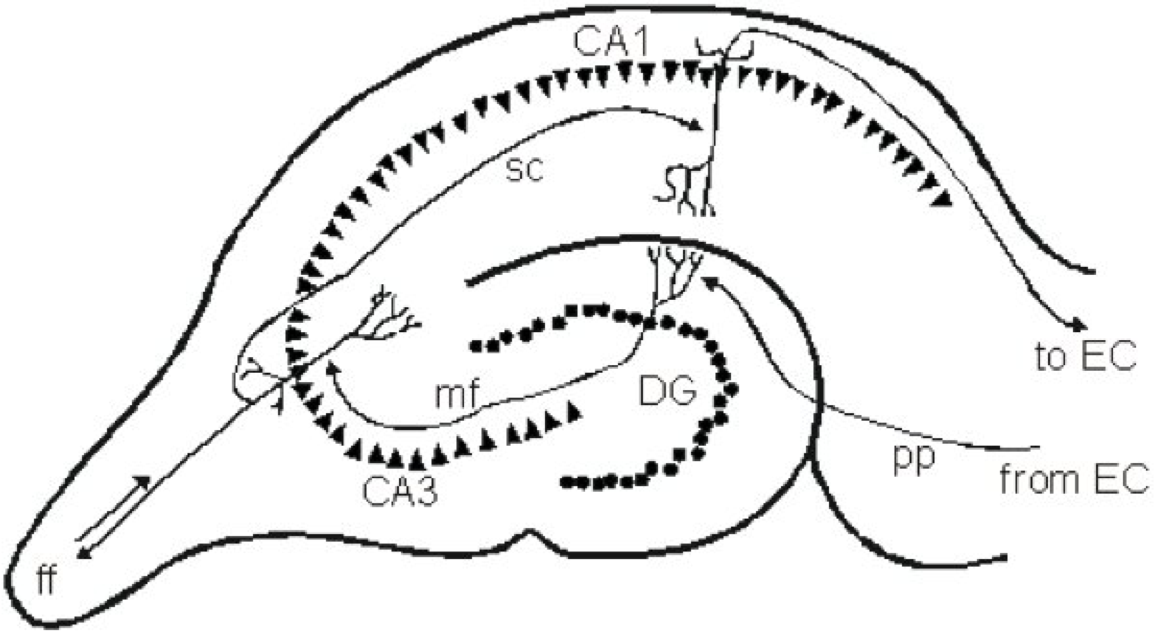


Figure 2.21. Diagram of the trisynaptic circuit within the hippocampus. The entorhinal cortex (EC) projects to the granule cells (depicted by black dots) in the dentate gyrus (DG) via the perforant path (pp). From there, DG sends mossy fibers (mf) to pyramidal cells (depicted by black triangles) in the CA3 subfield. Lastly, CA3 pyramidal cells project to CA1 subfield via the Schaffer collateral (sc). From the sc pathway, some of the projections branch off to the basal forebrain via the fimbria-fornix (ff), while some axons traverse to the CA1 subfield in the contralateral hemisphere via the Associational commissure (not shown). CA1 pyramidal cells complete the circuit by returning hippocampal outputs to the EC (Figure from Amaral and Witter, 1995 [2]).

---

[2] Copyright Elsevier (1995). Reprinted with permission.

2.3 Neurophysiology

This section reviews what is known from the neurophysiological studies of the hippocampus. The first topic presented is long term potentiation (LTP), a form of synaptic plasticity widely regarded as the physiological basis of learning and memory. Next, we examine findings from the activities of the hippocampal neurons in the freely moving rat via electro-encephalographic (EEG) recordings and single-cell recordings. EEG recordings monitor a population of neurons firing in the region, while single-cell recordings monitor the activity of a selective neuron. The patterns of EEG and single-cell activities during various types of behavior are reported.

Long term Potentiation

When an animal learns, its behavior is altered. The change in behavior as a result of learning is presumed to be preceded by a change in the animal's nervous system; the persistence of the learned behavior is memory. So how are changes from learning established in the nervous system? In 1949, Donald Hebb first postulated the following:

> When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased (Hebb, 1949, as cited in Bliss et al., 2007).

Thus, activity-driven changes at the synaptic level were proposed to be the basis of learning and memory.

In the 1960's, Lømo and Andersen discovered a long lasting form of synaptic plasticity termed long term potentiation (LTP) in the hippocampus of anaesthetized rabbits (Andersen, 1960 and Lømo, 1966; as cited in Bliss et al., 2007). While low frequency stimulations of 1 Hz or less elicit steady excitatory postsynaptic potentials (EPSP) in the dentate, high frequency stimulations of the perforant path yield much stronger, prolonged EPSP (see Figure 2.31). Specifically, when tetanic stimulations (i.e. a brief burst of high frequency stimulations of 100 Hz or more) are delivered to the perforant path inputs from the entorhinal cortex, a long-lasting increase in the strength of postsynaptic neurons in the dentate was observed in the form of increased EPSP (Bliss and Lømo, 1973). The increases in synaptic responses from LTP inductions are rapid and persistent; LTP can last for hours in slice preparations of hippocampal tissues or from days to months in a live animal.

Elsewhere, LTP has been found in the mossy fiber projections from the dentate to CA3, and in Schaffer collateral/ commissural fibers from CA3 to CA1 (Schwartzkroin and Wester, 1975; as cited in Bliss et al., 2007). Moreover, LTP has been induced in more natural conditions via briefer and higher frequency tetanus (for example, Douglas and Goddard, 1975).

It is beyond the scope of this introduction to cover the complex pharmacology of LTP. For our purposes of evaluating LTP as a valid cellular mechanism of learning and memory, however, it is necessary to review the parallels between the properties of LTP and that of learning and memory.

There are three defining properties of LTP that support its candidacy as a cellular mechanism for learning and memory: cooperativity, associativity, and input specificity. Cooperativity refers to the fact that low frequency stimulation of pathways that converge onto the same area on the postsynaptic membrane can collectively induce LTP (McNaughton et al., 1978). Associativity refers to a condition in which stimulation that induces a strong synaptic response is coupled with stimulation that induces a weak synaptic response (i.e. low frequency stimulation inadequate to induce LTP) on the same neuron. Eventually, the weak synapse is strengthened such that LTP is induced to it alone (Barrionuevo and Brown, 1983; Levy and Steward, 1979, 1983). Unlike cooperativity, associativity can occur in synapses that are far apart on a neuron. Both cooperativity and associativity, however, require temporal contiguity of events, as in Pavlovian conditioning. Finally, input specificity refers to the selectivity of LTP; LTP induced at one synapse will not arbitrarily propagate to adjacent synapses, but rather it propagates according to the rules of cooperativity and associativity (Andersen et al., 1977; Dunwiddie and Lynch, 1978). As such, the specificity of LTP likely results in the activation of the same neurons which may support memory retrieval.

Another feature of LTP that models learning and memory is the early- versus late- forms of LTP (Frey et al., 1988, 1993; Nguyen et al., 1994). Early-LTP refers to the increased efficacy of the synapses described above during the first 4 to 6 hours of LTP induction. Late-LTP involves gene transcription in addition to increase synaptic efficacy and typically lasts beyond 6 hours. Gene transcriptions in late-LTP result in increase of AMPA receptors (i.e. an ionotropic glutamate receptor) which increases the effectiveness of the synaptic connections, thus contributes to the long-lasting nature of LTP. The dichotomy of early- and late- LTP has been proposed as possible physiological evidence for short- and long- term memory, respectively.

Several lines of behavioral evidence support LTP as a cellular mechanism for learning and memory. For example, Whitlock and colleagues (2006) observed LTP during inhibitory avoidance learning in freely moving rats. The experimenters implanted multiple recording electrodes in the CA1 region and found enhanced field EPSP in some cells as a result of the behavioral training. In other studies, blocking LTP induction or maintenance via either pharmacological or genetic methods has been shown to hinder learning and memory. Recently, Pastalkova and colleagues (2006) demonstrated that late-LTP is required for long term spatial memory in an active place avoidance task. Intrahippocampal infusion of a synthetic peptide ZIP (which inhibits a cell permeable protein kinase PKMζ that is required for late-LTP maintenance) effectively obliterated long term spatial memory acquired either 22 hours or 30 days prior to infusion. Performance in short term memory and the capacity to learn were left intact, as ZIP spares early-LTP. These findings strongly support LTP as a cellular mechanism for learning and memory.

It is worth mentioning that the idea of LTP being the cellular model for learning and memory is not unequivocal. Counter evidence such as intact spatial learning after attempts of LTP blockade raises doubts as to the function of LTP (Saucier and Cain, 1995; Bannerman et al., 1995). In those studies, researchers attempted blocking LTP via the blockade of NMDA (N-methyl-D-aspartate) receptors that was previously believed to be essential for LTP onset. However, it was later found that a part of the trisynaptic circuit (namely the projections from the dentate to the CA3 subfield) exhibits NMDA-independent LTP or non-associative LTP. Other notable forms of synaptic plasticity include mossy-fiber potentiation, neurotrophin-induced potentiation, EPSP-spike potentiation, and long term depression (LTD). Clearly, LTP is but one form of synaptic plasticity that occurs during learning (Morris and Frey, 1997).

To summarize, although evidence for other forms of synaptic plasticity is emerging, LTP remains to be the most widely studied cellular mechanism in learning and memory. The main reason for its popularity is that LTP has many characteristics that suits our concept of learning and memory. LTP induction is rapid; once it is induced, it can last for hours or days. The induction of LTP requires temporal contiguity of activation amongst cells. Induction of LTP is known to be specific to the synapse; the same synapse is likely active for memory retrieval. Early- and late- LTP serve as potential cellular mechanisms for short- and long- term memory. Finally, behavioral data also support LTP as a cellular mechanism for learning and memory.

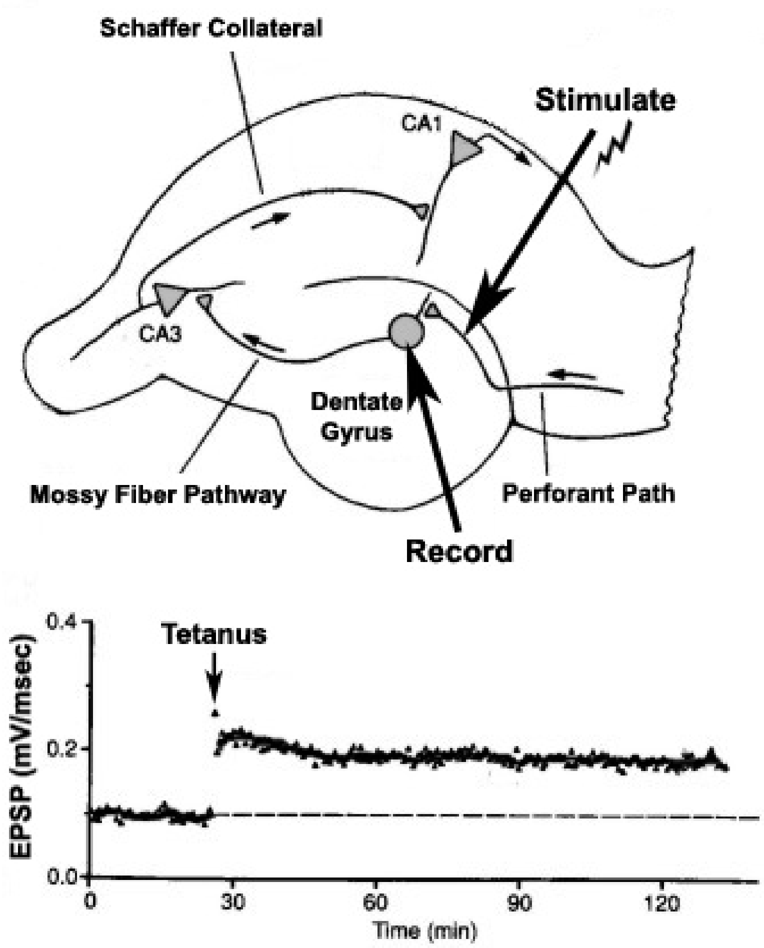


Figure 2.31. Long term potentiation. On the top, the figure illustrates how LTP is typically induced. Here, tetanus is delivered to the perforant path synapsing onto the granule cells in the dentate and EPSP recordings are monitored postsynaptically in the dentate. The large round circle denotes a granule cell soma in the dentate, large triangles represent pyramidal cell somas, and small triangles represent synapses. On the bottom, the figure shows an example of the EPSPs before and after tetanus during LTP induction (Figure after Kandel et al., 1995).

EEG recording.

Electro-encephalographic (EEG) recording is a technique used to monitor the field EPSP of a population of neurons in a region. Studies with EEG recordings in the hippocampus first began with Vanderwolf's (1969) observation of the behaving rat that the activities a rat engages in correlate with distinct moment-to-moment EEG pattern-changes in the hippocampus. According to Vanderwolf, there are three types of EEG activity: the rhythmic theta state (6-12 Hz), the large irregular amplitude (LIA) activity state, and the small irregular amplitude (SIA) activity state (Figure 2.32). Both irregular amplitude states have broad spectrums of frequencies.

Theta waves are generally found in the dentate gyrus and CA1 region. Theta waves can be further dichotomized into type I or translation-movement theta that occur during head direction changes in relation to the environment, and type II theta that occur during immobility while the animal is awake. Type II theta is known to be atropine-sensitive, whereas type I theta is not (Kramis et al., 1975).

Some evidence suggests that type I theta may be serotonergic and glutamatergic (for example, Gemma et al., 1999; Gallinat et al., 2006). The suggested functions of theta waves are that they provide 1) global synchrony of different parts of the hippocampus (Bullock et al., 1990), 2) a clocking system for hippocampal spikes (Jensen and Lisman, 2000), and 3) temporal control over the induction of LTP (Lømo, 1966, as cited in Bliss et al., 2007; Hasselmo, 2005). Bursts in theta firing of hippocampal afferent synapses have been known to induce LTP.

As in the case of theta waves, the occurrences of the two irregular amplitude states coincide with specific behaviors. The LIA state occurs during stationary activities that do not entail changes in location, such as grooming, sitting, eating, and drinking (Vanderwolf, 1969, 1971). It is classified by a behavioral state of immobility. The SIA state occurs when the animal is awakened from slow-wave sleep and rapid-eye-movement (REM) sleep (Jarosiewicz et al., 2002) or when the animal stops running abruptly (Whishaw and Vanderwolf, 1971, 1973). The exact function of SIA is not well understood.

The studies of EEG recordings provide information regarding the activity of the population of active neurons in the region of the recording electrode. One caveat of EEG studies is that the recordings reflect the overall function of the region from the activities of disparate cell types of both excitatory and inhibitory synaptic potentials. Thus, EEG recordings do not reflect the precise activities from active neurons at the cellular level. To better understand hippocampal activities during learning, individual neuronal responses are studied with single cell recording.

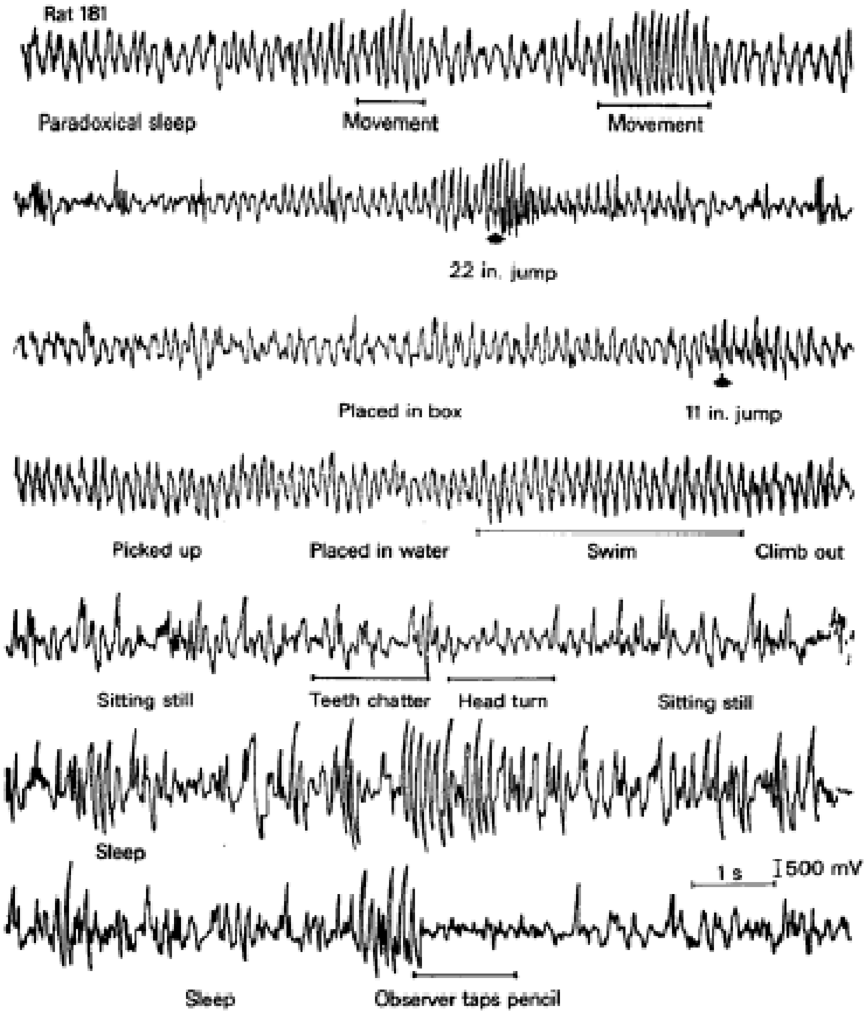


Figure 2.32. Seven traces of hippocampal EEG recordings of a behaving rat. Trace 1: theta waves during REM sleep. Traces 2 and 3: theta waves during jumping. Trace 4: theta waves during swims. Trace 5: LIA activity during quiet sitting. Traces 6 and 7: LIA during slow wave sleep and SIA during brief awakening from pencil tap. (Figure from Whishaw and Vanderwolf, 1973 [3]).

---

[3] Reprinted with permission from author.

Single cell recording.

The recording of activity of individual neurons is referred to as single cell recording. This is achieved by implantation of an extracellular microelectrode that can monitor firings of an adjacent single cell or “unit”. O’Keefe and Dostrovsky (1971; O’Keefe, 1976) found neurons in the CA3 region that fire only when the rat is in a certain location of an open field or maze. They labeled these neurons “place cells”; the specific location at which the cell fires was termed its “place field”. Place cells are found in the pyramidal cell layer in the CA3 and CA1 subfields. There is evidence that place cells are responsive to places marked by distal cues of the environment. Moreover, place cells form an abstraction of the environment that is flexible to change. For example, Muller and Kubie (1987) found that if a familiar environment with an established place field was scaled up in size with the relative position of the distal cues remain unchanged, a large subset of place cells also scaled to fire at the same angle and relative radial position to the cues (Figure 2.33a).

Since the discovery of place cells, two additional kinds of spatial cells have been found in the hippocampal system --- head direction cells in the subiculum (Ranck, 1984; Taube et al., 1990) and grid cells in the entorhinal cortex (Hafting et al., 2005). Figure 2.33b and 2.33c show examples of activity of these spatial cell types.

As suggested by the name, head-direction cells fire in response to changes in an animal’s head direction relative to the environment. For instance, a given head-direction cell may fire whenever the animal orients its head to the south, regardless of the animal’s location in the environment and its behavior. Like place cells, each head-direction cell fires to a “preferred” head direction specific to the distal cues in a given environment; the pattern of firing in the same environment remains stable over days. As the animal's head orients to the preferred direction, the firing rate of head-direction cells is increased linearly. The firing rate will remain high as long as the animal's head is oriented to the preferred direction (Taube and Muller, 1998; as cited in Taube, 2007) Head-direction cells are highly dependent on the vestibular system; inactivation of the vestibular hair cells abolishes head-direction cell signals (Stackman et al., 2002).

In contrast to place cells and head-direction cells, grid cells are less specific; each grid cell fires when the rat is in several locations in the environment. In the deeper layers of the entorhinal cortex (layers III to VI), grid cells are found to co-localize with head-direction cells and a large percentage of grid cells in these layers have head-direction preferences (Sargolini et al., 2006). Moreover, the firing rates of both grid cells and head direction cells in the deeper entorhinal layers are speed-dependent. The discovery of these direction-sensitive grid cells suggests they are part of a navigation system that integrates location, head direction, and speed to provide trajectories for the moving rat.

The discovery of place cells led to the emergence of O'Keefe and Nadel's *cognitive map theory* (1978) --- the most influential theory of hippocampal functions to date. In the next section, I will discuss this theory and others.

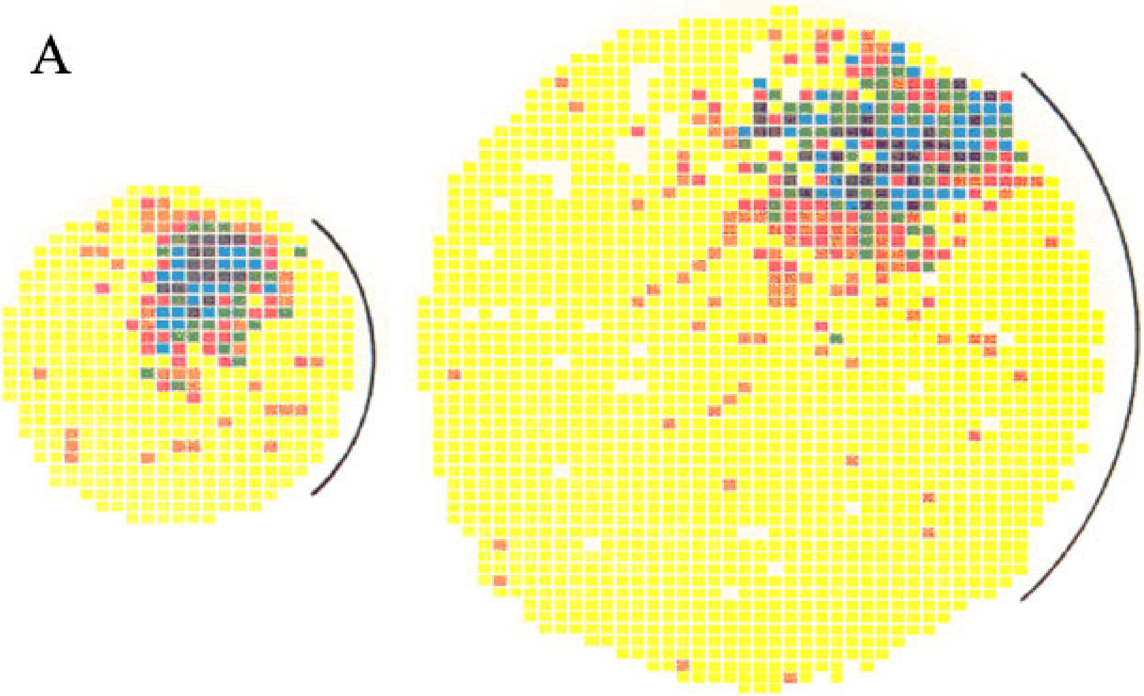


Figure 2.33a. Place cell recording. The colored grid shows the place cell activity of an animal in the place field relative to a visual cue in the environment (signified by the black arc). When the cue is moved, the place cell still fires to the animal's location in relation to the cue (not shown). When the environment is scaled up in size, a significant number of place cells also scaled up to retain firing at the same angular and relative radial position to the cue. (Figure from Muller and Kubie, 1987 [4]).

---

[4] Copyright 1987 by the Society for Neuroscience. Reprinted with permission.

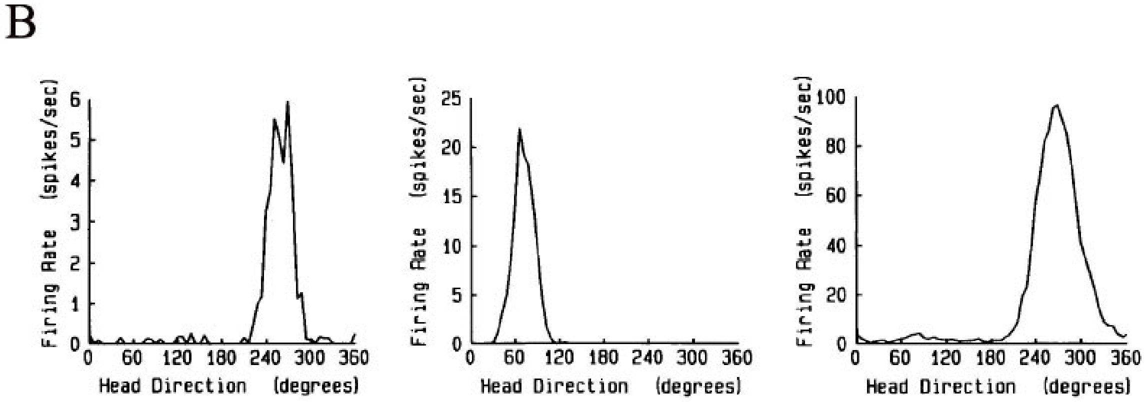


Figure 2.33b. Head direction cells firing patterns. The figure shows the firing of three representative head direction cells from three different animals plotted against the animals' head-direction angle. A given cell fires when the animal's head is at the "preferred" angle. (Figure from Taube et al., 1990 [5]).

---

[5] Copyright 1990 by the Society for Neuroscience. Reprinted with permission.

C

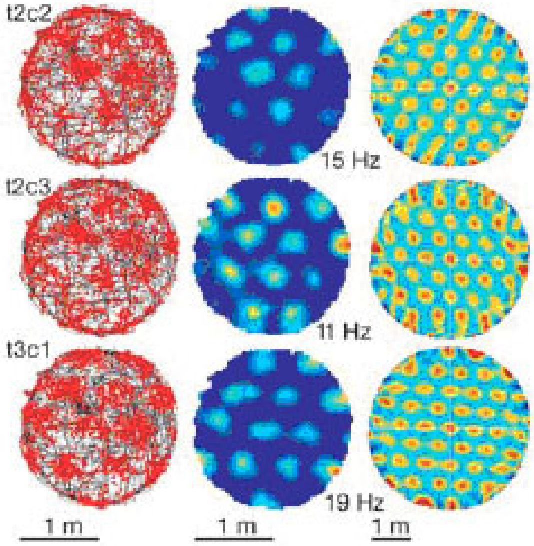


Figure 2.33c. Entorhinal grid cell firing patterns. The figure shows the firing of three cells (in rows) recorded simultaneously in the same animal during 30 min of activity in a circular open field. The left column represents the rat's trajectory, with red lines representing spiked locations. The middle column represents color-coded rate maps, with red indicating peak rates and blue indicating no activity. The right column represents spatial-autocorrelation of the rate maps. (Figure from Hafting et al., 2005 [6]).

---

[6] Reprinted by permission from Macmillan Publishers Ltd: Nature (Hafting et al., 2005), copyright (2005).

2.4 Theories

Thus far, this introduction has reviewed physiological evidence suggesting that learning and memory are related to LTP. The evidence for the correlation between hippocampal neuronal activity and behavior has also been provided by reviewing findings from EEG recordings and single cell recordings.

In this section, I will consider selected theories proposed to explain hippocampal function. I will discuss 1) O'Keefe and Nadel's cognitive map theory (1978), 2) Olton and colleague's theory of working versus reference memory (1979), and 3) Sutherland and Rudy's configural association theory (1989; Rudy and Sutherland, 1995). Although these theories are quite different, they were based on similar evidence from hippocampal studies.

To obtain a complete understanding of the functional significance of the hippocampus, selective lesions of the hippocampus in whole or in part are often induced; studying animals' behaviors after lesion damage helps to clarify the behavioral function of the structure. It should be cautioned that the behavior of animals after irreversible hippocampal lesions reflect post-recovery systems that may be different from that of a normal animal.

Cognitive Map Theory

In 1975, O'Keefe and colleagues discovered that lesions of the fimbria-fornix in rats impair place learning in a circular maze task while sparing cue learning (see Figure 2.41). This finding, along with the discovery of place cells, led to the emergence of O'Keefe and Nadel's cognitive map theory (1978).

O’Keefe and Nadel propose that the hippocampus contains a “locale” system, or a representation of the environment that navigation is dependent upon. They also proposed a separate “taxon” system responsible for simpler associative learning that is hippocampal-independent. Locale is allocentric --- it helps animals to form a mental map of the environment that is independent of the direction of the animal; in contrast, taxon is egocentric --- it serves to learn routes through the environment that results from using a series of stimulus and response associations. A common analogy that contrasts locale and taxon is “knowing where” and “knowing how”.

O’Keefe and Nadel defined the taxon system as goal-directive; it directs the animal’s attention to specific object or specific turns through egocentric space *en route* (i.e. turn left after *x* paces). Depending on the situation, the goal may be to approach or to avoid a positively or negatively perceived object. Taxons are slowly established and are inflexible; they must be used in a fixed sequence in a given environment. While simple associative learning is possible with the taxon system, it is susceptible to interference as it lacks a spatial / contextual component that can distinguish between different contexts. Conversely, the locale system is not goal-specific; it can be used to reach a variety of goals or places. Locales are quick to form and are flexible; they are not affected by any one particular landmark or cue but rather they are a representation of connected places in space. New routes can be readily formed via locales. Once mapping for an environment is established, animals can allocate attention away from predictable objects in the environment and attend to novelty. Distinct representations are formed even when a familiar object is displaced to an unfamiliar location in the same environment.

The cognitive map theory is the first comprehensive proposal of hippocampal function. It remains to be the most influential hippocampal theory to date.

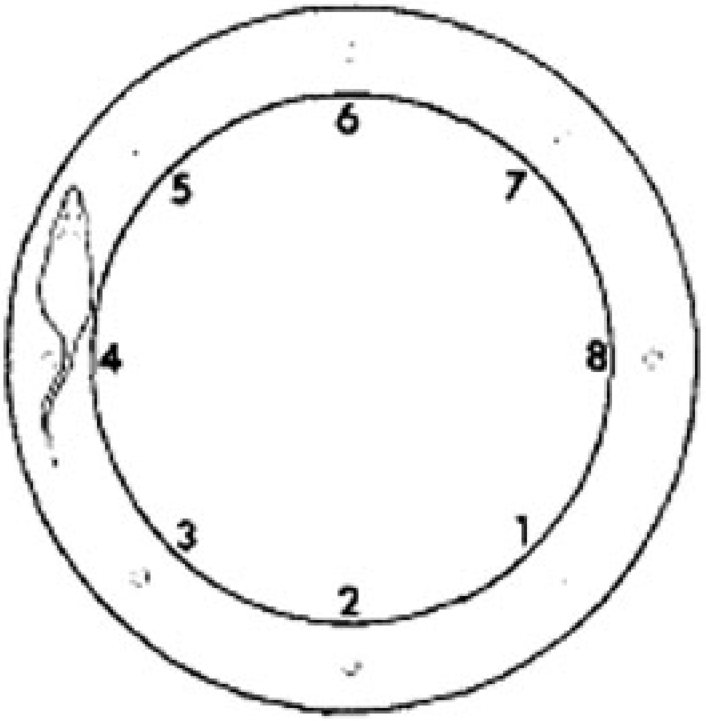


Figure 2.41. Circular maze task. Water deprived rats are trained to go to a water-well in one of eight locations to get water. In the place version of the task, the water-well position is in a fixed location in relation to distal cues in the test room. For a cued version of the task, a spot light shines onto random rewarded water-well locations, with the location of the reward changing on every trial. Fimbria-fornix lesions impair rats on the place but not the cued version of the task (Figure from O'Keefe et al., 1975 [7]).

---

[7] Reprinted from Experimental Neurology, 48 (1), O'Keefe, Nadel, Keightley, and Kill. Fornix lesions selectively abolish place learning in the rat. pp. 152-166, Copyright (1975), with permission from Elsevier.

Working/ Reference Memory Theory

In 1977, Olton reported an impairment in rats on the radial arm maze (RAM), a spatial task in which performance is dependent on extramaze visual cues. The rats had their septo-hippocampal connection severed. (Septal nuclei projections from the basal forebrain represent the major cholinergic input to the dentate.) A detailed description of the RAM task is provided in Figure 2.42. In an eight-arm version of RAM where all arms were baited, Olton (1977) noted that rats with hippocampal deafferentation/ deefferentation made more errors or re-entry to previously visited arms where the baits have been retrieved.

Subsequently, Jarrard (1978, 1986) found that fimbria-fornix and hippocampal lesion rats were impaired on the working-memory version of the RAM. In the working-memory version of RAM, four of the eight arms were never baited while the other four arms are consistently baited. To optimize performance during retrieval of baits, a rat must simultaneously remember the arms visited (i.e. working memory) and the four never-baited arms (i.e. reference memory) to avoid re-entry to emptied arms. While hippocampal-damaged rats have no trouble avoiding the never-baited arms, they commit more errors on baited arms.

In view of Jarrard's findings, Olton and colleagues (1979) proposed a theory of two simultaneous memory systems in rats --- working memory (i.e. short term memory with readily accessible information for the duration of one test trial) and reference memory (i.e. long term memory of information across a series of test trials). Working memory is deemed to be hippocampal-dependent, whereas reference memory is not. Olton et al.'s proposal of a hippocampal-independent reference memory system would explain the observation that hippocampal lesion rats can nevertheless remember which locations to avoid and thus reflect intact cognitive mapping abilities. Referring to O'Keefe and Nadel's cognitive map theory, while the impairment in working memory in RAM can be explained by the lack of a locale system for the integration of spatial and reward-retrieval information, the cognitive map theory cannot fully explain the sparing of reference memory.

Data from yet another spatial task, the Morris water maze (MWM), raises questions on the role of the hippocampus in reference learning. In the simplest form of MWM, rats acquire a fixed location of a hidden platform via distal cues and swim to it for escape (See Figure 2.43 for task description). Normal rats quickly learned to swim directly to the location of the platform regardless of the start location. In contrast, hippocampal lesion rats are severely impaired at acquiring the platform location, indicating that the hippocampus is necessary for reference memory learning (Morris et al., 1982; Steele and Morris, 1999). Figure 2.43 illustrates the MWM performance of a typical hippocampal lesion rat. With overtraining, however, rats can learn to compensate for their impairment by learning to swim to the platform quadrant via what Olton and colleagues claim to be working memory.

Is the integrity of the hippocampus not necessary for the reference memory of places as suggested by Olton and colleagues? Surprisingly, even though hippocampal lesion rats can learn the approximate location of the hidden platform on the MWM with overtraining, their learning is sensitive to novel start locations and the change of distal cues (Morris et al., 1990; Eichenbaum et al., 1990). These properties resemble those of O'Keefe and Nadel's hippocampal-independent taxon system, which may have mediated the compensatory response of swimming to the approximate hidden platform location. Subsequent studies with hippocampal-damaged rats show no deficit in non-spatial working memory, thus disputing Olton et al's view (Aggleton et al., 1986; Rasmussen et al., 1989). It is possible that the reference memory performance on the MWM task was based on the taxon system (e.g. by tending to one distal cue that is near the target quadrant) and therefore performances were reflective of a hippocampal-independent system. Thus, the working versus reference memory model Olton and colleagues proposed may not be in direct competition with the cognitive map theory.

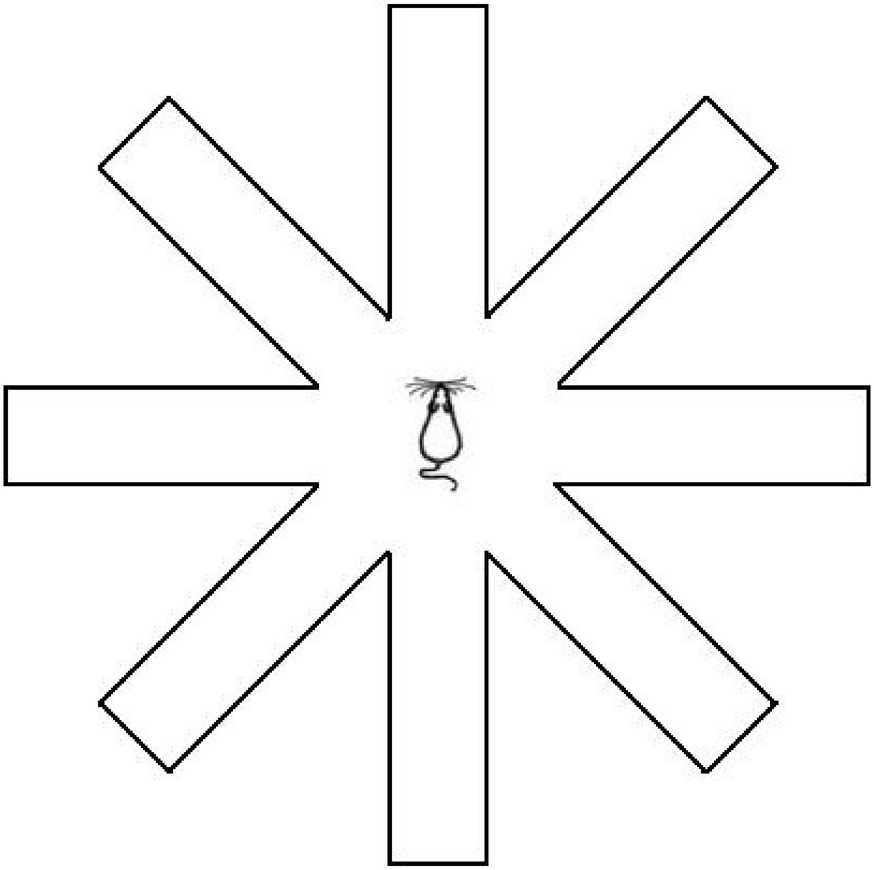


Figure 2.42. An eight-arm radial arm maze (RAM). The task was developed by Olton and Samuelson (1976). Food rewards are baited in wells hidden at the ends of the arms. A food-deprived animal is placed in the center of the maze to freely retrieve the baits. In the working-memory version of the task, four of the arms were never baited. Rats learn to remember which arms are consistently non-baited (i.e. reference memory) as well as which baited arms were visited (i.e. working memory) to avoid re-entry (Figure from http://www.ratbehavior.org/RatsAndMazes.htm [8]).

---

[8] Reprinted with permission from Anne Hanson, copyright (2003, 2004).

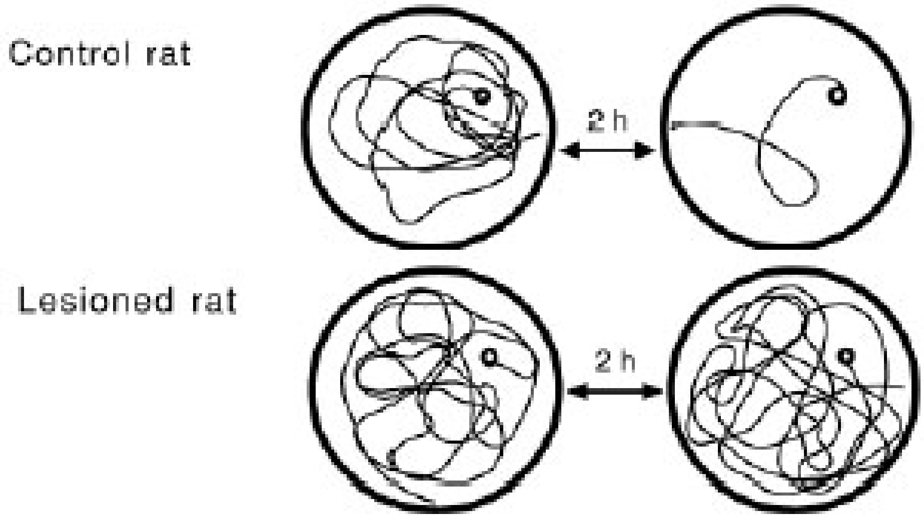


Figure 2.43. Representative swim paths on a delay-matching to place version of the Morris water maze task (Morris et al., 1982). The task consists of a maze that is a large round pool with opaque water containing a hidden platform below the water surface (location indicated by a small circle on each swim path). Rats or mice are released into the water to locate the platform for escape. They must learn to remember the location of the hidden platform via distal cues surrounding the pool. The typical swim paths for the acquisition and probe test of a control rat and a hippocampal lesion rat were shown. Control rats tend to swim directly to the hidden platform location, whereas hippocampal lesion rats swim haphazardly at the probe trial, indicating no memory of the hidden platform location (Figure from Steele and Morris, 1999[9]).

---

[9] Copyright 1999 Wiley-Liss Inc, U.S.A. Reprinted with permission.

Configural Association Theory

According to the cognitive map theory, associative learning is possible in the absence of hippocampus under the condition when the associations are simple. Simple associative conditioning refers to a situation in which a conditioned stimulus (e.g. tone) unambiguously predicts an unconditioned stimulus (e.g. shock). In cases where the association is complex or nonlinear, the hippocampus is required. This is the basis of Sutherland and Rudy's configural association theory (1989; Rudy and Sutherland, 1995).

Sutherland and Rudy's (1989) theory was initially devised to predict the function of the hippocampus for nonlinear associative problems, whereby the predictability of a conditioned stimulus (CS) to an unconditioned stimulus (US) is dependent on a qualifier, or the presence of a third factor. For example, a tone (CS) effectively predicts shock (US) only in the presence of light (qualifier); neither tone nor light unambiguously predicts shock, therefore this poses a nonlinear associative problem in which animals have to learn that the conjoint representation of light and tone predicts shock.

Four types of nonlinear tasks were used to test the configural association theory: negative patterning, transverse patterning, bidirectional discrimination, and feature-neutral discrimination. The configural association theory predicts impairment in all four tasks. A summary of the tasks is provided below:

Negative Patterning	A+, B+, AB-
Transverse Patterning	A+ B-, B+ C-, C+ A-
Bidirectional discrimination	AB+, CD+, AC-, DB-
Feature-neutral discrimination	AC+, C-, AB-, B+

A discussion of various findings from the four nonlinear tasks is reviewed by Rudy and Sutherland (1995). I will briefly review them here. In negative patterning, stimulus A and stimulus B are each rewarded when they appeared separately. However, when they appear in conjunction (i.e. AB), they are not rewarded. Empirical evidence on negative patterning with hippocampal lesion rats is inconclusive (Sutherland and Palmer, 1992; Davidson et al., 1993, McDonald et al., 1997). In transverse patterning, when A is presented with B, A is rewarded; when B is presented with C, B is rewarded; when C is presented with A, however, C is rewarded. Hippocampal lesion rats are able to perform the first two associations successively, but they have trouble with the third (Alvarado and Rudy, 1995a). In bidirectional discrimination whereby a given stimulus predicts reward and non-reward equally (i.e. A is rewarded when paired with B but not with C), hippocampal lesions seem to spare this type of discrimination (Whishaw and Tomie, 1991). Finally, in feature-neutral discrimination, combinations of stimuli (i.e. AC+, AB-) predict differently than the stimulus alone (C-, B+). Since each stimulus predicts reward and non-reward equally, the net valence of any stimulus would be “neutral”. Empirically, hippocampal lesions do not impair rats on feature-neutral discrimination (Gallagher and Holland, 1992; Alvarado and Rudy, 1995b).

In view of the mixed results from their predictions, Rudy and Sutherland (1995) revised their theory to state that the configural association of elemental stimuli is stored elsewhere in the neocortex, but the hippocampus remains important in enhancing associative strengths of the formed associations.

O’Reilly and Rudy (2001) later extended the configural association theory to conjunctive learning (i.e. learning of co-occurring stimulus features). They stated that the hippocampus is involved in pattern separation, and it serves to rapidly detect similarity in stimuli and to make the events different to minimize interference. In contrast, the neocortex establishes conjunctive learning over multiple episodes. Neocortical learning is believed to be deliberate and occurs when the animal is driven to problem-solve, as opposed to hippocampal learning that is automatic or incidental. Empirical evidence suggests that hippocampal lesions impair rats in spatial and temporal pattern separation (for review, see Kesner and Hopkins, 2006).

Applying the configural association theory to spatial tasks, a representation of space can be considered a special case of configuration in which multiple stimuli within a given context or environment guide the animal through space. Damage of the hippocampus would impair the animal's ability to form associations between multiple stimuli and hence impair navigation. This prediction fits that of the cognitive map theory. Multiple exposures of a given space would lead to eventual storage of stimuli that is hippocampal-independent, as is predicted by Olton and colleague's working memory theory. Thus, the configural association theory posits a proposal that broadens hippocampal functions to associative learning; it also proposes a hippocampal-mediated network of associations of configural memory in the neocortex.

2.5 Object memory

As discussed, the hippocampus has an established role in memory. A number of empirical findings and theories have implicated the hippocampus in spatial learning. Its role in another form of memory --- object memory --- is less clear. Structurally, the hippocampus has strong connectivity with the perirhinal cortex, a site in the adjacent parahippocampal region that is synonymous with object recognition. Given this connection, and given its general importance in mnemonic functions, many researchers have postulated that the hippocampus may contribute to object memory.

Animal studies, specifically in delay-matching-to-sample (DMTS) and delay-non-matching-to-sample (DNMS) tasks, implicate the hippocampus in object recognition, while the novel object recognition task produces no such evidence. Findings from both types of tasks are reviewed in this section.

Delay-matching-to-sample.

Data from DMTS and DNMS tasks in general supports a delay-dependent view of the hippocampal function in object memory. In particular, Clark and colleagues (2001) have found that bilateral lesions of the hippocampus render a delay-dependent impairment in rats in Mumby's DNMS task (see Figure 2.51 for task descriptions).

The performances of rats with hippocampal damage were temporally graded in the Mumby box, such that rats showed no impairment when the recall was immediately following the sampling of an object. Impairment was found when the retention interval was longer than one minute. Similar results were found in a swimming DMTS task developed by Prusky and colleagues (2004) in which rats were trained to recognize pictures on computer screens for escape (see Figure 2.52 for task descriptions). After performance reached the predetermined criteria, rats underwent either hippocampal or perirhinal lesions. While postoperative performances of both groups of lesion rats displayed impairments across various retention intervals, hippocampal lesion rats performed better than perirhinal lesion rats at intermediate retentions of thirty seconds to two minutes. Beyond two minutes, however, hippocampal lesion rats were unable to perform the task. Thus the results from DMTS literature supports a delay-dependent role of the hippocampus in object recognition.

Novel object recognition. Contrary to DMTS findings, results from novel object recognition (also known as spontaneous object recognition) indicate that rats with hippocampal damage can recognize objects at much longer delays. For example, Forwood and colleagues (2005) were able to show recognition of objects in hippocampal lesion animals after up to 48 hours of retention. In the standard version of the novel object recognition task developed by Ennaceur and Delacour (1988), rats were given one trial to be familiarized with copies of the sample objects in a non-reinforced "spontaneous" manner during the sample phase. After a delay, rats were presented with a copy of the sample object and a novel object at the choice phase. Control animals typically prefer to explore the novel object thereby demonstrating their memory for the sample object. By comparing the time rats spent exploring the familiar sample object versus the novel object, the memory for the sample object can be inferred.

Some studies attribute the sparing of recognition memory for hippocampal lesion rats in the novel object recognition experiments to be a function of lesion size (for example, Broadbent et al., 2004). This view, however, has scant support. For example, in a double dissociation study of the hippocampus and the perirhinal cortex, Winters and colleagues (2004) demonstrated that extensive hippocampal damage in rats obliterated spatial memory, while sparing object memory. The opposite impairment was found with perirhinal cortex lesions, in which perirhinal damage impaired object memory but not spatial memory. Thus, the discrepancy observed in the DMTS and novel object recognition tasks is likely due to the differences in task demands and cannot be explained by lesion size alone.

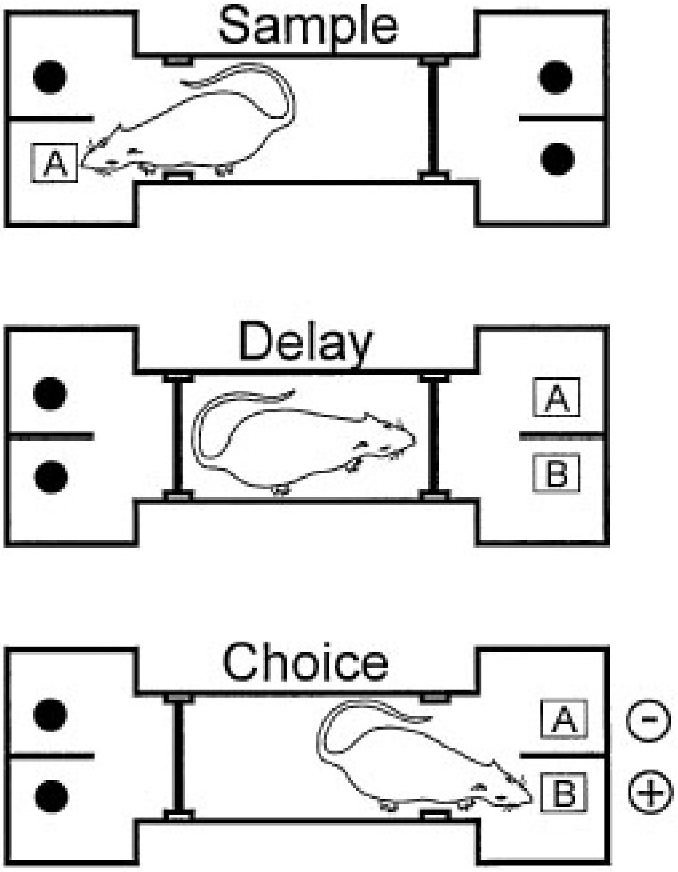


Figure 2.51. Mumby’s delay-non-matching-to-sample apparatus for object recognition. In this task, rats were trained to recognize a sample object (i.e. object A) by displacing it for food reward during sample. After a delay, rats were shown a copy of the sample object (i.e. object A) and a novel object (i.e. object B) at the opposite end of the apparatus. Rats were required to displace the novel object (hence the object “non-matching” to sample) at the choice phase for food reward (figure from Clark et al., 2001[10]).

---

[10] Copyright 2001 Wiley-Liss Inc, U.S.A. Reprinted with permission.

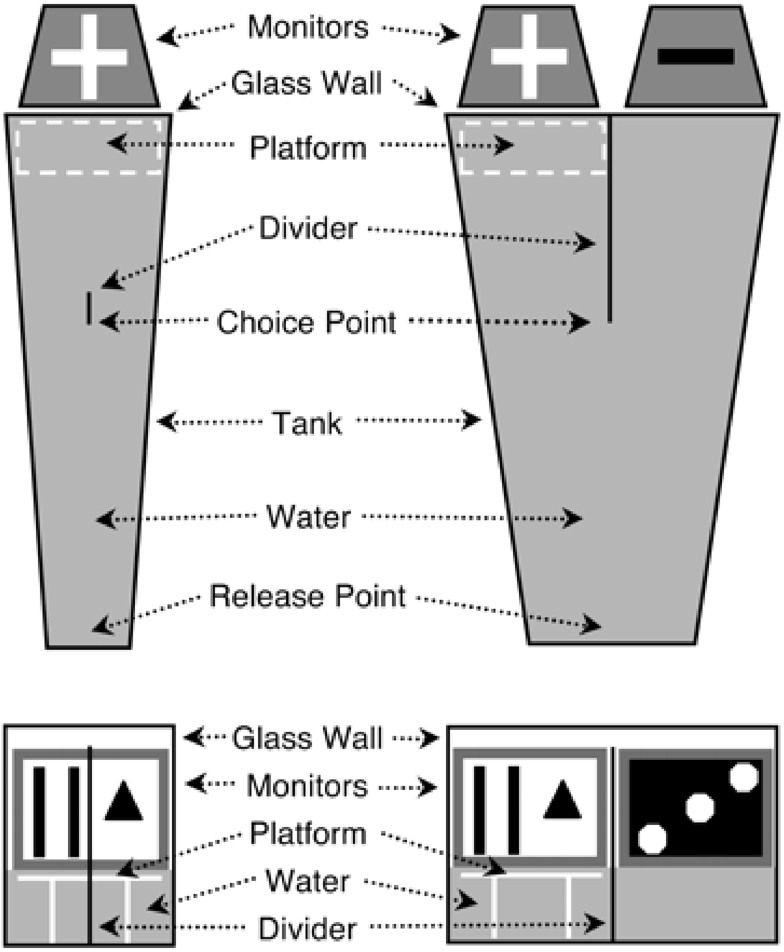


Figure 2.52. Apparatus of the delay-matching-to-sample visual water task. Rats were trained to swim to a sample picture (+) for escape in the sample pool (top left on the figure). After a delay, rats were given a choice to swim towards the previously seen sample picture (+) and a novel picture (-) in the choice pool (top right). Rats were required to swim to the sample picture to escape from the cold water. The bottom half of the diagram shows the rats' view of the pictures during the swims (Figure from Prusky et al., 2004 [11]).

---

[11] Copyright 2004 National Academy of Sciences, U.S.A. Reprinted with permission.

3. DMTS VERSUS NOVEL OBJECT RECOGNITION

The controversy surrounding the role of the hippocampus in object memory is perplexing. As reviewed in the previous section, findings from the DMTS task suggest the hippocampus is involved in object memory in a temporally graded manner, whereas findings from the novel object recognition task suggest that hippocampus is not involved in object memory.

The explanation for the conflicting findings likely resides in the task differences. It is possible that performance on the relatively complex DMTS paradigms is confounded by an animal's ability to remember the task rules (i.e. sequences of learned associations for task completion). Since DMTS requires deliberate learning and problem solving skills, performance on this task is likely neocortical dependent (O'Reilly and Rudy, 2001). In addition, performance on the DMTS task is inherently affected by motivation to external incentives (i.e. stress to escape aversive situations or the desire for reward). Recent evidence also suggests that place cells in the hippocampus signal goal expectancies in operant tasks and may further impact motivation (Hok et al., 2007). With these potential issues in mind, the novel object recognition task is better suited to test the memory of objects.

Behaviorally, the novel object recognition task provides the means of testing long term object memory that is otherwise impossible with the DMTS task. DMTS paradigms are extremely difficult for rodents to learn; the demonstrated retention on DMTS for normal rats is generally within 5 minutes but no longer than 16 minutes (for example, Prusky et al., 2004; Wood et al., 1993). By comparison, retentions of up to 48 hours of novel object recognition have been shown in rodents (Forwood et al., 2005). Thus, the novel object recognition task and its variants provide the means of testing long term object memory and selective stages of such memory. In addition, since learning on the novel object recognition task is incidental, performance on this task is likely hippocampal dependent (O'Reilly and Rudy, 2001), rendering it a more suitable method for our investigation.

Although novel object recognition provides a good alternative method for testing object memory, the task has its limitations. The non-rewarded, internally-motivated nature of the task renders the performance more sensitive to any factor that can impact explorations on or prior to a test. To compensate for this problem, a larger sample size is usually required for novel object recognition experiments. Care must also be given when handling rats at all times so as to reduce the stress and any other factor that may potentially affect rats' motivation to explore.

4. RESEARCH OBJECTIVES

In the current literature, most object memory studies on the role of the hippocampus are focused solely on investigating the hippocampus' involvement in the recognition of a familiar object from novelty. Beyond that, evidence for hippocampal involvement in object memory is lacking.

A few studies have investigated the role of the hippocampus pertaining to the associations of objects and the environmental context surrounding the object encounter. For example, Mumby and colleagues (2002) found that rats with hippocampal damage were unable to recognize sample objects if the object were presented in a place or in a context different from the initial object encounter. The same group of researchers also found impairment in novel object recognition regardless if the choice trial took place in a novel or in a familiar context (O'Brien et al., 2006). Thus, object memory of rats after hippocampal lesions becomes highly dependent on the context.

The present study aims to elucidate the role of hippocampal function by challenging hippocampal involvement in various aspects of object memory. In the first part of this thesis, using the novel object recognition task and its variants, rats with hippocampal damage were tested on object recognition, object recency discrimination (i.e. memory for the order of objects seen), and object context-recency association (i.e. memory for the context and the order the objects were seen). Rats with partial or complete hippocampal lesions were compared to examine whether regions of the hippocampus differ in object memory contribution.

The second part of this thesis investigated the mechanism of object memory impairment in rats with hippocampal damage. Based on the results from the first part of the study, interference was deemed to be a possible cause of object memory impairment in rats without the hippocampus. The second part of the study examined the types of interference and the direction of interference affecting object memory. Interference is operationally defined as any stimulus encountered close in time with the sample object that can potentially interfere with the memory of the sample object. It is inferred via measuring rats' memory of the sample object. Rats with complete hippocampal damage were tested on the effects of object- and context-type interference, encountered before or after a sample object.

According to cognitive map theory, although spatial memory requires the hippocampus, discrimination of the novel object is possible in its absence as both the taxon and locale system could direct an animal to novelty. With the hippocampus damaged, the goal-directive taxon system would direct rats' attention to the novel object as it is innately more interesting than the familiar object. With the hippocampus intact, the locale system could direct attention to the novel object as the rat notices both its novelty and that the space the novel object occupies is where the familiar object used to be. However, the theory would predict that only the hippocampal-dependent locale system can discriminate the relative recency of two sample objects in which both objects would be familiar and the objects would only differ in the order they were seen. To discriminate the objects based on recency, the rat likely forms a mental "map" of when an object was seen. Similarly, the theory predicts that the hippocampus would be required for associating objects with the context and the order they were seen, as rats are required to retain a mental "map" of where and when an object was seen. Finally, the cognitive map theory would predict rats with hippocampal damage to be impaired in both object-and context- interference regardless if the interference occurred prior to or after encoding, as the taxon system is susceptible to interference.

The working / reference memory theory would predict similar results as the above. Since novel object recognition likely utilizes reference memory to remember one sample object, no impairment is to be expected in the absence of the hippocampus. In contrast, both the object recency discrimination and the object context-recency association would require rats to remember and compare multiple objects for the duration of the task. In other words, both tasks require working memory. For the object interference tasks, since the memory for both the sample object and the interference object is required for successful discrimination of the sample object against an entirely novel object, the task would require the hippocampus. Context interference would likely not impair novel object recognition in hippocampal lesion rats, as the memory of the context is not required for recognizing the sample object against the novel object.

Lastly, the configural association theory, which states the hippocampus is important for moderating associative strengths of stimuli, would predict similarly that the hippocampus is required for performing object recency discrimination and object context-recency association. The theory would argue that both of these tasks require rats to remember the conjunctive representations of objects and their order, or objects with their contexts and their order. O'Reilly and Rudy's (2001) extension of the theory, which states that the hippocampus is required for conjunctive learning, would predict that the object interference will impair object recognition of rats with hippocampal damage, as the memory of both the sample object and the interference object may be considered as a conjunctive representation of objects seen prior to choice. Impairment may or may not be observed following context interference, for this would depend on whether rats would perceive the novel context as a conjunction to the sample object.

5. THE HIPPOCAMPUS AND OBJECT RECOGNITION, OBJECT-RECENCY DISCRIMINATION, AND OBJECT-CONTEXT RECENCY ASSOCIATION

ABSTRACT

A number of studies have shown that novel object recognition is independent of the hippocampus (HPC). Whether the HPC is involved in object memory beyond the recognition of familiarity from novelty, however, is unclear. In the present study, rats were tested on novel object recognition and two modified versions of the task which were designed to test the memory for the order of objects (i.e. object recency) and the association between objects and contexts, as well as the order of objects (i.e. object context-recency). Rats with complete and partial HPC lesions were tested on all three tasks. Complete HPC lesion rats were not impaired on novel object recognition, but were impaired on object recency discrimination as well as object context-recency association. Dorsal HPC lesion rats behaved very similarly to complete HPC lesion rats, whereas ventral HPC lesion rats displayed intact object recency memory in addition to intact object recognition. Ventral HPC lesion rats showed some signs of object context-recency association, possibly due to intact object recency memory. The observed impairments are speculated to be due to interference between the memories of objects.

INTRODUCTION

The hippocampus is a prominent mnemonic structure in the medial temporal lobe with well-established roles in spatial memory and plasticity (for review, see Martin and Clark, 2007). Anatomically, it has strong connectivity with the perirhinal cortex, a structure in the adjacent parahippocampal region that is implicated in visual processing and object memory (Mumby and Pinel, 1994; Buckley and Gaffan, 1998; Murray and Richmond, 2001; Bartko et al., 2007; Murray et al., 2007). Given its connectivity with the perirhinal cortex and its general importance in mnemonic functions, many have postulated that the hippocampus plays a role in object memory.

It is known that complete lesions of the hippocampus in rats produce delay-dependent deficits on object memory in the delay-matching-to-sample (DMTS) task (Clark et al., 2001; Prusky et al., 2004) while having no impact on novel object recognition (Winters et al., 2004; Forwood et al., 2005). The discrepant findings are likely due to different demands posed by the two types of tasks; specifically the more complex DMTS paradigms require rats to remember task rules and are confounded by motivational factors. From this perspective, findings from the novel object recognition task is a more appropriate measure of object memory.

At present, the investigations on the role of the hippocampus in object memory are limited to the recognition of familiarity from novelty. Whether the HPC is involved in object memory beyond recognition is unclear. Whereas the encounter of objects in the novel object recognition experiments occurred in a controlled environment with objects being the only salient cue, in actuality the memory of an object may be dependent upon factors in the environment or the circumstance of the encounter. Factors that can affect object memory may include the relative recency, the context, and/or the location where the object was seen (Mumby et al., 2002; O'Brien et al., 2006). As is known, the hippocampus is not necessary for novel object recognition but is important for spatial navigation. Thus, it would be of interest to investigate if the hippocampus is necessary for various types of object memory in a non-spatial version of the novel object recognition task.

The present study proposed to examine the effects of hippocampal damage on object recognition, object recency discrimination, and object context-recency association. Rats with complete and partial hippocampal lesions (hereon referred to as HPC lesions) were tested on modified versions of the novel object recognition task derived from Ennaceur and Delacour’s (1988) original one-trial object task. The study began with an investigation on novel object recognition as a baseline of object memory which investigated rats’ ability to discriminate a novel object from the familiar. The next task investigated rats’ ability to discriminate the relative recency of two objects, seen one before the other (i.e. object recency discrimination). The final task investigated rats’ ability to associate two objects with their respective contexts as well as the order the objects were seen (i.e. object context-recency association). In addition to using complete HPC lesion rats, two small groups of partial HPC lesion rats --- dorsal and ventral lesions --- were piloted to investigate a possible dichotomy of object memory function within the hippocampus. Dorsal and ventral HPC lesions (hereon refer to as dHPC and vHPC lesions) were chosen as this dichotomy has repeatedly shown different patterns of cognitive performance in the literature (for example, Broadbent et al., 2004; Pouthuisen et al., 2004; Stouffer and White, 2007).

Inferences from prominent theories of hippocampal function such as the cognitive map theory (O’Keefe and Nadel, 1978), the working / reference memory theory (Olton et al., 1979), and the configural association theory (Sutherland and Rudy, 1989; Rudy and Sutherland, 1995) suggest that the integrity of the hippocampus is critical for integrating multiple pieces of information. Based on these theories, the present study predicts that HPC lesions will 1) spare novel object recognition, as suggested by the literature; 2) impair rats' recency discrimination of objects; and 3) impair rats' ability to form associations between object, contexts, and recency.

MATERIALS AND METHODS

Rats

Fifty-one Long Evans female rats were obtained from the local colony at the Canadian Centre for Behavioural Neuroscience. Eighteen rats received complete HPC lesions, 15 received partial HPC lesions (8 dorsal and 7 ventral lesions), and 18 rats received sham surgeries. From our sample, a large number of rats were dedicated to complete HPC lesion surgeries compared with the number of rats that had partial lesions as the partial lesions were intended as pilot projects to observe a possible object memory dichotomy between the dHPC and vHPC regions. The majority of the sample (n = 42 from all 4 lesion types) had previously participated in a contextual fear experiment receiving one trial of tone and shock while the remainder were naïve rats. Of the 18 sham rats, 13 served as control subjects for complete HPC lesion rats and 5 were control rats for partial lesion rats.

Rats were approximately 300g at the time of surgery. They were housed in either pairs or in threes in standard colony housing, with food and water available *ad libitum*. Rats were maintained on a 12-hour dark/light cycle at 20-21°C. Testing took place during the light period of the cycle. All procedures were conducted in accordance with the guidelines provided by the Canadian Council on Animal Care (CCAC) and were approved by the local animal care committee.

Surgery

Surgery was performed under Isofluorane anaesthesia in a standard stereotaxic apparatus. In preparation for surgery, ophthalmic liquid gel was applied to a rat's eyes for protection, the hair was shaved from the top of the rat's head with an electric shaver, the scalp was cleaned with 70% alcohol and Hibitane, and an analgesic was given to the back of the neck (0.07ml of buprenorphine (Temgesic) at 0.3mg/ml, s.c.). A midline incision was made and the fascia (periosterum) was cut and pushed to the edges of the skull with a sterile gauze swab. The skin was retracted with 4 mosquito forceps to expose the skull, and holes were drilled into the skull using a 1mm drill bit and high speed drill at predetermined coordinates for the different types of HPC lesions (see Table 5.1).

The excitotoxin *N*-methyl-D-aspartate (NMDA, 7.5µg/ µl PBS) was loaded into 30-gauge injector needles, each needle consisting of 30-gauge stainless steel tubing (Smallparts, Inc) glued onto PE20 polyethylene tubing. The tubing was connected to a Hamilton 10µl syringe mounted on a Harvard mini-pump. For each injection site, the injector needles were lowered bilaterally wherever possible and 0.4µl of NMDA solutions were delivered at a rate of 0.15µl/ min. The needles were left in place for an additional 2.5 min to allow for diffusion.

To alleviate convulsions following surgery, rats were each given 0.2ml of diazepam (i.p.), with an additional 0.2ml of diazepam if convulsions persisted. Rats were monitored after surgery until they became active. Rats were then housed individually for three days to allow for recovery, following which they were group-housed at least a week prior to behavioral testing.

		AP	ML	DV
Complete HPC lesions	dHPC lesions:			
	Injection 1	-3.0	± 1.0	-3.6
	Injection 2	-3.0	± 2.0	-3.6
	Injection 3	-4.0	± 2.0	-4.0
	Injection 4	-4.0	± 3.5	-4.0
	Injection 5	-4.9	± 3.0	-4.1
	vHPC lesions:			
	Injection 6	-4.9	± 5.2	-7.2
	Injection 7	-4.9	± 5.2	-5.0
	Injection 8	-5.7	± 4.4	-4.4
	Injection 9	-5.7	± 5.1	-7.3
	Injection 10	-5.7	± 5.1	-6.0

Table 5.1. The coordinates for NMDA injections for HPC lesions. Injections 1-5 produced dHPC lesions, injections 6-10 produced vHPC lesions, while injections 1-10 produced complete HPC lesions. Measurements were taken from bregma in mm. AP: anterior-posterior; ML: medial-lateral; DV: dorsal-ventral.

Apparatus

All tests were conducted in a 13’ by 8.5’ room that was illuminated by fluorescent lights on the ceiling. Field boxes (hereon referred to as the Y-mazes) used for object trials were constructed with sheets of ¼ inch thick Plexiglas held together by acrylic and latex adhesives. The Y-mazes, as described in Winters et al. (2004), were especially designed to test object memory in HPC damaged rats. The use of the Y-mazes had the following advantages: 1) shape restricts spatial exploration ideal for HPC lesion rats; 2) its tall walls minimize the viewing of confounding spatial information outside the apparatus. The walls of the Y-maze were 40 cm high with 27 cm long arms that were 10 cm wide. As an additional precaution, three pieces of white-corrugated plastic boards (two pieces above the top arms 5 cm wide, one piece above the start arm 10 cm wide), were taped on top of each box at the end of the arms to restrict the view of the ceiling. Two different colors of Y-mazes were constructed to create two distinctly different contexts in which object exploration would occur. One type of Y-maze had white Plexiglas all around and the other had black Plexiglas walls with white floors (Figure 5.1). To further increase contrasts between the two contexts, the floors of the black Y-mazes were lined with corncob bedding. All tests of novel object recognition and object recency discrimination were conducted in the white Y-mazes, while the object context-recency association tests were conducted in both the white and black Y-mazes.

A radio situated at the north end of the test room provided background noise to minimize the effects of potential noise disturbance during object trials. All trials were video-recorded by a camera mounted on a tripod over the apparatus.

Transport

For each test, animals were singly housed in the waiting room in transport cages lined with bedding, food and water. The trials began with the experimenter placing a rat at the start arm of the Y-maze facing the wall (Figure 5.2). As soon as the rat's limbs landed on the floor of the maze, the experimenter would exit the test room, close the door, and allow the rat to freely explore the Y-maze for 3 min. At the end of the trial, the experimenter would re-enter the room to retrieve the rat.

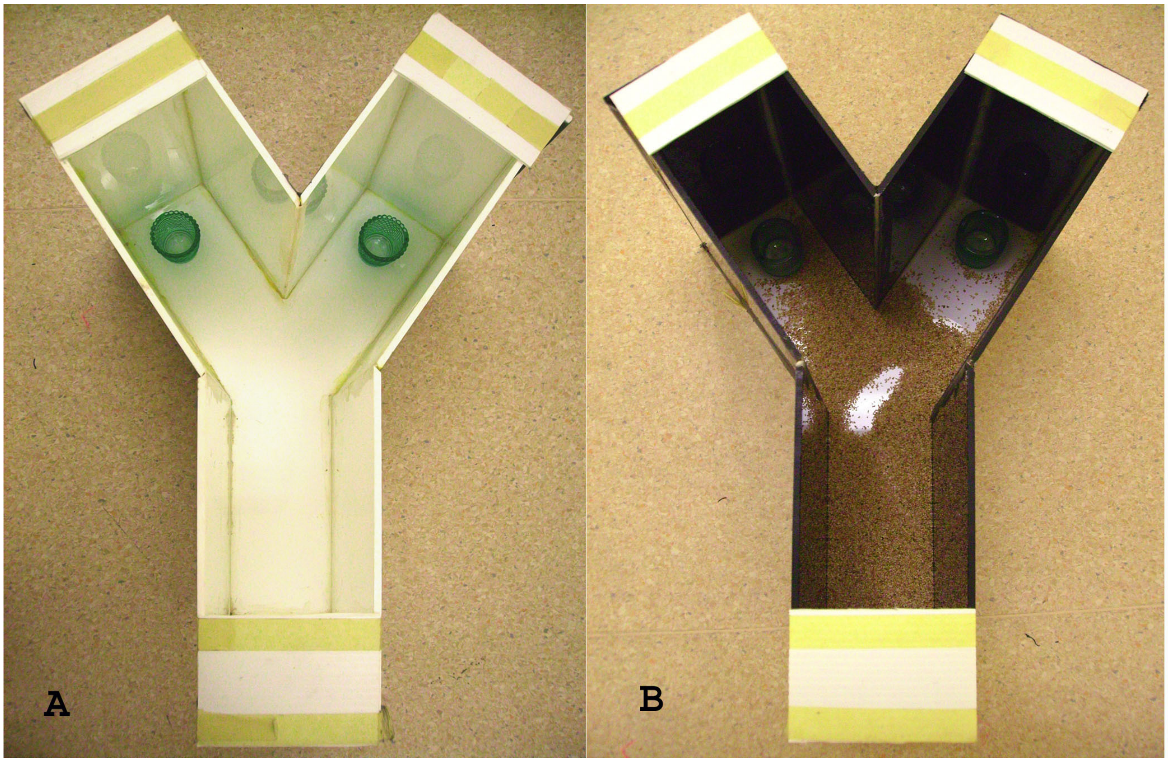


Figure 5.1. Photograph of (a) the white Y-maze and (b) the black Y-maze, with two copies of each of the sample objects shown.

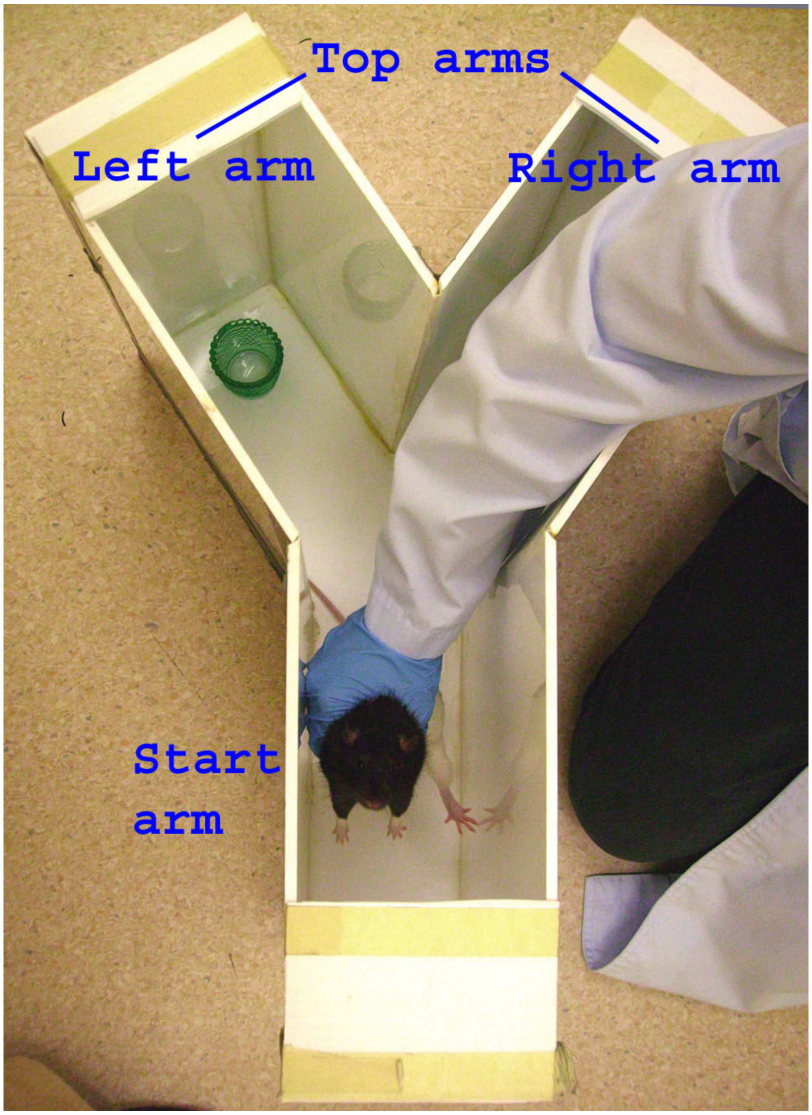


Figure 5.2. Photograph showing the components of the Y-maze and the position of the rat at the start of a trial.

Stimuli

Objects used for the memory tests were acquired from local dollar-stores. Each object-pair consists of objects made with the same material (i.e. plastic, glass, or metal). All items were previously piloted on naïve rats to ensure good baseline discrimination. Prior to all object trials, objects were cleaned in 50% ethanol then rinsed with water and patted dry. The objects were adhered to the floor of the Y-maze using white non-toxic adhesive putty.

Histology

Upon the completion of the experiment, rats were euthanized with an overdose of euthansol (0.4 ml per rat administered i.p.). Rats were transcardially perfused with 60 ml of 0.9% saline, followed by 40 ml of 4% formalin. Their brains were removed and post-fixed in a solution of 30% sucrose in 4% formalin for a minimum of 3 days. The brains were then frozen and coronal sections of 40µm thickness were cut on a cryostat at -21ºC. The brains were cut with the aid of Paxinos and Watson's atlas (1998). Every fourth section beginning from -1.6 to -7.0 mm bregma was mounted. All sections were stained in Cresyl violet (0.1%).

Video Analysis

Videos of all the sample and test trials were scored by two experienced scorers who were blind to the subjects' group- and object- assignments. The interscorer reliability was r = .943. Using stopwatches, the videos were scored on the real time rats spent on exploring each object. Object exploration is defined as head-directed active investigation within one inch proximity of the object. Chewing, sitting on top of an object, staring, and investigation of negative spaces between the object and the walls were not considered object exploration. All sample trials were scored once from 0 to 3 min, and all test trials were scored twice by the minute for the duration of each trial (i.e. 0-1min, 1-2min, and 2-3 min). For accuracy, data analysis was performed on the scores taken from the second time the test trials were scored. Scores were recorded to 0.1 sec accuracy.

Habituation

All of the rats were well-handled at the start of habituation, which began at least a week and a half post-surgery. The rats were habituated to the white Y-mazes in the test room once daily on two consecutive days prior to the first test. During each habituation session, rats were individually placed in an empty white Y-maze and were allowed free exploration of the apparatus for 5 min. No reagent was used to clean the apparatus to allow for the saturation of rat odour. In between trials when cleaning was necessary, the Y-mazes were wiped clean with dry paper towels. In the case of severe soiling, the apparatus was wiped clean with damp paper towels. All habituation sessions were video-recorded and reviewed to verify for exploratory behavior before conducting the first object test.

Behavioral procedure

The detailed test protocol for all three experiments for this chapter is summarized in Figure 5.3 and is described below:

Experiment 1: Novel object recognition

The first test of novel object recognition took place approximately 24 hours after the last habituation trial. The purpose of this task is to establish a baseline of object memory for the entire experiment. This task consists of a sample trial and a test trial. On the sample trial, rats were placed in the start arm in the white Y-maze with two identical copies of an object at the two top arms, placed one inch from the end wall (see Figure 5.2). Rats were allowed free exploration of objects in the maze for 3 min. At the end of the 3 min, rats were returned to their transport cages for the retention. Complete HPC lesion rats and their sham rats were given 15 min and 4 hr retention intervals. Partial lesion rats and their sham rats were tested on 4 hr and the 1 hr retention intervals. The 1 hr retention interval was chosen as the short retention interval for partial rats as it was deemed to be more comparable to the intervals present in experiment 2 and 3.

Following retention, a test trial commenced. For the test trial, rats were returned to the same white Y-maze they were in during the sample phase, with a novel object as well as a third copy of the sample object they have encountered earlier. Normal rats would typically prefer the novel object as it is more interesting. Based on the time rats spent on exploring each object, recognition of the relatively familiar sample object is inferred. This test was repeated with the different retention intervals conducted 48 hours apart, the order of the retention intervals was counterbalanced amongst rats.

Experiment 2: Object recency

The object recency task is designed to test the memory for the order the objects were seen. This test, similar to the one used in Hannesson et al. (2004), consists of sample trials of two objects, seen one after the other (i.e. object C in sample trial 1 and object D in sample trial 2). After retention, rats were presented with new copies of both objects (i.e. objects C and D). Normal rats would prefer to explore the object that was seen further back in time (i.e. the remotely familiar object) as it is less familiar and hence it is more interesting to investigate. The sample trials for this task were 15 min apart; the retention intervals were 1 hr and 4 hr, beginning from the end of the second sample trial.

The test for each retention interval was spaced 48 hours apart to avoid interference from the previous test. The order of the retention intervals tested was counterbalanced amongst rats.

Experiment 3: Object context-recency

This task was designed to investigate if rats were capable of associating objects with the physical contexts and the order with which they were seen. Rats were presented with two objects in two distinct contexts. The white and the black Y-mazes served as the two contexts (Figure 5.2). Prior to tests, rats were habituated to the black Y-mazes. On test day, rats were exposed to two objects, one in each context (i.e. object E in the white Y-maze, object F in the black Y-maze). After retention, a third copy of each of the objects was presented in one of the two contexts (i.e. objects E and F in either the white or the black Y-maze), rendering one of the objects in a context mismatched from the sample trial exposure. Normal rats that remember the sample objects with their paired contexts would prefer the object in the mismatched context on the test trial. For instance, on a test trial with objects E and F in the black Y-maze, rats would prefer object E as it has not been seen in the black Y-maze before. The sample trials are 15 min apart, the retention interval was 1 hr.

The three-trial design of this paradigm renders two scenarios: the “target” object (i.e. the mismatched object) is either remotely familiar or recently familiar, depending on the order the target object was seen during the sample phase. For instance, the remotely familiar target object would have been the sample object seen further back in time (i.e. during sample trial 1) and thus this target object is both contextually- and temporally-favored. Conversely, the recently familiar target object is the mismatched object encoded more recently (i.e. during sample trial 2) and thus this target object is contextually- but not temporally- favored (see Figure 5.3 for clarification). To account for this difference in design, all rats were tested once in each condition, 5-7 days apart. The order of the objects and the contexts they were tested in were counterbalanced across rats.

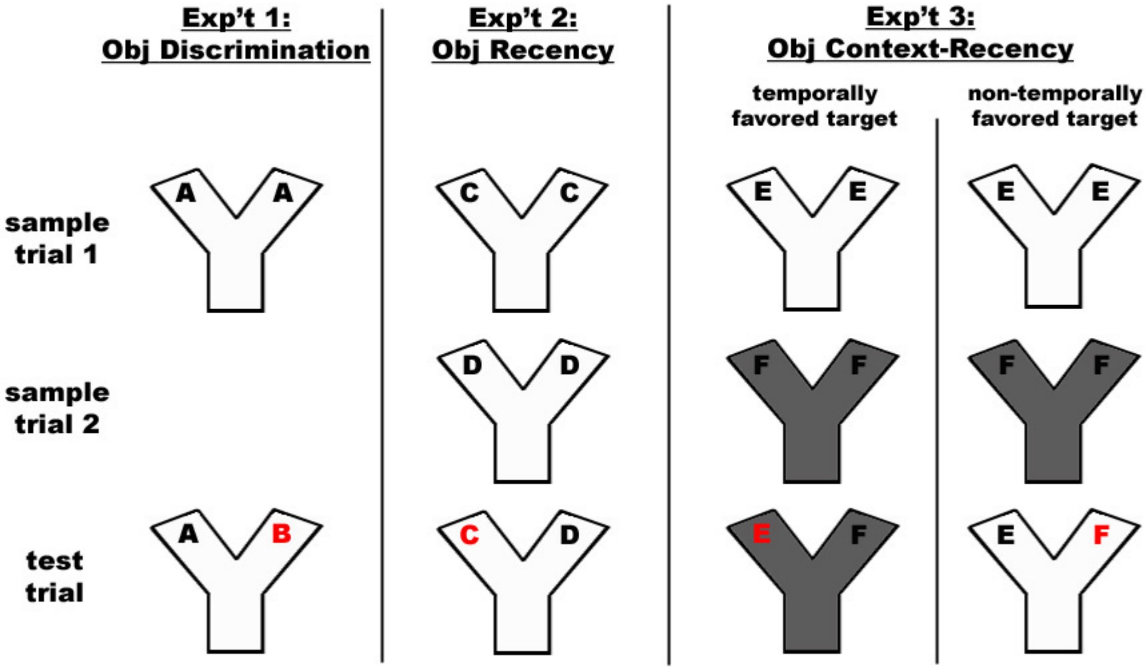


Figure 5.3. Summary of test protocols for experiments 1-3 in Chapter 5. Objects in the Y-maze apparatus are represented by letters; red colored letters denote the target object for each experiment at test trial.

RESULTS

Histology Results

Coronal sections of the rat brains were viewed under a light microscope to verify the extent of the lesion damage. Estimated percentage of HPC tissue damage was calculated based on counts of crosses superimposed on photomicrographs of coronal sections from each rat at -2.3, -3.3, -4.3, -5.3, and -6.3 mm bregma. The crosses were superimposed using ImageJ © software (version 1.37). Figure 5.4 shows images of cresyl stained brain tissues of the extent of HPC tissue damage for each type of lesion. Table 5.2 shows the estimates of mean percentage tissue damage for each lesion group.

	HPC % damage		
Lesion Type	**Average**	**Smallest**	**Largest**
Complete HPC	89.61	58.15	97.75
dHPC	30.56	22.75	46.24
vHPC	54.61	31.76	63.30

Table 5.2. Estimated percentage of HPC damage for each lesion type.

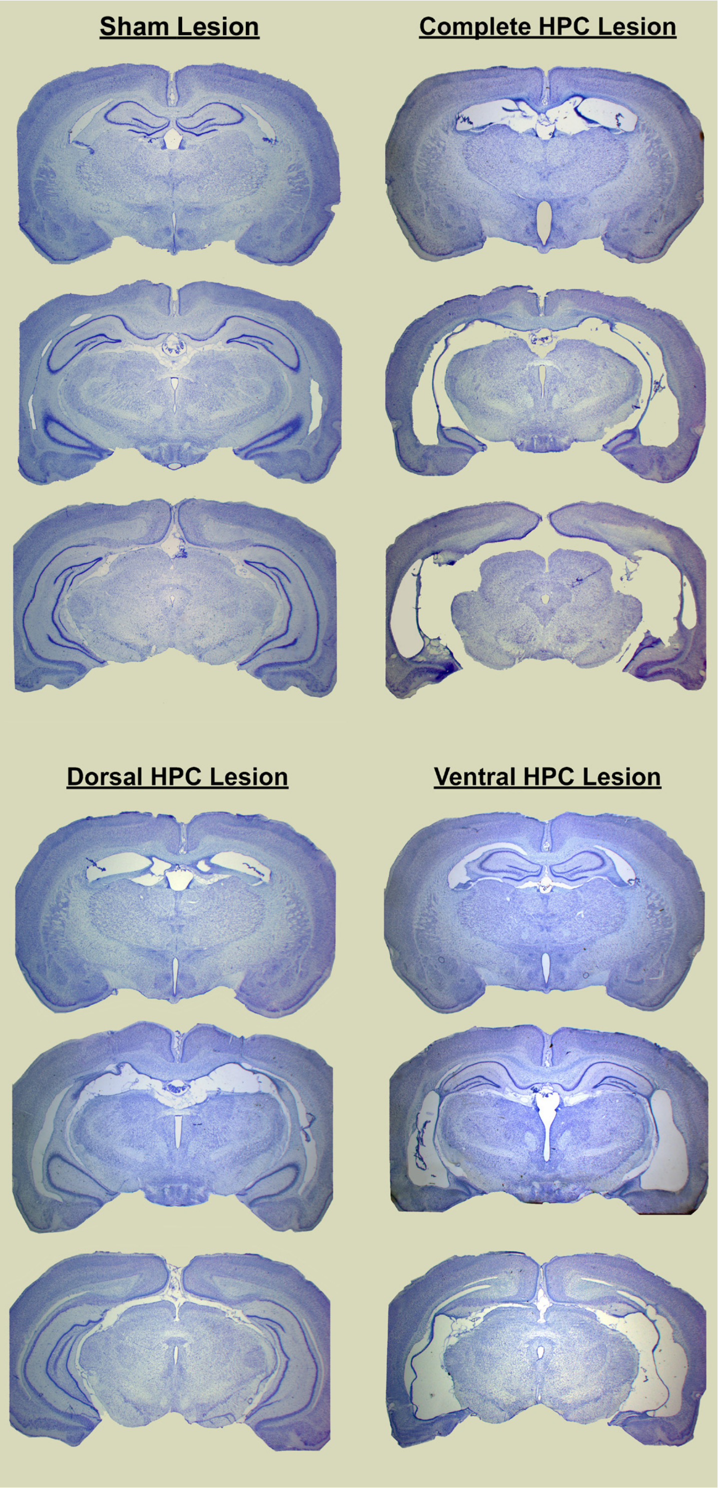


Figure 5.4. Photomicrographs of cresyl stained coronal sections for each HPC lesion type. Coronal sections show the extent of HPC damage at -2.3, -4.3, and -5.3mm from bregma.

Data Analysis

For the analysis of object exploration, the total amount of time rats spent investigating object at the sample trials was analyzed against the total amount of time rats spent investigating objects during the test trials. Repeated measures analyses were performed with TRIALS as the within-subject variable and LESIONS as the between-subjects factor for the analysis of object exploration for each of the three experiments.

For the analyses of object recognition, object recency discrimination, and object context-recency association, data for the time spent investigating the target object during the test trial were analyzed against the time spent investigating the non-target object. The analyses were performed on the object discrimination ratio (as used by Mumby and colleagues, 2002), derived from the following formula (T stands for time):

$$T_{\text{target}} / (T_{\text{target}} + T_{\text{non-target}})$$

Thus, based on this formula, a discrimination ratio of 0.5 would indicate a level of preference for the target object at chance. To determine if a discrimination ratio was different from chance, a one-sample t-test (one-tailed, against a value of 0.5) was conducted on the mean discrimination ratio from each lesion type at various retention intervals for each experiment. A ratio significantly above 0.5 would indicate a preference for the target object and thus imply object recognition or object discrimination.

Experiment 1: Novel object recognition

a) Object exploration

Figure 5.5 shows the object exploration patterns across sample and test trials according to lesion type and retention intervals tested. Complete HPC lesion rats and their shams were tested at 15 min and 4 hr retention intervals, while partial lesion rats and their shams were tested at 1 hr and 4 hr retention intervals. Since all rats were tested on the 4 hr retention interval, the two groups of sham rats were combined for that analysis. One rat from the sham group was excluded from the analysis at the 15 min retention interval as she ripped off an object during her test trial.

Analysis on the total amount of time rats spent on exploring objects during the sample and the test trials at the 15 min retention interval revealed no significant difference amongst complete lesion rats and their shams. There was no evidence of a main effect of TRIALS (F (1, 28) = 1.030, p = .319), LESIONS (F (1, 28) = 2.258, p = .144), or an interaction (F (1, 29) = 1.130, p = .297). Figure 5.5a shows the mean amount of time spent on investigating objects at both trials for complete HPC lesion and sham rats.

At 1 hr retention interval, partial lesion rats and their shams showed no main effect of LESIONS (F (2, 17) = .939, p =.411), nor an interaction (F (2, 17) = .874, p =.435). A main effect of TRIALS, however, was found (F (1, 17) = 10.314, p = .005). Referring to Figure 5.5b, exploration at the test trial was generally lower than at the sample trial. There appears to be a huge drop in test trial exploration for the sham rats. Inspection of the data's scatter plot revealed that the mean exploration of the group was affected by an outlier at the sample trial (with exploration almost two standard deviations above the mean) thus rendering the impression of a significant drop in exploration at test trial. Due to the small sample size of this sham group, outliers exert a greater weight on the group's mean. The object exploration for the majority of the sham rats at sample trial were clustered at around 20 sec.

At 4 hr retention, main effect of TRIALS (F (1, 47) = 4.081, p = .049) and a main effect of LESIONS (F (3, 47) = 6.510, p = .001) were found. Posthoc analysis (Tukey) revealed significant difference between dHPC and sham rats (p =.004) as well as dHPC and complete HPC lesion rats (p = .001). As shown in Figure 5.5c, dHPC rats have an elevated level of exploration at the sample trial compared to rats of other lesion types. Inspection of the sample trial data on scatter plot revealed the majority of explorations of the dHPC lesion group were clustered around 38 sec. However, this high level of sample object exploration was not replicated for dHPC lesion rats at 1 hr retention (Figure 5.5b). Thus, the greater level of exploration observed at the 4 hr retention is likely due to random test conditions for dHPC lesion rats on that particular test and not a reflection of heightened exploration for dHPC rats at the sample trial in general. No interaction between TRIALS and LESIONS was observed (F (3, 47) = 1.844, p = .152).

In summary, the analysis on object exploration between the sample and the test trials revealed that for novel object recognition of 1 hr and beyond, rats have a tendency to explore objects less at test trial compared to the sample. There was no strong evidence for any difference in exploration between lesion types across the sample and the test trials.

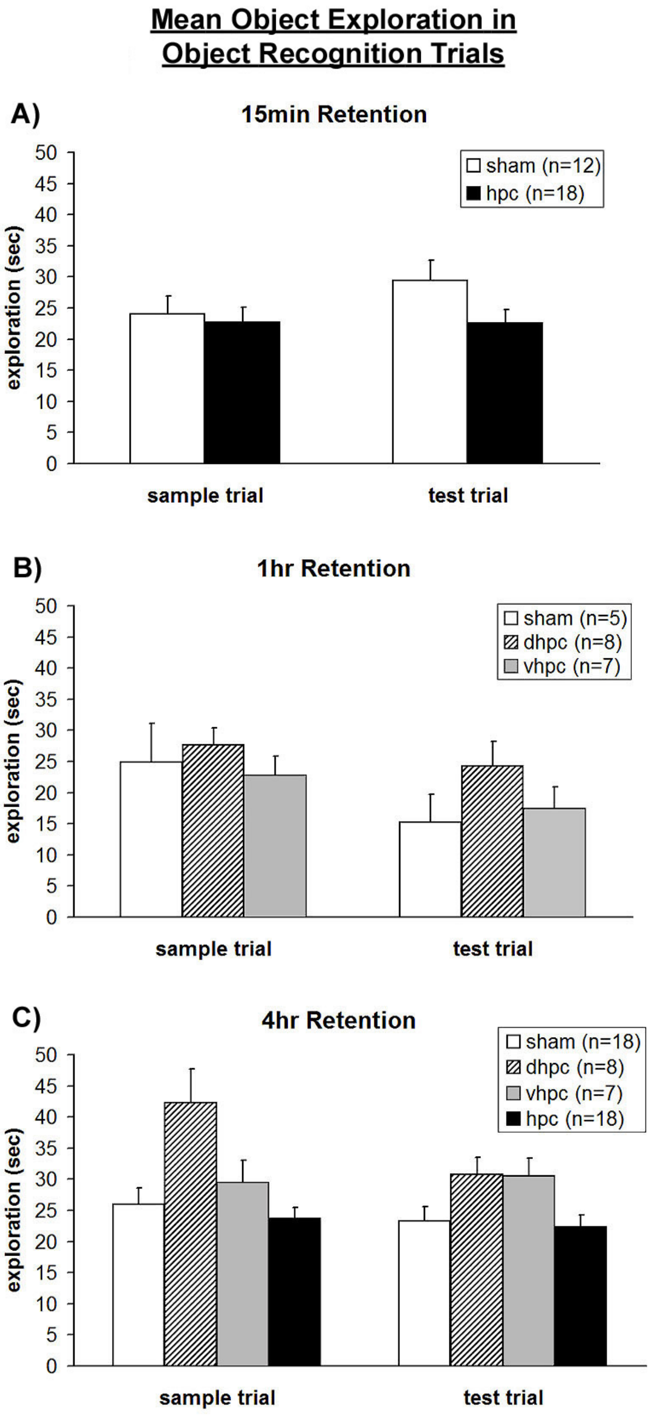


Figure 5.5. Mean object exploration at the sample and the test trials for each lesion type at a) 15 min, b) 1 hr, and c) 4 hr retention intervals on novel object recognition. Data expressed as ±SEM.

b) Object discrimination

Figure 5.6 shows the novel object discrimination ratios by lesion types on 15 min, 1 hr, and 4 hr retention intervals. Recalling that a discrimination ratio of 0.5 represents a lack of object preference, sham rats showed significant preference from chance for the novel object (i.e. the target object) at retentions of 15 min (t (11) = 7.338 , p = .00000736) and 4 hrs (t (17) = 3.310, p = .002). At 1 hr retention, sham rats' preference for the novel object was not significantly different from chance (t (4) = 1.056, p = .175). This was likely due to the small sample size of this group (i.e. n = 5). Complete lesion rats showed preferences for the novel object at both 15 min (t (17) = 3.549, p = .001) and 4 hr (t (17) = 4.014, p = .00045) retentions. DHPC lesion rats showed near significant preference for the target object at 1 hr retention interval (t (7) = 1.816, p = .056), the near-significance was again likely due to the small sample size of this pilot group (i.e. n =8). Despite the greater amount of object exploration at sample, dHPC rats showed no preference for the target at 4 hr retention. Finally, vHPC lesion rats showed preferences for the novel object at both 1 hr (t (6) = 2.038, p = .044) and 4 hr retention intervals (t (6) = 3.198, p = .010).

Taken together, experiment 1 showed that both complete HPC lesion rats and sham rats showed spontaneous preference for the novel object at both the short retention of 15 min and the long retention of 4 hr. For partial lesion rats, vHPC rats displayed object recognition at 15 min and 4 hr retentions. Although dHPC lesion rats did not show a preference for the novel object at the 4 hr retention, they had a near-significant level of recognition at 1 hr indicating robust memory of the sample object at this interval.

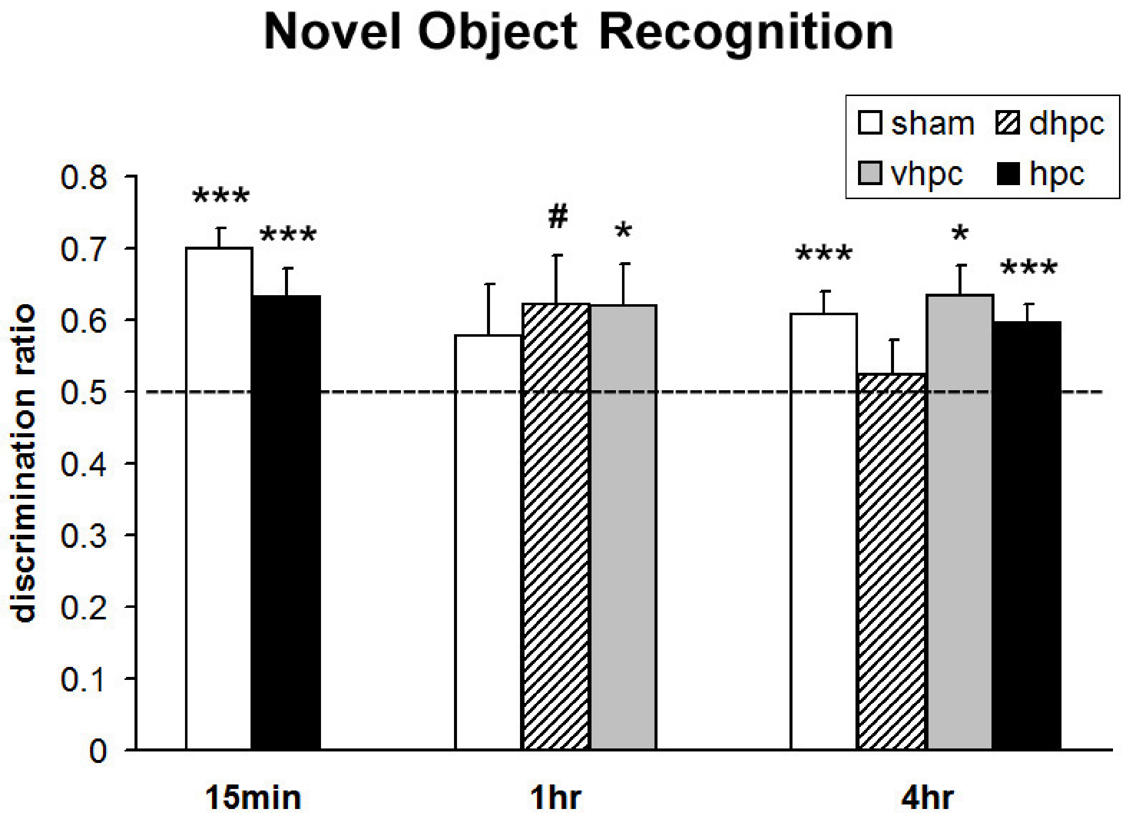


Figure 5.6. Novel object recognition, expressed by the proportion of time spent investigating the novel object versus total object exploration during the test trial. Positive values above chance (dotted line) indicate preference for the novel object. * $p < .05$, *** $p \leq .01$, and # $p < .06$ (near significance) compared to the preference for the novel object at chance level. Data expressed as ±SEM.

Experiment 2: Object recency

a) Object exploration

Repeated measures analysis of the total object explorations at 1 hr retention interval indicated no main effect of TRIALS (F (2, 92) = 1.131, p =.327), no main effect of LESIONS (F (3, 46) = 1.282, p = .292), and no interaction between the two (F (6, 92) = .509, p = .800). One sham rat was excluded from this analysis as she ripped off an object during her test trial. The mean object exploration for the three trials at 1 hr retention is shown in Figure 5.7a.

At 4 hr retention, a main effect of TRIALS was found (F (2, 94) = 4.321, p = .016). Pairwise comparisons of trials revealed test trial explorations to be significantly lower than exploration at the sample trial 1 (p = .038) and at the sample trial 2 (p = .002). Referring to Figure 5.7b, a trend of decrease object exploration, with the exception of the dHPC rats, was evident across successive trials. No main effect of LESIONS (F (3, 47) =1.639, p = .193) nor any interaction (F (6, 94) = 1.730, p = .123) was observed.

To determine if the main effect of TRIALS at 4 hr retention was due to the long delay introduced for this task, repeated measures analysis was performed separately on the total object exploration for each trial type across retentions, with trials of the two RETENTIONS as the within-subject variables and LESIONS as the between-subjects factor. A significant main effect of RETENTIONS was found for the exploration at the test trial (trial 3) (F (1, 46) = 4.386, p = .042). No main effect of LESIONS (F (3, 46) = 1.477, p = .233) nor any interaction (F (3, 46) =.785, p = .508) was found.

To summarize, object exploration analysis across the three trials for the recency test revealed that total object explorations were comparable across trials at 1 hr retention.

However, when the retention interval was increased to 4 hrs, rats showed decreased object exploration across successive trials. In addition, rats exhibited significantly lower levels of total exploration at the test trial for the 4 hr retention interval compared with the test trial at 1 hr retention.

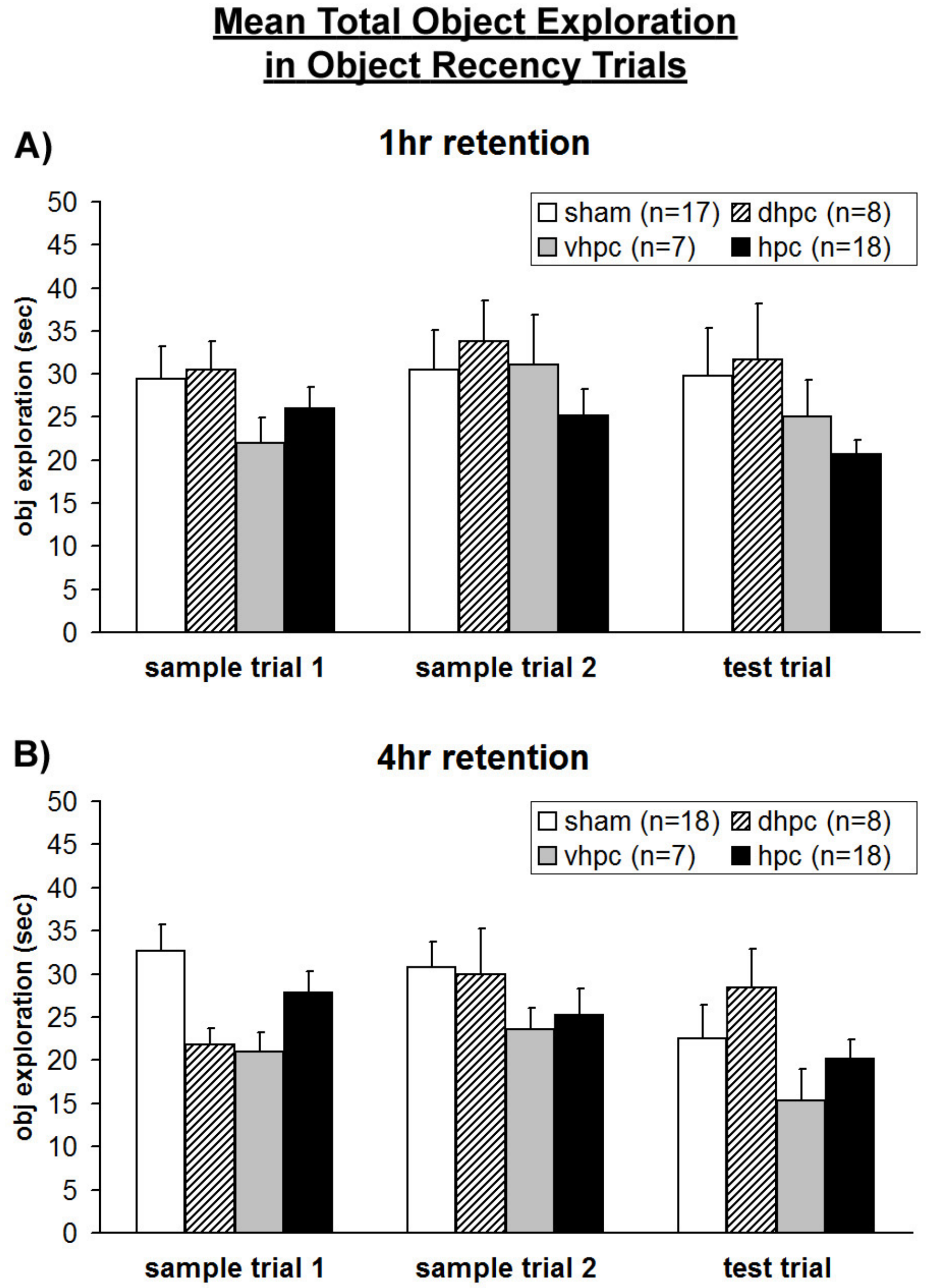


Figure 5.7. Mean object exploration during the two sample trials and test trial for each lesion type at a) 1 hr retention interval, and b) 4 hr retention interval in object recency discrimination. Data expressed as ±SEM.

b) Object recency discrimination

Figure 5.8 shows the discrimination ratios of different HPC lesion types on the two retention intervals tested. At 1 hr retention, sham rats showed significant preference for the target object (i.e. the remotely familiar object) from chance (t (16) = 2.583, p = .010); none of the lesion groups displayed any preference for the target although vHPC lesion rats had near significant preference for the target object (t (6) = 1.829, p = .059). At 4 hr retention, none of the rats showed significant preference for the target object.

To summarize, at 1 hr retention, sham and likely vHPC lesion rats were able to distinguish the relative recency of two familiar objects by showing their preference for the remotely familiar object. Neither dHPC nor complete HPC lesion rats showed any hint of recency memory at 1 hr retention. At 4 hr retention, the retention interval of the recency test proved to be too difficult for rats to establish object recency memory, as none of the rats showed any significant preference for the remotely familiar object.

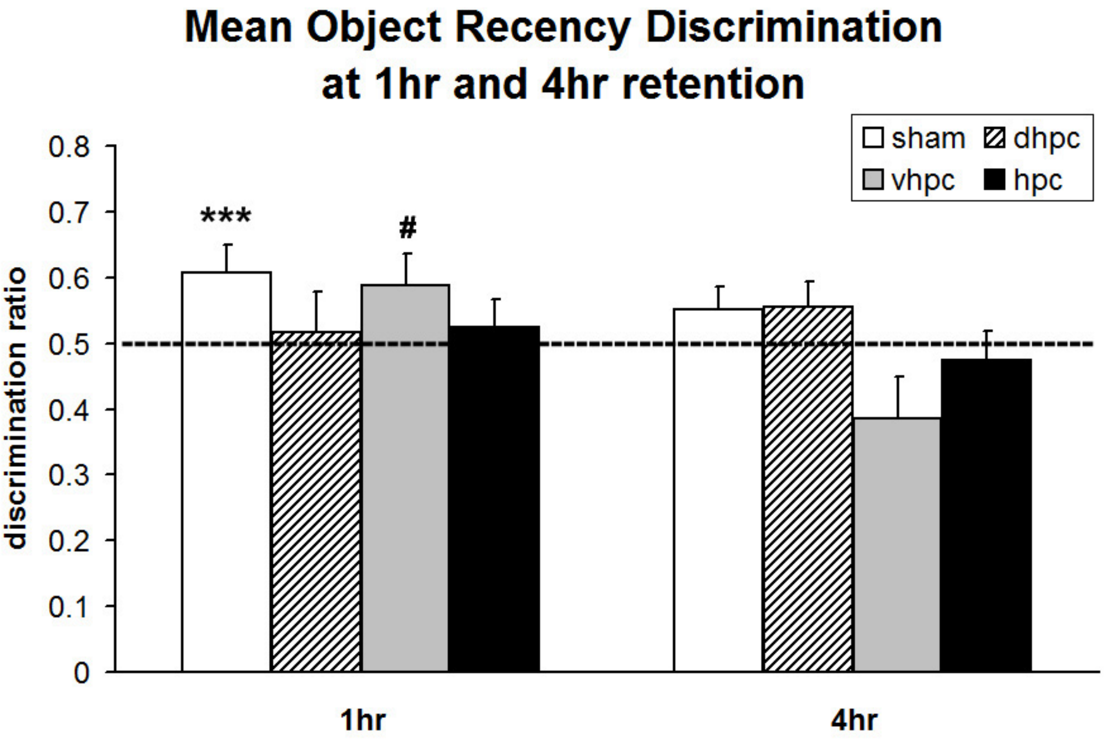


Figure 5.8. Object recency discrimination, expressed by the proportion of time spent on investigating the remotely familiar object (i.e. the target object) versus total object exploration during the test trial. Positive values above chance (dotted line) indicate the preference for the target object. * $p < .05$, *** $p \leq .01$, and # $p < .06$ (near significance) compared to investigations at chance level. Data expressed as ±SEM.

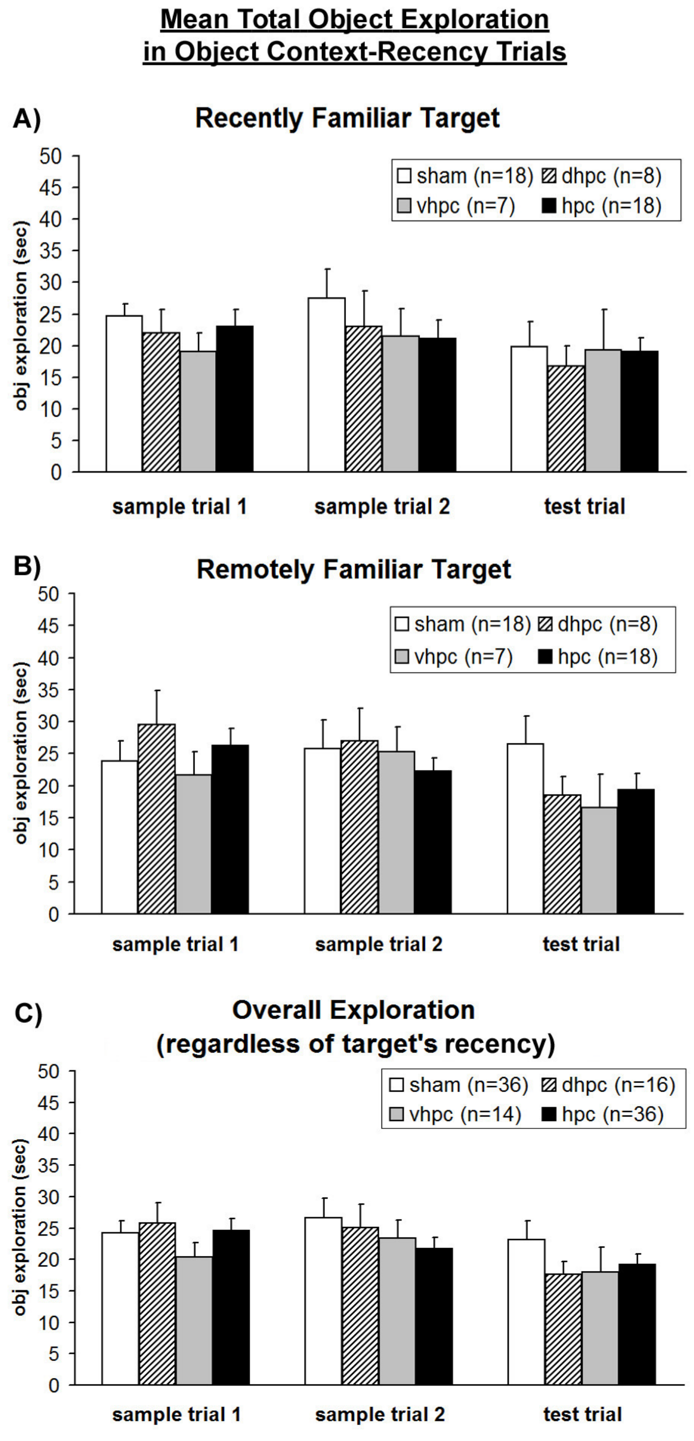
Experiment 3: Object context-recency

a) Object exploration

For this experiment, total object explorations during the sample and test trials were analyzed according to the relative recency of the target object. As mentioned in the methods section, the target object for this task is the object in the mismatched context. Due to the three-trial nature of this paradigm, the target object can be either 1) recently familiar (hence contextually but not temporally favored), or 2) remotely familiar (hence both contextually and temporally favored). Figure 5.9a and 5.9b shows the mean object exploration across trials for the two recency scenarios. Figure 5.9c shows the overall exploration for the target object regardless of recency. The data for this figure were derived from averaging each rat's exploration from the two recency scenarios.

Repeated measures analysis of the total object exploration on the three trials when the target object was recently familiar (Figure 5.9a) indicates no significant effect of TRIALS (F (2, 94) = 1.617, p = .204), no main effect of LESIONS (F (3, 47) = .559, p = .645), and no interaction (F (6, 94) = .273, p = .948). No main effect was observed when the target object was remotely familiar (Figure 5.9b), with F (2, 94) = 2.096, p = .129 for the main effect of TRIALS and F (3, 27) = .545, p = .654 for the main effect of LESIONS). No interaction was evident (F (6, 94) = .845, p = .539). Analysis on the overall exploration (Figure 5.9c), however, indicate a main effect of TRIALS (F (2, 94) = 3.486, p = .035) but not a main effect of LESIONS (F (3, 47) =.705, p = .554) nor an interaction (F (6, 94) = .553, p = .767). Pairwise comparisons of the trials for the overall exploration revealed the test trials yield significantly lower exploration than sample trial 1 (p = .043), and sample trial 2 (p = .024).

In sum, object exploration analysis for the context-recency tests revealed no significant difference in exploration when the target object was recently familiar or remotely familiar. However, when the data were combined to evaluate object exploration regardless of how recently the sampled objects were seen, a decrease of exploration during the test trial was found, indicating a normal pattern of decrease exploration from the sample to the test trial.



<u>Figure 5.9.</u> Mean object exploration during the two sample trials and test trial in object context-recency association when the target object was a) recently familiar, b) remotely familiar, and c) regardless of target's recency. Data expressed as <u>+</u>SEM.

b) Object context-recency association

Figure 5.10 shows the object discrimination ratios for when the target object was recently familiar and remotely familiar. Sham rats displayed preferences for the target object when the target was remotely familiar (t (17) = 1.752, p =.049) but showed no preference for the target when it was recently familiar (t (17) = 1.402, p = .09). Interestingly, complete HPC lesion rats showed a reversed pattern of preference; they displayed a near significant preference for the target object when it was recently familiar (t (17) =1.703, p =.054), but they showed a preference for the non-target object when it was remotely familiar (t (17) = -2.163, p =.023). Collectively, complete HPC lesion rats appear to direct their attention to the object they have seen more recently, regardless if it was a target object. Finally, neither types of partial lesion rats showed any significant preference for the target object in either target condition.

Figure 5.11 shows the overall object discrimination ratios of different lesion types when objects are presented in a mismatched context from the sample trial, regardless of the recency factor of when objects were sampled. This ratio reflects rats' ability to associate objects with the contexts. Sham rats showed significant preference for the target object compared to the non-target object (t (17) = 2.208, p = .021). All in all, only sham rats showed the ability to associate objects with the contexts they were presented in. None of the lesion groups displayed such an association.

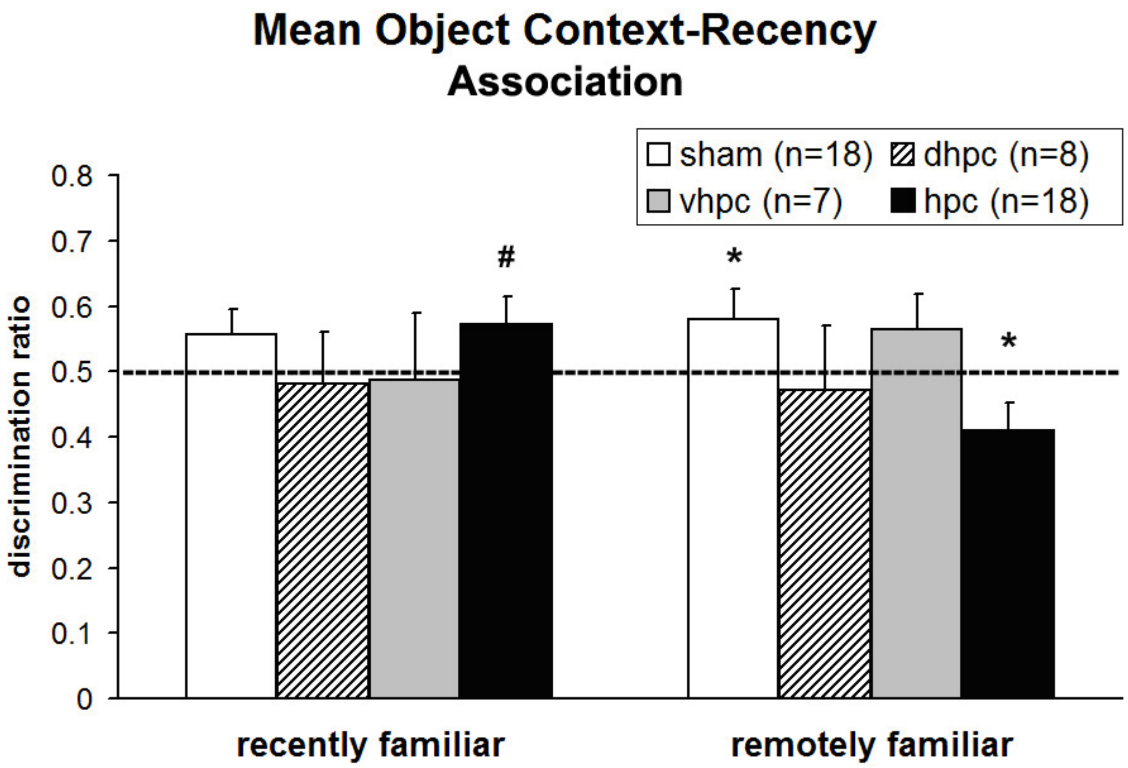


Figure 5.10. Object context-recency association, expressed as the proportion of time spent on investigating the target object in the mismatched context versus the total object exploration on the test trial according to recency. Positive values above chance (dotted line) indicate a preference for the target object. * $p < .05$, *** $p \leq .01$, and # $p < .06$ (near significance) compared to the investigation at chance level. Data expressed as ±SEM.

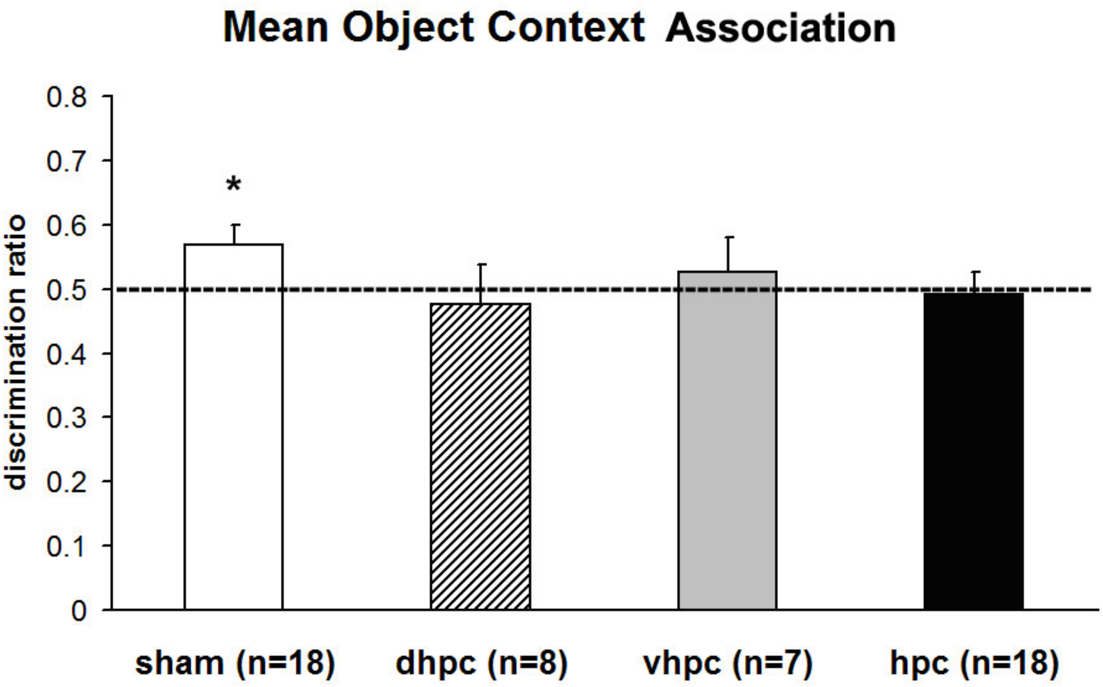


Figure 5.11. Object context association, regardless of the recency of the target object. Positive values above chance (dotted line) indicate preference for the target in the mismatch context. * $p < .05$, compared to investigation at chance level. Data expressed as ±SEM.

DISCUSSION

Using the novel object recognition task and its variants, the present studies have demonstrated after complete obliteration of the hippocampus, rats were able to discriminate a novel object from a familiar sample object, as suggested by the literature. However, they did not demonstrate the ability to discriminate the relative recency of seen objects as they failed to display preference for the less recently seen (i.e. remotely familiar) object. They also did not demonstrate normal association of objects, context, and/or the relative recency of the objects they saw, as they failed to show a preference for the object in the mismatched context. All in all, it appears that complete damage of the hippocampus renders rats unable to demonstrate object memory beyond novel object recognition.

Interestingly, the pilot findings from partial lesion rats showed a similar pattern of impairment as rats with complete HPC lesions. Both dHPC lesion and vHPC lesion rats demonstrated novel object recognition and showed no significant preference for the target object in both the object recency task and the object context-recency task. Interestingly, there is some indication of intact object recency memory in vHPC rats at 1hr retention while dHPC rats showed no evidence of such memory. Studies of partial HPC lesions support that vHPC damage producing comparatively less memory impairment than dHPC or complete HPC damage (Broadbent et al., 2004). Due to the small number of partial lesion rats in our sample, however, our findings should be interpreted with caution.

The most unexpected finding of the present study resides in the results of the object context-recency task, in which sham rats were able to demonstrate preference for the remotely familiar target in the mismatched context, but showed no significant preference when the target was recently familiar. In contrast, complete HPC lesion rats showed the opposite preference; specifically, complete HPC lesions led to rats preferring the recently familiar target object while not preferring the remotely familiar target. In fact, when the target object was remotely familiar, complete HPC lesion rats actually preferred the non-target object. It appears that when HPC lesion rats see two objects presented in two distinctly different contexts, at recall, they prefer the object that was most recently seen, regardless of the context-change.

The preference for the recently seen object in the object context-recency task is contrary to prediction. Recall that in the remotely familiar target condition, the target object was the object in the mismatched context that was both temporally- and contextually- favored. Temporal-favoritism refers to the target object seen further back in time and therefore its memory was more susceptible to decay. Thus, based on the premise of novel object recognition whereby the object not remembered is more interesting to the rat, one would expect rats to prefer the temporally favored target object as it is favored by memory decay as well as mismatched context.

Referring to Figure 5.10, complete HPC lesion rats showed a preference for the non-target object. This preference was the opposite of what is predicted by recency or contextual factors. In the same task, however, complete lesion rats displayed no significant preference for the target object when the target was recently familiar. In this condition, the target object was in the mismatch context but was not temporally favored. In other words, the memory trace for context and recency both compete for object preference. In this scenario, sham rats showed no significant preference for the target object, which was likely due to the competition of the two memory traces. Surprisingly, complete HPC lesion rats showed a significant preference for the recently familiar target object despite this competition.

Detailed examination of complete HPC lesion rats' pattern of preference in both temporally favored and non-temporally favored target object scenarios suggests that these rats prefer the object they have seen in the second sample trial (i.e. the recently familiar object), regardless if the object is in a mismatched context.

One possible explanation for rats' pattern of object preference after complete HPC lesions is that the change of context in the last experiment induced anxiety and thus rats feel safer to explore the object they were more familiar with. However, analysis of the exploratory behavior at test trial in the object context-recency experiment did not reveal any difference amongst the lesion types. Informal observation of the rats' behavior shows no indication of anxiety. Rats were seen to have fed and slept during retention and explored normally on object trials. Considering that these rats were highly familiarized with the apparatus and procedures, it is unlikely that their object preference pattern in this task was instigated by fear.

Another possible reason for the observed object preference in complete HPC lesion rats is that the three-trial test protocol induced interference of object memory in these rats. Recall that after complete HPC lesions, rats were impaired in object recency and object context-recency tasks, both of which require rats to remember more than one sample object. It is likely that in both experiments, information from the two sample trials were interfering with each other. In the object recency task, after complete HPC lesions, rats did not show any preference for the remotely familiar (i.e. target) object nor the recently familiar (i.e. non-target) object, indicating the memory trace for both objects were equally weak or equally strong.

If interference was indeed the reason for the observed impairment in object recency discrimination and object context-recency association for rats without the hippocampus, which direction does interference act in? From the object recency data, one would deduce that the memory of sample trial 2 was interfering with the memory of sample trial 1 in a retrograde manner to render an absence of a recency effect for complete HPC lesion rats. From the object context-recency data, however, complete HPC lesion rats prefer the recently familiar object regardless of context change, indicating that they remembered the remotely familiar object while forgetting the recently familiar object. In this case, interference is occurring in the anterograde direction in which information from sample trial 1 interferes with information seen at a later time on sample trial 2. Clearly, our results cannot determine in which direction object interference occurs.

As a final point, analysis on the target object preference for the object context-recency experiment showed no evidence of object context memory for complete and partial HPC lesion rats when recency was factored out. Averaging the performances for remotely- and recently- familiar target scenarios for complete HPC lesion rats render an overall target object preference at chance. Only sham rats were able to demonstrate the ability to associate objects with the context in which they were seen in a spontaneous manner.

In conclusion, the present study has shown that the integrity of the hippocampus, although not important for object recognition, is important for discriminating the order of objects and/or associating the objects with their contexts. Pilot results indicate the dorsal hippocampus to be more important than the ventral hippocampus both in discriminating the order of objects and associating objects with their contexts. Ventral hippocampus is not required for discriminating the order of objects. The present study conjectures that the observed impairment in hippocampal lesion rats may be due to interference of object information amongst sample object trials, which may have occurred in both retrograde and anterograde directions. Further investigation is required to determine if interference is in fact the cause of the impairment.

6. THE HIPPOCAMPUS AND THE EFFECTS OF INTERFERENCE ON OBJECT RECOGNITION

ABSTRACT

In a series of experiments in chapter 5, complete lesions of the hippocampus (HPC) in rats spared novel object recognition while impaired object recency discrimination and object context-recency association. As speculated in the discussion of the previous chapter, the observed impairments may be due to retrograde and/or anterograde interference inherent in both the object recency and the object context-recency tasks. The present study aims to investigate the validity of this claim. Rats with complete HPC lesions and sham lesions were tested on novel object recognition and the effects of retrograde and anterograde interference of objects and contexts on novel object recognition. Anterograde interference was induced by presenting an interference stimulus before the rat sees the sample object; retrograde interference was induced by presenting the interference stimulus after the rat sees the sample object. Both retrograde and anterograde novel object interference affected object recognition in complete HPC lesion rats. Context interference, on the other hand, affected object memory similarly in both sham and HPC lesion rats. While anterograde context interference did not affect the memory of objects, retrograde context interference rendered rats with no significant preference for the novel object. This non-significant result is likely due to over-training. Thus, in the absence of a functional HPC, object memory is resilient to context interference but is susceptible to object-specific interference in both the anterograde and the retrograde directions. The present study concludes that the HPC serves to maintain the memories of multiple objects through reducing interference amongst them.

MATERIALS AND METHODS

Rats

Sixteen naïve Long Evans female rats were obtained from the local colony at the Canadian Centre for Behavioural Neuroscience. Eight rats received complete HPC lesions and 8 rats received sham surgeries.

Rats were approximately 300g at the time of surgery. They were housed either in pairs or in threes under standard colony housing with food and water available *ad libitum*. Rats were maintained on a 12-hour dark/light cycle at 20-21°C with testing that took place during the light period of the cycle. All procedures were conducted in accordance with the guidelines provided by the Canadian Council on Animal Care (CCAC) and were approved by the local animal care committee.

Surgery

Surgical procedures for complete HPC lesions and sham lesions were identical to the procedures in chapter 5. Surgery was performed under Isofluorane anaesthesia in a standard stereotaxic apparatus. In preparation for surgery, ophthalmic liquid gel was applied to a rat's eyes for protection, the hair was shaved from the top of the rat's head with an electric shaver, the scalp was cleaned with 70% alcohol and Hibitane, and an analgesic was given to the back of the neck (0.07ml of buprenorphine (Temgesic) at 0.3mg/ml, s.c.). A midline incision was made and the fascia (periosterum) was cut and pushed to the edges of the skull with a sterile gauze swab. The skin was retracted with 4 mosquito forceps to expose the skull, and holes were drilled into the skull using a 1mm drill bit and high speed drill at predetermined coordinates (see Table 5.1).

The excitotoxin *N*-methyl-D-aspartate (NMDA, 7.5µg/ µl PBS) was loaded into 30-gauge injector needles, each needle consisting of 30-gauge stainless steel tubing (Smallparts, Inc) glued onto PE20 polyethylene tubing. The tubing was connected to a Hamilton 10ul syringe mounted on a Harvard mini-pump. For each injection site, the injector needles were lowered bilaterally wherever possible and 0.4µl of NMDA solutions were delivered at a rate of 0.15µl/min. The needles were left in place for an additional 2.5 min to allow for diffusion.

To alleviate convulsions following surgery, rats were given 0.2ml of diazepam (i.p.), with an additional 0.2ml of diazepam if convulsions persisted. Rats were monitored after surgery until they became active. Rats were then housed individually for three days to allow for recovery, following which they were group-housed a week prior to the start of behavioral testing.

Apparatus

Object tests were conducted using the same test room, apparatus, and stimuli as the experiments in chapter 5. All tests were conducted in the same test room that measured 13’ by 8.5’ with fluorescent lights illuminating the room from the ceiling. Field boxes or Y-mazes used for the object trials were constructed with sheets of ¼ inch thick Plexiglas held together by acrylic and latex adhesives. The walls of the Y-maze were 40 cm high with 27 cm long arms that were 10 cm wide; three pieces of white-corrugated plastic boards (two pieces above the top arms 5 cm wide, one piece above the start arm 10 cm wide, see Figure 6.1) were taped on top of the ends of the arms to restrict the view of the ceiling. Two different colors of Y-mazes were constructed to create two distinctly different contexts. One Y-maze was constructed with white Plexiglas all around and the other had black Plexiglas walls with white floors. To further increase contrasts between the two contexts, the floors of the black Y-mazes were lined with corncob bedding (Figure 6.1a and 6.1b).

In addition to the black and the white Y-mazes, a third type of field box constructed from 4 mm thick white corrugated plastic sheets was introduced as a novel context for the present study. Figure 6.1c shows the photograph of one of the white corrugated plastic boxes, which measured 42 X 42 X 41 cm (length X width X height). The edges of the corrugated plastic boxes were held together by latex adhesives and duct tape, and the floors were lined with corncob for ease of cleaning. All object interference tests were conducted in the white Y-mazes, and all context interference tests were conducted in the white Y-mazes in combination with the black Y-mazes or with the corrugated plastic boxes.

A radio situated at the north end of the test room provided background noise to minimize the effects of potential noise disturbances during the object trials. All trials were video-recorded by a camera mounted on a tripod over the apparatus.

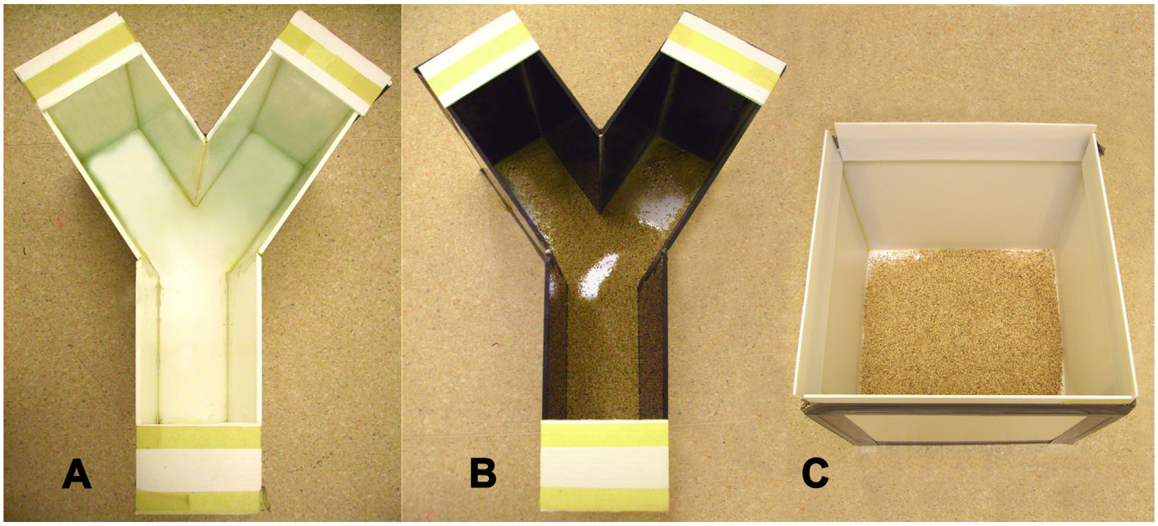


Figure 6.1. Photograph of (a) white Y-maze, (b) black Y-maze, and (c) white corrugated plastic box. Objects were sampled and tested in the white Y-mazes only while the black Y-mazes and the white corrugated plastic boxes served as novel contexts.

Transport

For each test, animals were singly housed in the waiting room in transport cages lined with bedding, food and water. The trials with the Y-mazes began with the experimenter placing a rat at the start arm of the Y-maze facing the south wall of the apparatus (Figure 5.2); the trials with the corrugated plastic boxes began with a rat being placed at the south end of the box facing the wall. As soon as the rat's limbs landed on the floor of the maze, the experimenter would exit the test room, close the door, and allow the rat to freely explore the Y-maze for 3 min. At the end of the 3 min, the experimenter would re-enter the room to retrieve the rat.

Stimuli

Objects used for the present study were the same objects used in the previous set of experiments in chapter 5. Objects were acquired from local dollar-stores with each object-pair consisting of objects made with the same material (i.e. plastic, glass, or metal). For experiment 2, a same material object was selected as the interference object for a given object-pair. All items were previously piloted on naïve rats to ensure good baseline recognition. Prior to the start of every object trial, objects were cleaned in 50% ethanol then rinsed with water and patted dry. In preparation for each trial, the objects were adhered to the floor of the Y-maze using white non-toxic adhesive putty.

Video Analysis

All sample and test trials were video-recorded by a camera mounted above the field boxes. Trials with the objects in the white Y-mazes were scored by an experienced scorer who was blind to the subjects' group- and object- assignments. Videos were scored on the real time rats spent on exploring each object to a precision of 0.1 sec.

All sample trials were scored once from 0 to 3 min, and all test trials were scored twice by the minute for 3 min (i.e. 0-1min, 1-2min, and 2-3 min). For accuracy, data analysis was performed on the scores taken from the second time the test trials were scored.

Habituation

Habituation to the white Y-mazes and the test room began a week and a half post-surgery. Rats were well-handled prior to the start of habituation. The rats were habituated to the white Y-mazes in the test room once daily on two consecutive days prior to the first test. During each habituation session, rats were individually placed in an empty white Y-maze and were allowed free exploration of the apparatus for 5 min. No reagent was used to clean the apparatus to allow for the saturation of rat odour. In between trials when cleaning was necessary, the Y-mazes were wiped clean with dry paper towels. In the case of severe soiling, the apparatus was wiped clean with damp paper towels. Rats were not habituated to the black Y-mazes nor the corrugated plastic boxes as those contexts were to be kept novel for the context interference experiments. All habituation sessions were video-recorded and reviewed to verify for exploratory behavior before conducting the first object test.

Behavioral procedure

The test protocol for all three experiments of this study is summarized in Figure 6.2. The details for each experiment are described below.

Experiment 1: Novel object recognition

This first experiment took place approximately 24 hrs after the last habituation trial. As with the previous experiments in chapter 5, the purpose of this task was to establish a baseline of object memory with all of our rats. This task consists of a sample trial and a test trial. On the sample trial, rats were placed in the start arm in the white Y-maze with two identical copies of an object at the two top arms, placed one inch from the end wall (see Figure 5.2). Rats were allowed free exploration of objects in the maze for 3 min. At the end of the 3 min, rats were returned to their transport cages for a 4 hr retention interval.

Following retention, a test trial commenced. For the test trial, rats were returned to the same white Y-maze they were in during the sample phase, with a novel object as well as a third copy of the sample object they had encountered earlier. Normal rats would typically prefer the novel object as it is more interesting. Based on the time rats spent investigating each object, recognition of the sample object is inferred. The order of the objects was counterbalanced amongst rats.

Experiment 2: Object interference

The second experiment of object interference commenced approximately 48 hrs after the novel object recognition task. The purpose of this experiment was to investigate if seeing another object before or after encountering a sample object would affect the memory of the sample object. Similar to experiment 1, the sample trial consists of exposing rats to two copies of a novel sample object (i.e. object C) in the white Y-maze to be recognized at the test trial. The interference trial consists of rats seeing two copies of a second novel object (i.e. object D) that would not be seen again. Finally, the test trial consists of a third novel object alongside a copy of the sample object (i.e. object E with object C). Normal rats whose memory of the sample object is unaffected by the event of seeing object D would prefer the novel object during the test trial. Interference can occur either forward in time (i.e. “anterograde” interference, in which the interference object is seen before the sample object), or backwards in time (i.e. “retrograde” interference, in which the interference object is seen after the sample object). Sample trials and interference trials occurred 15 min apart; test trials occurred 1 hr after the end of the second trial. All rats received one anterograde interference test and one retrograde interference test conducted approximately 48 hrs apart, with the order of the tests balanced across rats.

Experiment 3: Context interference

The final experiment on context interference took place approximately 72 hrs after the end of the object interference test. The purpose of this experiment was to investigate if non-object stimulus can interfere with object recognition. Rats were tested on an interference paradigm similar to experiment 2. For the sample trials, rats encountered two copies of a novel sample object in the white Y-maze. For the interference trials, rats encountered a novel context (either the black Y-maze or the corrugated plastic box), either before or after seeing the sample object. After retention, rats encountered a novel object alongside a copy of the sample object in the white Y-maze. Normal rats whose memory of the sample object is unaffected by seeing a novel context would typically prefer the novel object. Sample trials and interference trials occurred 15 min apart; test trials occurred 1 hr after the end of the second trial. As with experiment 2, all rats received one anterograde context interference test and one retrograde context interference test. The two tests were conducted 48 hrs apart with the order of tests and the two novel contexts balanced across rats.

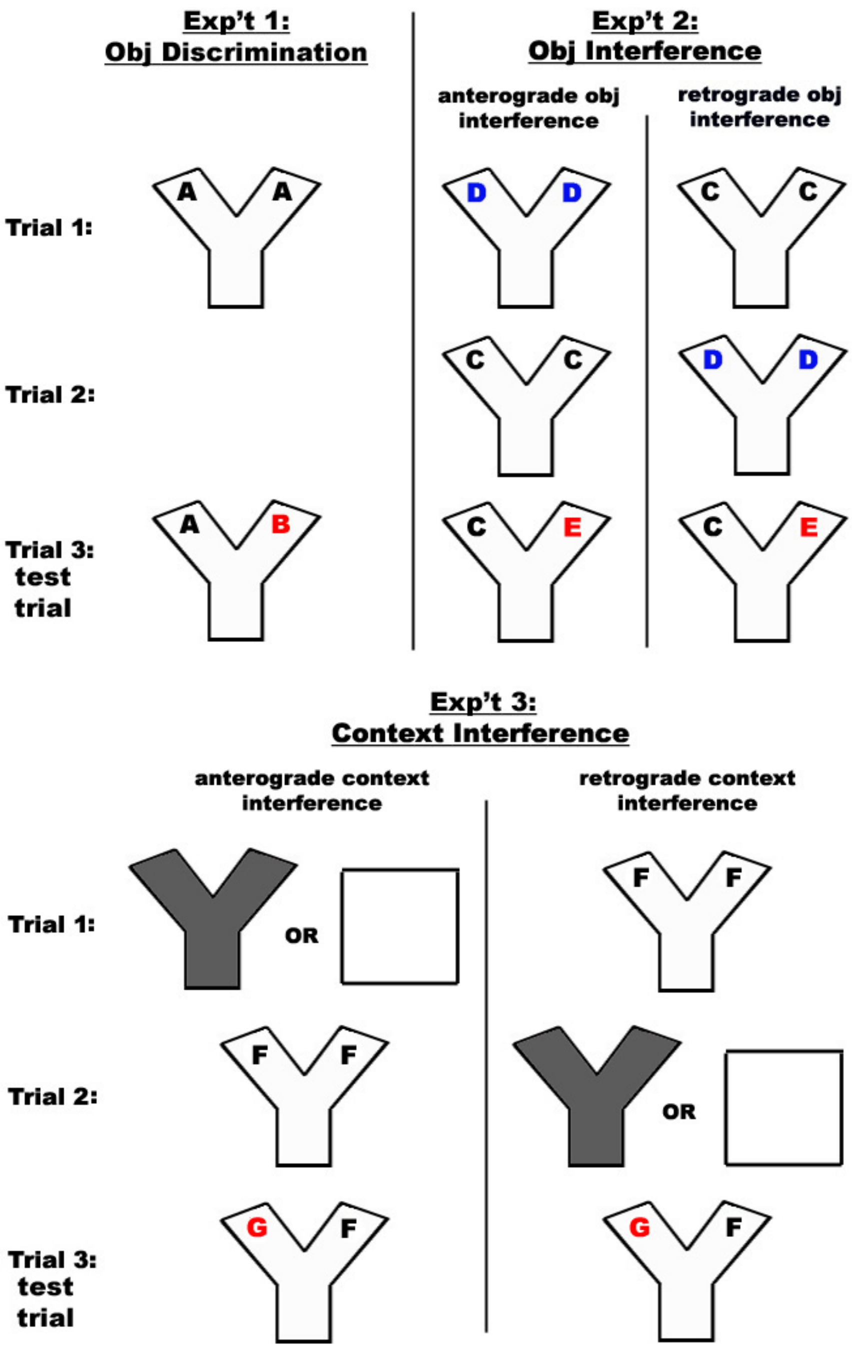


Figure 6.2. Summary of test protocols for experiments 1-3 in Chapter 6. Objects in the apparatus are represented by letters; blue colored letters denote the interference objects and red colored letters denote the target objects for each experiment.

RESULTS

Histology Results

Histology results for the rats in this study are not available as they are involved in an ongoing experiment. Please refer to *Histology Results* section in chapter 5 for examples of HPC damage sustained by sham and complete HPC lesions.

Data Analysis

For the object exploration analysis, to determine if lesion types affect object exploration differently in the different types of trials, the total exploration times in the sample, interference, and test trials were analyzed against each other. Repeated measures analyses were performed with TRIALS as the within-subject variable and LESIONS as the between-subjects factor for each of the three experiments.

As described in chapter 5, data collected on the test trials of the time rats spent exploring the target object was analyzed against the time spent on the non-target object by the derivation of an object discrimination ratio. The formula for the object discrimination ratio, as used by Mumby and colleagues (2002), is as follows:

$$T_{\text{target}} / (T_{\text{target}} + T_{\text{non-target}})$$

Based on this formula, a discrimination ratio of 0.5 would indicate a level of exploration for the target object at chance. To determine if the discrimination ratios were different from chance, one-sample t-tests (one-tailed, against a value of 0.5) were conducted on the mean discrimination ratios from each lesion type and delay for all test trials at each experiment. A ratio significantly above 0.5 would indicate a preference towards the target object and thus imply object recognition.

Experiment 1: Novel Object Recognition

a) Object exploration

Figure 6.3 shows the object exploration patterns of both the sham and the complete HPC lesion rats during the sample and test trials on the novel object recognition task. Analysis on the total amount of time spent on exploring objects during the two trials revealed a significant main effect of TRIALS (F (1, 14) = 16.150, p =.001), but no main effect of LESIONS (F (1, 14) = .339, p =.570) nor an interaction between the two factors (F (1, 14) = 2.374, p =.146). In summary, rats spent less time exploring objects during the test trial than the sample trial, regardless of the type of lesions they had.

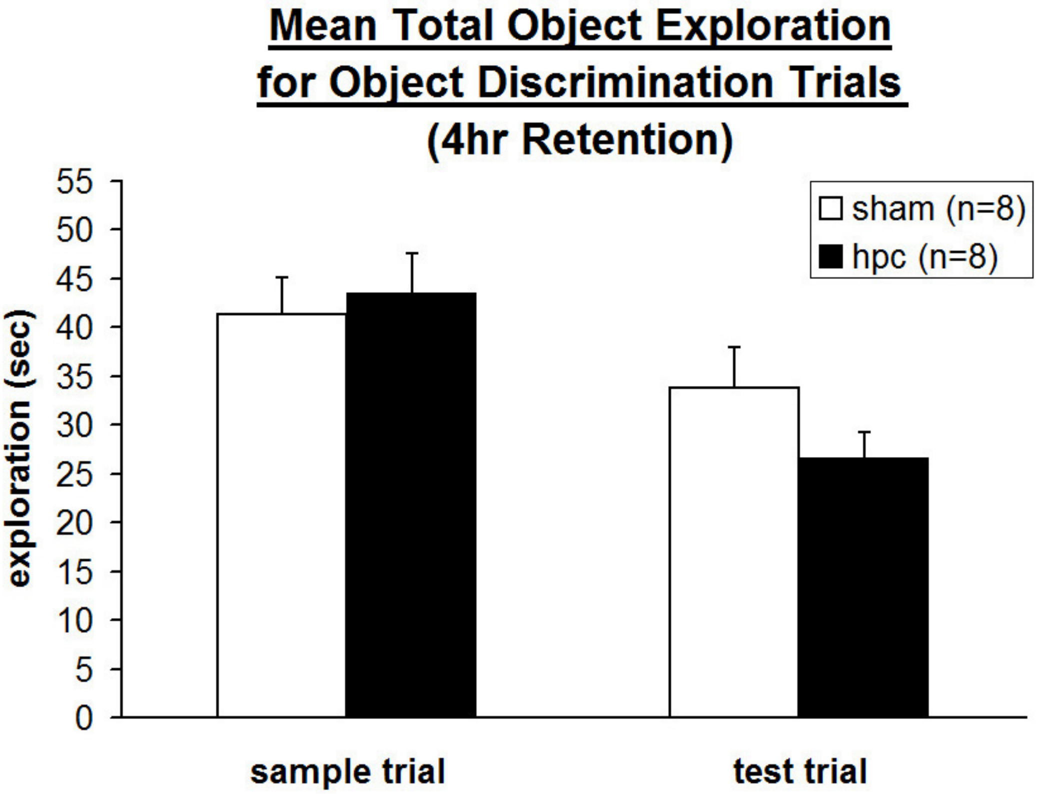


Figure 6.3. Mean object exploration at sample and test trial for sham and complete HPC lesion rats on the novel object recognition task. Data expressed as +SEM.

b) Object discrimination

Figure 6.4 shows the novel object recognition of sham and complete HPC lesion rats during the test trial 4 hrs after sample. Recall that chance level preference of the target object would represent an object discrimination ratio of 0.5; this is illustrated by a dotted line on the figure. One-sample t tests of the target object preference against the chance value of 0.5 revealed significant preference for the target object in both sham and complete HPC lesion rats (t (7) = 2.562, p =.019 and t (7) = 3.068, p =.009, respectively). Thus, both groups of rats showed intact recognition of the sample object and displayed a preference for the novel target object 4 hours later.

Taken together, the results of this first experiment established that both sham and complete HPC lesion rats are capable of recognizing a familiar object up to an interval of 4 hrs after sampling.

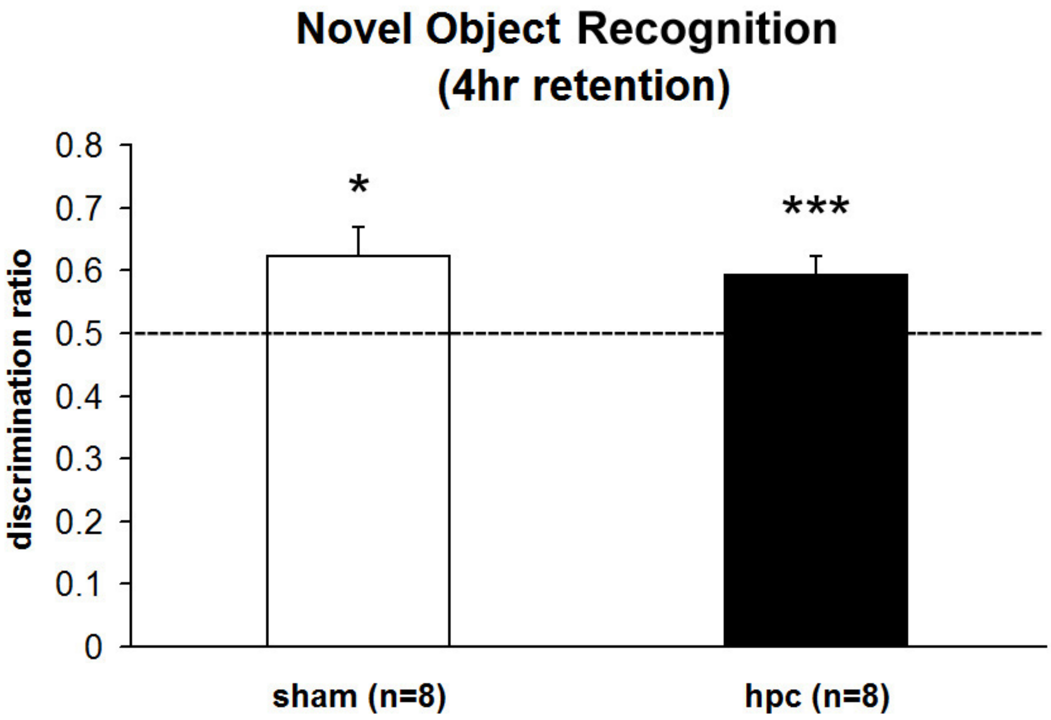


Figure 6.4. Novel object recognition, expressed by the proportion of time rats spent investigating the novel object versus their total object exploration during the test trial. Positive values above chance level of 0.5 (dotted line) indicate a preference for the target object. * p < .05, *** p ≤ .01, compared to investigation of target object at chance level. Data expressed as ±SEM.

Experiment 2: Object interference

a) Object exploration

Figure 6.5 shows the object exploration during the sample, the interference, and the test trials of anterograde and retrograde object interference tests. Repeated measures analysis of object explorations with TRIALS as within-subjects variable and LESIONS as the between-subjects variable following anterograde object interference revealed no main effect of TRIALS (F (2, 28) = 1.298, p =.289), no main effect of LESIONS (F (1, 14) = 1.881, p =.192), nor an interaction between the two factors (F (2, 28) = .813, p =.454). Analysis for object explorations following retrograde object interference revealed a significant main effect of TRIALS (F (2, 28) = 5.873, p =.007). No main effect of LESIONS (F (1, 14) = .001, p =.975) nor an interaction between the two factors (F (2, 28) = .779, p =.469) were found.

Referring to Figure 6.5b, the high levels of sample trial exploration in the retrograde object interference test for both sham and complete HPC lesion rats was the major reason for the main effect of TRIALS observed. Examination of the rats’ raw exploration data on scatter plots revealed no outlier for both groups of rats on that trial. The high explorations observed were likely due to random factors affecting exploration; the factors can be anything ranging from colony conditions to specific sample objects used on that test. Verification of the exploration data on scatter plots also revealed an outlier in the sham lesion group during the sample trial of the anterograde object interference task (Figure 6.5a). One of the sham rat’s explorations was around 60 sec (i.e. two standard deviations above the mean, compared to the rest of the group which clustered at around 30 sec). Due to the small sample size, the outlier had a greater weight on the group mean and thus gives the impression that sham rats had greater levels of object exploration during the sample trial.

To summarize, analysis on object exploration for the object interference test revealed a decrease in exploration across trials for retrograde- but not for anterograde-object interference paradigm. This main effect of TRIALS is likely a random variation and not a result of differences in exploratory behavior induced by the retrograde interference paradigm.

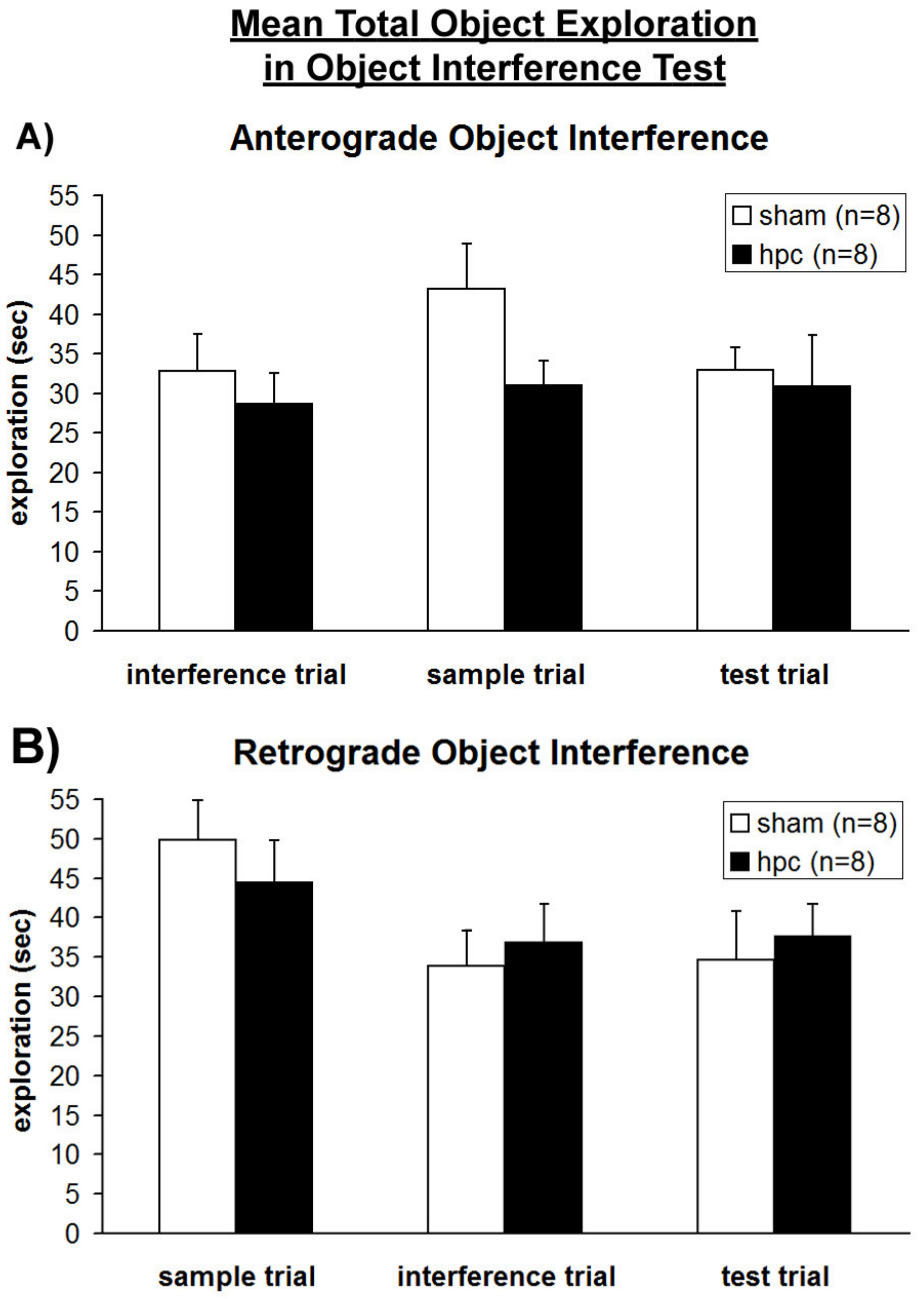


Figure 6.5. Mean object exploration during the interference, the sample, and the test trial for sham and complete HPC lesion rats in a) anterograde object interference task, and b) retrograde object interference task. Data expressed as ±SEM.

b) Object discrimination

Figure 6.6 shows the novel object recognition of both the sham and the complete HPC lesion rats on the test trial following anterograde and retrograde object interference. One-sample t tests of the target object preferences during the test trial revealed that sham rats retained significant preference for the target novel object regardless of when the interference occurred (t (7) = 4.170, p =.002 and t (7) = 2.334, p =.026 for target object preference following anterograde and retrograde object interference, respectively). In contrast, rats with complete HPC lesions showed no preference for the target object following either types of object interference; the t test results for complete HPC rats after anterograde and retrograde object interference were t (7) =.598, p =.285 and t (7) = .618, p =.556, respectively.

In sum, both anterograde and retrograde object interference have no effect on sham rats' novel object recognition. For complete HPC lesion rats, however, the memory of a familiar sample object is disrupted by both anterograde and retrograde object interference.

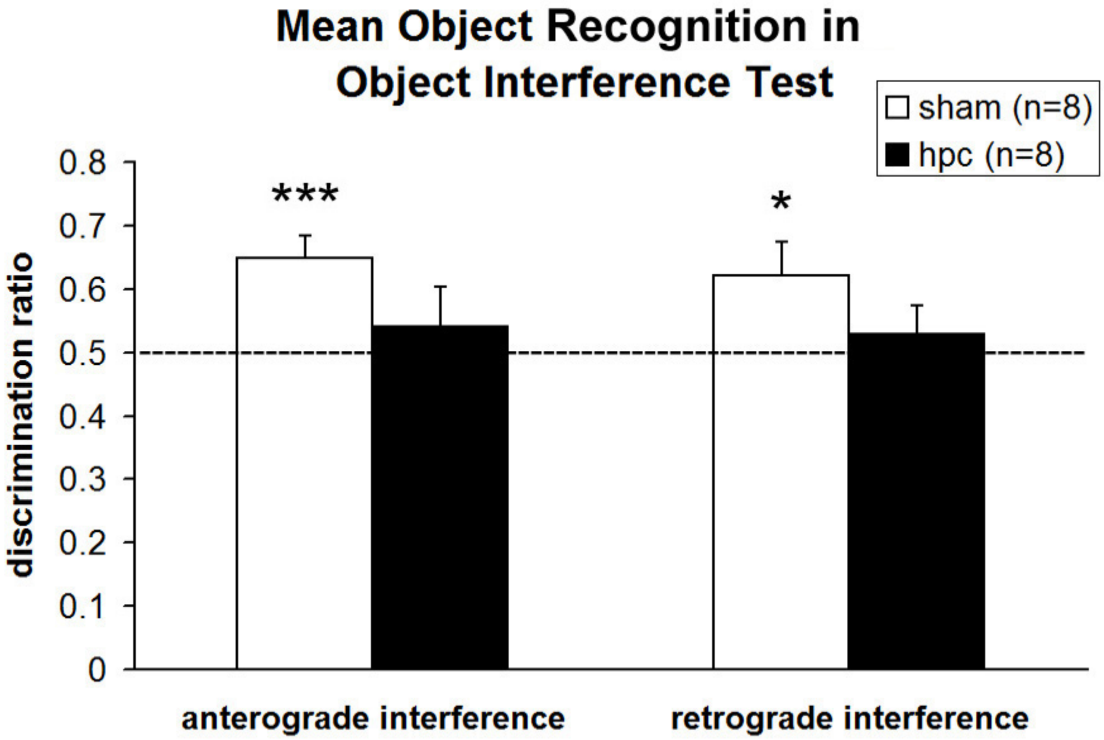


Figure 6.6. Object recognition of sham and complete HPC lesion rats during test trial following anterograde or retrograde object interference. * p < .05, *** p ≤ .01, compared to investigation of target object at chance level (dotted line). Data expressed as ±SEM.

Experiment 3: Context interference

a) Object exploration

Figure 6.7 shows the object exploration of sham and complete HPC lesion rats during the sample and the test trials of anterograde and retrograde context interference tests. Since the interference trials consist of rats encountering a novel context in the absence of any object, analysis of object explorations was performed on the data derived from the sample trials and the test trials only.

Repeated measures analysis of object exploration during the sample and test trials for anterograde context interference revealed no significant main effect of TRIALS (F (1, 14) = 1.775, p =.204), no main effect of LESIONS (F (1, 14) = 1.043, p =.324), and no interaction between the two factors (F (1, 14) = 2.371, p =.146).

Analysis of the object exploration during the sample and the test trials for retrograde context interference revealed a main effect of TRIALS (F (1, 14) = 5.217, p =.038), showing that rats (especially complete HPC lesion rats) spent considerably more time exploring the objects during the test trial than the sample trial (Figure 6.7b). This is a reversal of exploratory behaviors seen thus far. Examination of the raw data at the sample trial revealed that three of the HPC lesion rats had uncharacteristically low levels of exploration. Two of these rats also happened to be exposed to the same object pair on the same test day, which would likely have contributed to the low mean explorations of complete HPC lesion rats and the main effect of TRIALS observed. No main effect of LESIONS (F (1, 14) = 2.114, p =.168) nor an interaction of the two factors were found (F (1, 14) = .347, p =.565).

In summary, context interference was found to disrupt the normal decrease in object exploration from sample to test trial --- a phenomenon that is typical in spontaneous object trials. Moreover, following retrograde context interference, both sham and complete HPC lesion rats spent considerably more time exploring the objects at the test trial than at the sample. There is a possibility that the main effect of TRIALS was a result of random factors occurring for the retrograde interference test that affected exploration (i.e. the conditions in the colony or the use of specific sample objects which elicited low exploration). Alternatively, it is more likely that rats tested under the retrograde context interference paradigm --- rats that were over-trained from a series of object-related trials --- experienced renewed interest in explorations as a result of seeing a novel context after the sample trial. The encounter of a novel context prior to the test trial may have encouraged object exploration and thus rats spent more time exploring objects during the test trial than at sample. The same effect on explorations may have occurred for rats tested under the anterograde context interference paradigm; the encounter of a novel context prior to the sample object may have generated renewed interest in exploring objects on both the sample and test trials following the context interference trial. The net effect of the anterograde novel context interference therefore would be an overall heightened object explorations with no main effect of TRIALS.

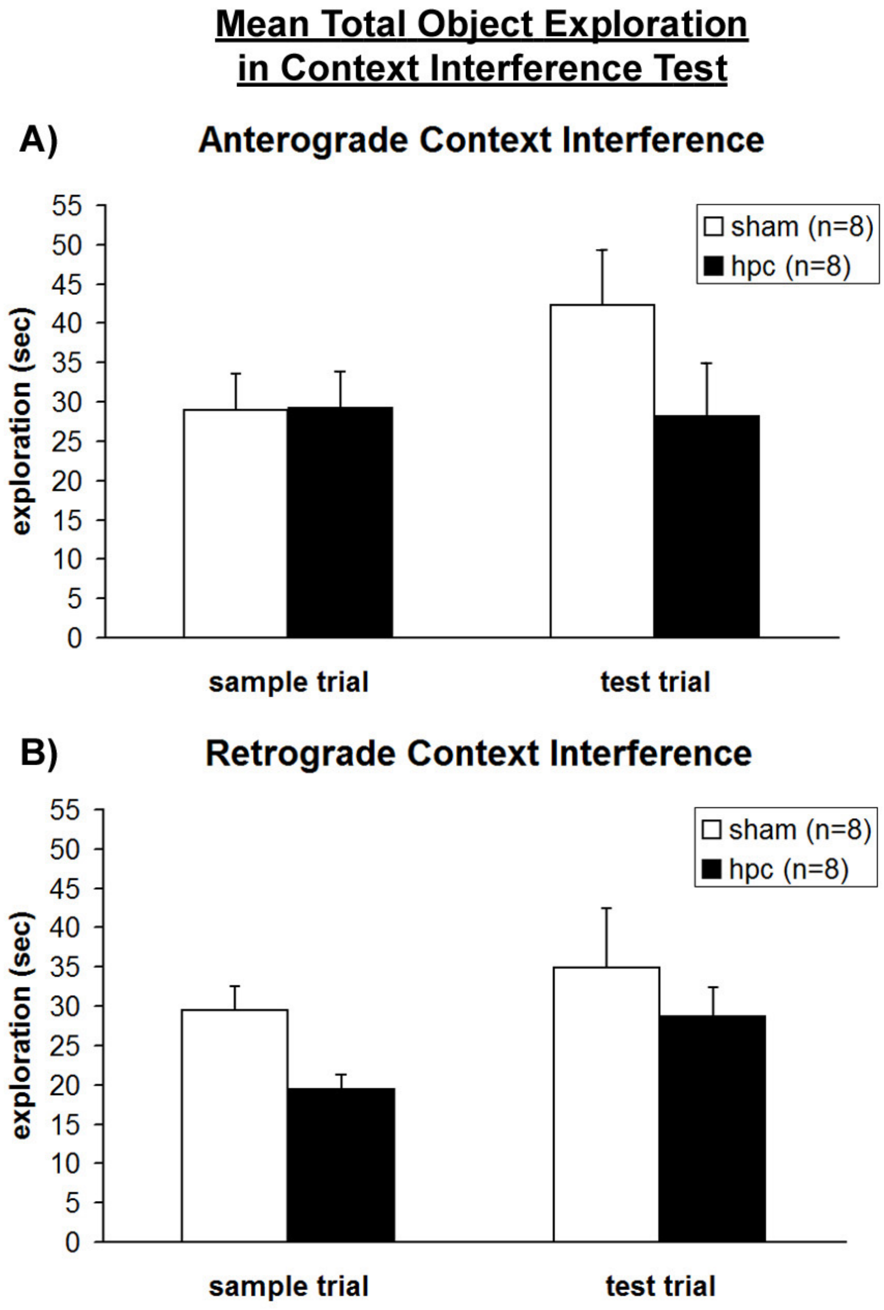


Figure 6.7. Mean object exploration during sample and test trial for sham and complete HPC lesion rats in a) anterograde context interference and b) retrograde context interference tasks. Data expressed as +SEM.

b) Object discrimination

Figure 6.8 shows the novel object recognition of sham and complete HPC lesion rats following anterograde and retrograde context interference. One-sample t test results of the novel object preference after anterograde context interference revealed a significant preference for the target object from both sham and complete HPC lesion rats (t (7) = 2.346, p = .026 and t (7) = 4.612, p =.001, respectively). Following retrograde context interference, however, both sham and complete HPC lesion rats showed non-significant preferences for the target object at test trial. T test results were t (7) = 1.334, p = .112 for sham rats and t (7) = 1.585, p =.079 for complete HPC lesion rats, compared to target preference at chance. Examination of the discrimination ratios from individual rats in retrograde context interference revealed that the majority of rats from each of the two groups showed a preference for the target object, thus indicating both groups retained a non-significant preference for the target object.

In summary, anterograde context interference has no effect on rats' spontaneous recognition of the sample object; both sham and complete HPC lesion rats showed significant preference for the target object at test trial. Retrograde context interference, on the other hand, affected rats' spontaneous recognition of the sample object in both sham and complete HPC lesion rats as both groups displayed non-significant preference for the target object. Since retrograde context interference affected both the sham and complete HPC lesion rats similarly, and since the sample trial exploration for HPC lesion rats were uncharacteristically low, there is no strong evidence of retrograde context interference affecting object recognition in HPC lesion rats.

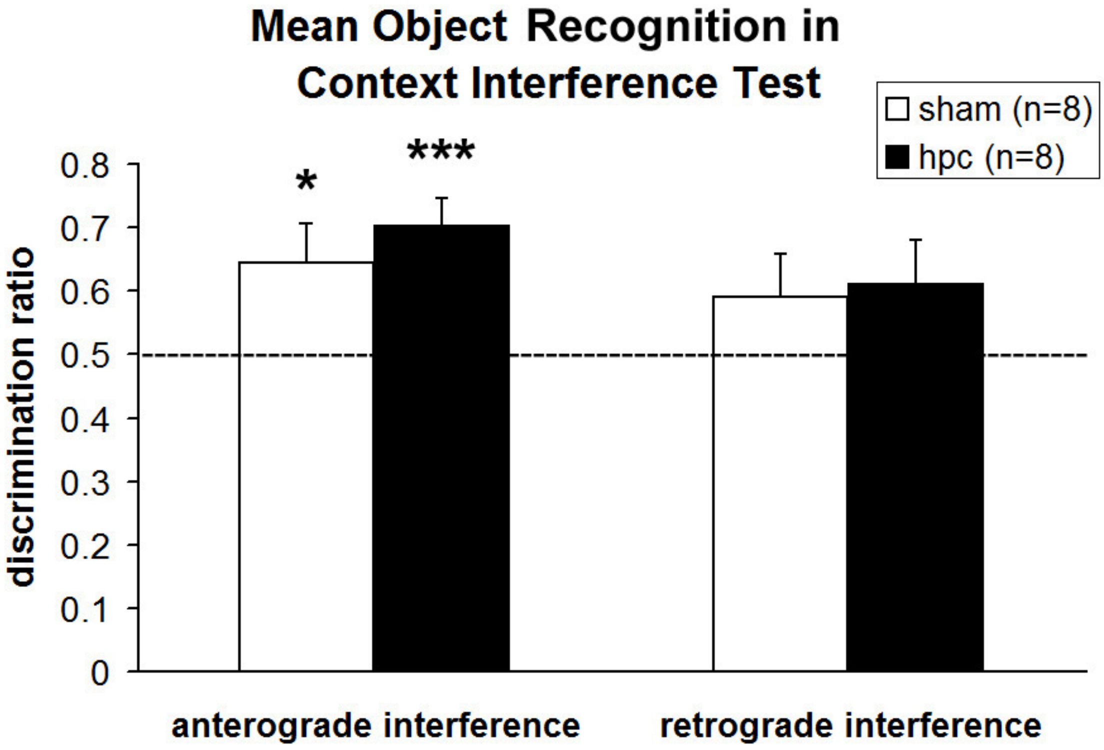


Figure 6.8. Object recognition of sham and complete HPC lesion rats during test trial following anterograde or retrograde context interference. * $p < .05$, *** $p \leq .01$, compared to investigation of target object at chance (dotted line). Data expressed as ±SEM.

DISCUSSION

The present study sought to test the hypothesis that interference may account for the impairments in object memory observed in rats' ability to discriminate object recency and associate objects with the contexts they were seen in after HPC damage. To this avail, the results have confirmed our hypothesis. Firstly, it was observed that after complete HPC lesions, rats' memory of the sample object is impaired by object interference. Secondly, object interference was found to be bidirectional; the encounter of another object, both prior to and after the encounter of a sample object, disrupted the memory of the sample object for rats with HPC damage. Thirdly, interference to object memory via non-object stimuli appears to be independent of HPC lesions. Interference to object memory via encounter of a novel context affected object memory in the same way for both sham and complete HPC lesion rats; specifically, both groups of rats retained novel-object preference following anterograde context interference, while both groups displayed non-significant novel object preference after retrograde context interference. In conclusion, the findings from the present study support object interference as a possible mechanism of object memory impairment for HPC lesion rats.

The present study began with a novel object recognition task intended to establish a baseline of object recognition memory in all rats. Rats were given free explorations to a novel sample object and after a delay of 4 hrs; the rats encountered a copy of the sample object and a novel object. As expected, both sham and complete HPC lesion rats showed significant preference for the target novel object thereby demonstrating intact memory for the sample object seen earlier.

Next, rats were tested on a three-trial object interference task in which a second novel object (i.e. the interference object) was seen by the rats either prior to or after seeing the sample object. After a delay, rats' preference for the first sample object against a third novel (i.e. target) object was observed. Interference of the memory of the sample object, therefore, can potentially occur in two directions. When the interference object was seen prior to the sample object, the memory of the second novel object (i.e. the interference object) would disrupt the memory of the sample in the anterograde direction (i.e. forward in time); when the interference object was seen after the sample object, the memory of the interference object would disrupt the memory of the sample object in the retrograde direction (i.e. back in time). The present study found that while sham rats' memory of the sample object was unaffected by object interference in either direction, HPC lesion rats were equally affected by object interference in both directions.

Lastly, rats were tested on a context "interference" task to examine whether the induced impairment in object recognition was limited to object-specific interference or if it can be induced by non-object interference as well. Novel context was chosen as the non-object interference stimulus to test whether encountering a different context during the course of the object memory task would impact object recognition. Similarly to the paradigm of object interference, here, rats encountered a novel context either before or after seeing a sample object. After a delay, rats' preference for the sample object against a novel object was observed. The results indicate no difference in novel object preference amongst sham and lesion rats. Both groups of rats displayed significant preference for the target object after anterograde context interference and showed non-significant preference for the target object after retrograde context interference. These results indicate that context interference does not disrupt novel object recognition in HPC damaged rats.

Although the data on retrograde context interference showed a non-significant preference for the target object for all rats, this is unlikely a reflection of an actual mnemonic impairment induced by context interference. Rather, this result is likely due to poor-discrimination from over-trained rats, as is evident from the extremely low levels of object exploration of HPC lesion rats at the sample trial. Inevitably during the course of spontaneous object tasks, since the task is not rewarded, rats will become over-trained. Alternatively, we could have scheduled more days in between tests . However, this represents a tradeoff between overtraining rats and having to re-habituate our rats. Another possible reason for the non-significance in our data may be our small sample size owing to the fact that the present study was intended to be a pilot investigation on the effects of interference on object memory.

One other possible explanation for the context interference data is that retrograde interference may create greater mnemonic disruption than anterograde interference as memory consolidation occurs during the period between encoding and recall and during this time, object memory may be more sensitive to disturbances compared with the period prior to encoding. Why then, one may ask, was this difference in the relative strengths of the two directions of interference not observed in the object interference experiment? Perhaps in instances in which the interference stimulus is very similar to the sample stimulus (as in the object interference experiment); interference of the memory for the sample stimulus is strong in both anterograde and retrograde direction, masking any difference in their relative strengths (see Figure 6.6). Conversely, when the interference stimulus is dissimilar to the sample stimulus, interference is weak and a difference between the two directions of interference may emerge (see Figure 6.8).

Exploration analyses of object trials in all three experiments revealed that rats' explorations of objects during sample trials are generally greater than, or at level with, explorations at test trials. An exception to this trend was the retrograde context interference trials in which the test trial exploration was significantly greater than that of the sample trial for both the sham and complete HPC lesion rats. One reason for this anomaly may be that the retrograde context interference paradigm affected explorations at test trial. Typically, the low level of object explorations during a sample trial indicates over-training. Recall that in retrograde context interference paradigm, rats encountered a novel context after the sample trial. The encounter of a novel context in between sample and test trial may have encouraged increase exploration via renewing rats' general interests to explore. As a result, rats' object exploration during the test trial was increased.

All in all, the data from the interference experiments suggests HPC has a unique role in object memory. Firstly, object memory is not stored in HPC, as HPC lesion rats can remember the sample object in novel object recognition. Secondly, while HPC is not involved in the memory of a single object, it modulates the memory of multiple objects. Recall the data from the object interference experiments, rats with HPC damage showed memory impairment following interference in both the anterograde and retrograde direction. Retrograde interference implies the memory of an object is forgotten after seeing a second object at a later time. Anterograde interference implies the memory of a latter seen object is forgotten or not encoded due to the memory of an object seen earlier. Object interference occurring in both directions suggests that memory traces of both early- and later- seen objects might have co-existed at some point in time and were interfering with each other. Had there been no interference, both objects would have been remembered and rats would have displayed normal discrimination of either one of the seen objects from a completely novel third object. Had there been preservation of the memory for one of the objects but not the other, one would expect there to be interference in one direction and not in both. Thus, it appears that rats with HPC damage could not retain memories of two or more objects due to interference. As a result, after HPC damage, rats were not capable of remembering more than one object in a given test. In intact rats, the HPC likely served the function of managing and maintaining the memories of multiple objects.

Thirdly, interference of object memory in HPC lesion rats can only occur with the memory of another object; context interference has little or no effect on object memory. Lastly, HPC does not regulate exploratory behavior. Rats with HPC lesions showed object exploration levels comparable to sham lesion rats.

In conclusion, the present study supports object interference as a viable mechanism for the impairments of HPC damaged rats in object recency and object context-recency tasks. Without a functional HPC, object memory is somewhat resilient to contextual interference but is susceptible to object-specific interference that occur prior to or after encoding. The present data suggests that HPC acts to maintain the memory of multiple objects, specifically through controlling for object-related interference.

7. GENERAL DISCUSSION

This thesis investigates the contribution of the hippocampus to object memory. Object memory is a form of memory with which objects can be discriminated, remembered, or chosen for their reinforcing value. The role of the hippocampus in object recognition is currently unclear, with some studies demonstrating a delay-dependent impairment after hippocampal damage while other studies show no impairment. The present thesis used the novel object recognition task and its variants to investigate object memory in rats with hippocampal lesions. In the first set of experiments, impairments were observed in some task variations but not in others. In the second set of experiments, it was found that encountering another object shortly prior to or after encountering a target object impairs the recognition of the target. In a control procedure, encountering a novel context did not impair object recognition. Thus, this thesis demonstrates that the intact hippocampus reduces interference between memories of objects encountered close in time. This finding clarifies inconsistencies in previous work and posits a role for the hippocampus in object memory. The following discussion will: 1) provide the rationale for studying hippocampal involvement in object memory, 2) describe the procedures and the present results, 3) present a model for the role of the hippocampus in remembering multiple objects, and 4) discuss the findings in relation to theories of the hippocampal function.

7.1 Rationale for Studying Hippocampal Involvement in Object Memory

The hippocampus is essential for declarative memory, a form of memory with which previous experiences are remembered (Squire, 1992). Because the hippocampus has connections with the adjacent perirhinal cortex, a brain region strongly implicated in object recognition, it might also be involved in object memory. In rats, object memory is studied via the delay-matching-to-sample (DMTS) task and the novel object recognition task. DMTS tasks train animals to encode a sample object. After a delay, rats are presented with the sample object and a novel object. Rats are rewarded upon choosing the sample object (hence "matching-to-sample"), or for choosing the novel object for delay-non-matching-to-sample (DNMS) version of the task. Novel object recognition tasks are based on the premise that novel objects are more interesting than familiar objects. The task requires no training. Rats are free to explore a sample object and after a delay, rats are presented with both the sample object and a novel object. Normal rats would investigate the novel object thereby demonstrating recognition of the sample. To date, studies using both tasks have produced mixed results with respect to a role for the hippocampus in object memory.

Findings from DMTS tasks show a delay-dependent impairment in object recognition for rats with hippocampal lesion. Particularly, the longer the delay between the sample and choice, the more impaired rats are at recognizing the sample object (Clark et al., 2001; Prusky et al., 2004). Conversely, findings from the novel object recognition tasks show no impairment up to a delay of 48 hours (Winters et al., 2004; Forwood et al., 2005). A handful of studies have found that without the hippocampus, object recognition becomes dependent on environmental cues. Specifically, recognition in hippocampal damaged rats is impaired if the context or object location during recall is different from that during encoding. In comparison, normal rats show intact recognition when encountering context or location change (Mumby et al., 2002; O'Brien et al., 2006). Thus, these discrepancies in recall in different tasks present a problem in defining the role of the hippocampus in object memory.

7.2. Procedures and Results

The novel object recognition task was chosen to test object memory for this thesis. The advantage of this task is that it is not confounded by rats' motivation for reward and rats' memory for task rules (see chapter 3 for an in-depth argument). Two sets of experiments were performed.

The first set of experiments examined the effects of complete and partial hippocampal damage on object memory. Using the novel object recognition task and two other variations of the task, rats were investigated on their ability to 1) recognize a sample object, 2) discriminate objects according to the order in which they were seen (i.e. object recency), and 3) associate objects to the contexts and the order in which they were seen (i.e. object context-recency). As described, the novel object recognition task involves letting rats freely explore a sample object. After a delay, rats are presented with both the sample object and a novel object. Normal rats would investigate the novel object, thereby demonstrating recognition of the sample. For the object recency variation of the task, rats encounter a sample object. After a short delay, rats encounter a second sample object. After a long delay, rats see both sample objects. Normal rats would prefer the remotely familiar sample object, thereby demonstrating discrimination of the order of objects seen. For the object context-recency variation of the task, rats encounter a sample object in one context and after a short delay, they encounter a second sample object in another context. After a long delay, they encounter both objects in one of the two contexts. Normal rats would prefer the object in the mismatched context (i.e. the target object) thereby demonstrating their memory for the objects and their respective contexts. Normal rats would show an even greater preference for the target object if it was also sampled further back in time (i.e. a remotely familiar target object), thereby demonstrating their memory for the order as well as the respective contexts of the objects.

The second set of experiments investigated object recognition memory using a variation of the task in which successive presentations of objects or non-object stimuli could potentially interfere with memory. First, rats were tested on novel object recognition. Next, rats were tested on anterograde and retrograde object interference. The object interference task is similar to the novel object recognition task, except rats are presented with a second sample object either shortly prior to or after seeing the sample object. Since the second sample object is not to be seen again, it acts as interference to the memory of the first sample object. For anterograde interference, the interference trial takes place shortly before seeing the sample object; for retrograde interference, the interference trial takes place shortly after seeing the sample. Normal rats unaffected by the interference would display intact novel object recognition at recall when they see the first sample object paired with a novel object. Lastly, as a control measure, rats were tested on anterograde and retrograde context interference. The paradigm of context interference is very similar to object interference except instead of an interference object, rats see a novel context either shortly prior to or after seeing a sample object. Again, normal rats that are unaffected by the interference would show intact novel object recognition.

The results from the first part of this thesis show that rats with complete hippocampal damage, while having intact novel object recognition, have impaired object recency discrimination and object context-recency association. As well, the pilot results with partial hippocampal lesion rats indicated a dichotomy of function related to lesion location. Rats with ventral hippocampal lesion retained object recency memory and showed signs of object context-recency memory, whereas rats with dorsal hippocampal lesions were impaired in both types of memories. The results from this first set of experiments, although seemingly variable, could be explained by supposing that hippocampal damage impairs rats' ability to form object recency discrimination and object context-recency association due to interference. That is, in the absence of the hippocampus, memories of objects from the two sample trials may be interfering with each other rendering rats unable to discriminate either the relative recency of objects or associate objects with their presented contexts.

The results from the second set of experiments confirm an object-interference hypothesis; rats with complete hippocampal lesions were found to be impaired in object recognition following both anterograde and retrograde object interference. Object recognition was largely intact following context interference. Thus, these findings indicate that the hippocampus is involved in the memory of multiple objects, and it likely does so by reducing interference between successive objects encoded.

Taken altogether, the present investigation revealed a unique profile of object memory impairment in rats following hippocampal damage. This profile has five main characteristics, which are discussed below.

First, object recognition is independent of the hippocampus. Rats with complete hippocampal lesions are unimpaired in novel object recognition. This finding replicates a number of previous studies (for example, Winters et al., 2004; Forwood et al., 2005).

Second, memories of multiple objects are dependent on the hippocampus. Results from the object interference tasks showed that hippocampal lesion rats were unable to recognize the sample object if it was seen prior to or after the interference. Since on any given object trial during the sample phase of the object interference task, it was impossible for rats to know whether an object were to be recognized at choice trial, rats must remember both the sample object and the interference object in order to display novel object preference at choice. Although recognition of the interference objects was not tested, since rats did not remember the sample object regardless of the order in which it was seen, it is likely that rats do not remember either object. Moreover, object recognition was not disrupted by context interference, indicating the effects of interference are object-specific. Thus, the hippocampus is required for memories of more than one object.

Third, object recency memory is dependent on the hippocampus. Rats with complete hippocampal lesions showed equal preference for the remotely familiar object and the recently familiar object. The lack of recency discrimination can result from: 1) both objects being equally well-remembered, or 2) both objects being forgotten. In view of the data from object interference, it is likely that the rats have forgotten both objects and therefore showed no object recency discrimination. Thus, without a functional hippocampus, rats cannot remember the order of the objects.

Fourth, object context-recency association is dependent on the hippocampus. Rats with complete hippocampal lesions showed an abnormal pattern of object context-recency discrimination. Not only do hippocampal lesion rats not show a preference for the mismatched object, they prefer the recently familiar object regardless of the context at choice. This means: 1) these rats do not take into account of context and/or recency information when recognizing objects and 2) they show a primacy effect of remembering the first object when the objects were presented in distinctly different context, presumably due to reduced interference. In agreement with these findings, studies on the hippocampal relational network have described rat without the hippocampus as rigid and selectively pays attention to the most predictive stimuli while ignore the associations of surrounding cues (Moses et al., 2002; 2005).

Fifth, the dorsal hippocampus may be more important for object memory than the ventral hippocampus. Pilot results indicate that after dorsal hippocampal damage, rats cannot discriminate object recency while after ventral hippocampal damage, rats may have no impairment. Additionally, neither dorsal nor ventral hippocampal lesion rats showed intact object context-recency association. Since both object recency memory and object context-recency memory require rats to discern multiple objects, and since our findings showed ventral hippocampal lesions to produce relatively less impairment than dorsal hippocampal lesions, the dorsal hippocampus may be more important for the memories of multiple objects. Our results is consistent with the literature, which finds ventral hippocampal lesions to produce comparatively less object memory impairment than dorsal or complete hippocampal lesions in spatial memory (see Broadbent et al., 2004 for review). It would have been ideal to increase our sample sizes for partial hippocampal lesions and test them on object interference task.

Summarizing the object impairments observed thus far, hippocampal lesions in general impairs rats in tasks in which they are required to remember two objects presented in the same context, likely due to interference of memory. No impairment is seen when rats are required to remember only one object. In addition, encounters with a novel context --- a non-object stimulus --- does not impair object memory. When two objects are presented in two distinct contexts, rats retain memory for the first object seen. The preserved memory of the first object is likely due to reduced interference: that is, the object memory of hippocampal lesion rats exhibited a strong context- and place-dependency in which the contexts affected the memory of the objects (Moses et al., 2002; Mumby et al., 2002; O'Brien et al., 2006). If the object trials are made different by contrasting their contexts, the memory of the object trials will become less prone to interference and hence some part of the object memory will be preserved.

Elsewhere, evidence for the hippocampus managing spatial (i.e. both context and location) and semantic (Marr, 1971; Zola-Morgan et al., 1986; Shapiro and Olton, 1994; Leutgeb et al., 2006) memory interference have been found. In particular, interference of similar memories is known to occur as a function of increased overlap of spatial or semantic elements. To minimize interference, the hippocampus encodes and separates similar events in space and time to make the events more different by a process called pattern separation (see Kesner and Hopkins, 2006 for review).

The present results can be explained by positing that the hippocampus mediates pattern separation. As described above, the results indicate that object impairment in rats with hippocampal damage varies as a function of feature overlap between object trials (Figure 7.21). The next section introduces a model for object memory that incorporates pattern separation.

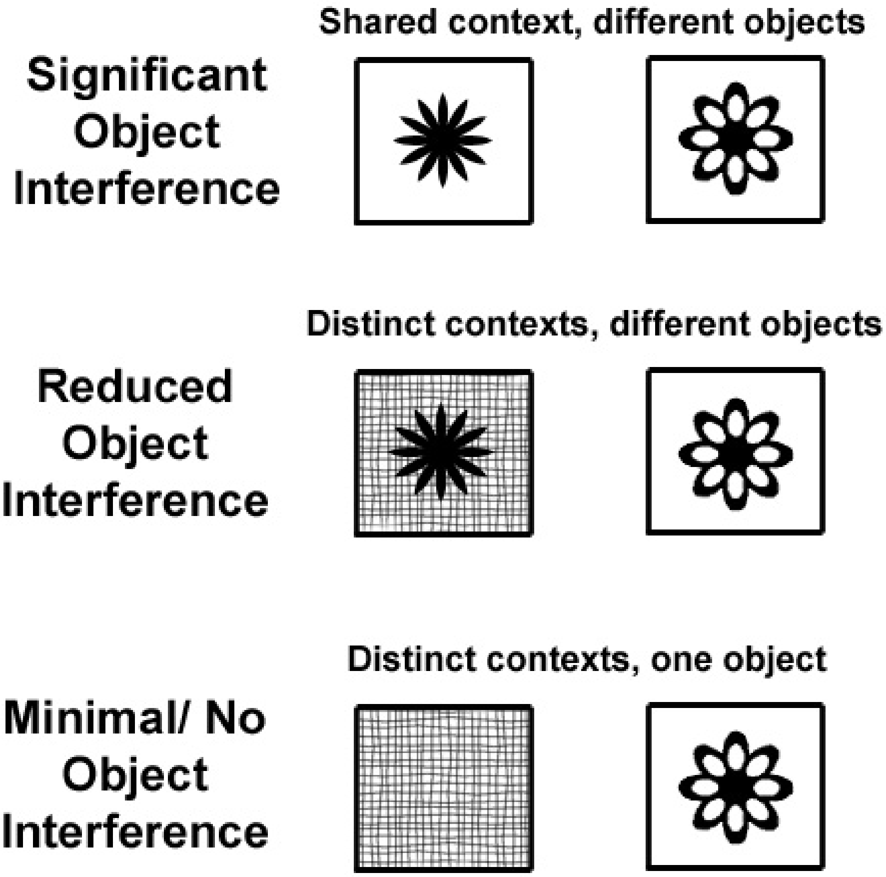


Figure 7.21. Interference as a function of feature overlap between object trials. Object interference becomes apparent in the absence of the hippocampus, supporting rats' use of the hippocampus to pattern-separate the memories of multiple objects. Contexts are represented by the white and hashed square boxes; objects are represented by the floral symbols inside the contexts.

7.3 A Model for the Role of the Hippocampus in Reducing Object Interference

Figures 7.31 and 7.32 present two parts of a stimulus-response model that accounts for how rats remember single and multiple objects during spontaneous, one-trial encoding. This model, based loosely on Hirsh's model of contextual retrieval (1974, as cited in Morris, 2007), proposes that the hippocampus is actively involved in the encoding, the consolidation, and the retrieval of multiple objects. When an animal sees an event, first, the hippocampus establishes a flexible representation for the event. Second, the hippocampus pattern-separates similar events to allow for subsequent event-encoding and the consolidation of encoded events. Third, the hippocampus compares the familiarity of events at recall to generate appropriate motor output such as directing the exploratory behavior of the animal.

In the first part of the model (Figure 7.31), recognition of a single object is possible via both the hippocampal (HPC) and the non-hippocampal (non-HPC) system. At encoding, the hippocampal (HPC) system forms a flexible representation of the event by encoding the object, the context, and any additional information contingent with the event. The non-HPC system, on the other hand, encodes the event as a scene by encoding the object and its context as whole; this representation therefore is inflexible and is vulnerable to change. At recall, the HPC system discriminates objects via comparing for their relative familiarity (i.e. object A in context 1 versus object B in context 1: which one is (more) familiar?). The non-HPC system would recognize parts of the scene as familiar or novel (i.e. object B was not in the scene initially encoded). Although both systems would render the same motor output of directing exploration to the novel object, the non-HPC system is more efficient for this type of memory as the system would require less resources at encoding and it would be faster at recognizing novelty at recall.

The second part of the model (Figure 7.32) illustrates the discrimination of multiple objects or events. Again, the HPC system forms flexible representations of the events while the non-HPC system forms an inflexible representation. Upon encoding of the second event, the HPC system immediately engages in pattern separation if the second event was deemed similar to the first. Pattern separation minimizes any interference between the memories of the events encoded and thus both events are remembered. The process of pattern separation will be repeated if there are subsequent events to encode. The non-HPC system, however, does not support pattern separation. Thus, the representations of events encoded by the non-HPC system are prone to interference. Pattern separation is not required if the events are deemed dissimilar. At recall, the HPC system again discriminates objects based on their relative familiarity to determine the appropriate motor output. The non-HPC system would not support any process of object discrimination since encoded events are no longer remembered due to interference.

This stimulus-response model of object memory accommodates all of the results of this thesis. For example, it explains the impairment we observed on the object recency task and the object interference task in which rats see two objects in the same context (i.e. object A in sample trial 1 then object B in sample trial 2, both objects presented in context 1). According to the model, the HPC system forms flexible representations of each object trial and engages in pattern separation upon encountering the second object. Both objects are then remembered. At recall, rats see either objects A and B for the object recency discrimination or objects A with C for the object interference task. The HPC system then compares the objects for their relative familiarity. For the object recency discrimination, object A is compared against object B, and since object B was seen more recently, object A would be judged as less familiar. For the object interference task, object A would be compared against object C for familiarity. Since object C is entirely novel, it would be deemed as unfamiliar. Applying the model to the object context-recency task, in which rats see two objects in distinctly different contexts (i.e. object A in context 1, then object B in context 2), the HPC system would encode flexible representation of the object and its respective context for each trial. Pattern separation here is less critical as the events are made different by the different contexts. At recall, both objects are seen in one of the contexts (i.e. objects A and B in context 2). The HPC system would once again compare for familiarity between the objects, taking into account of the contexts (i.e. object A in context 2 versus object B in context 2). Object A which has not been seen in context 2 would be deemed less familiar and rats would respond by directing the motor output of exploring the less familiar object.

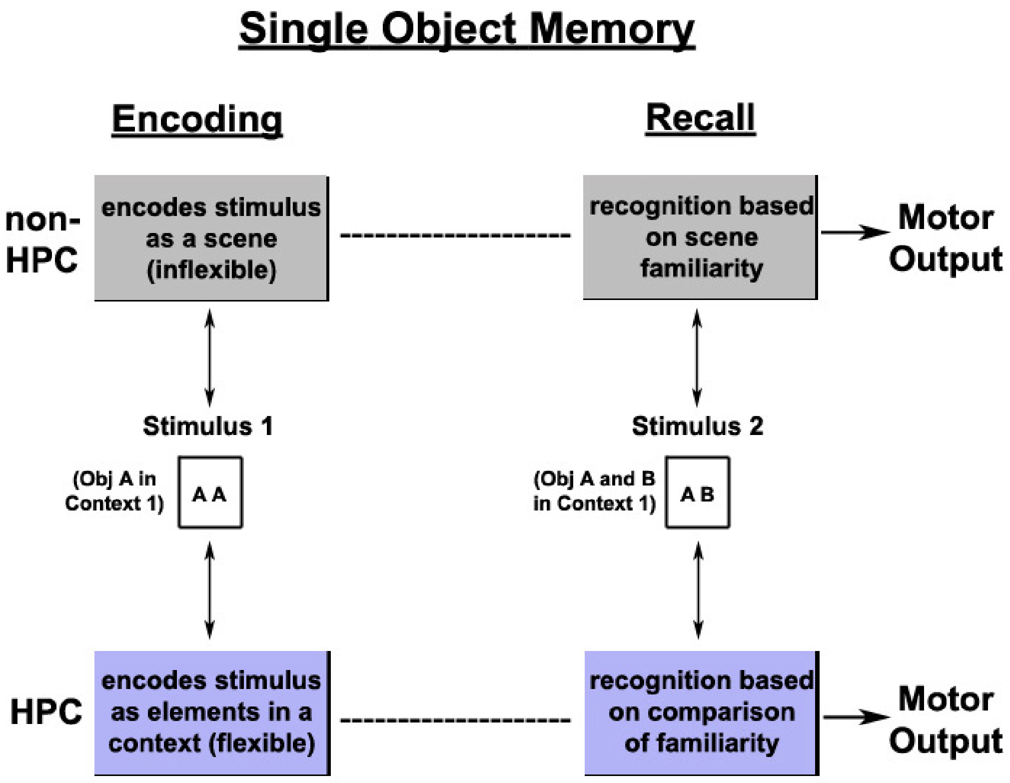


Figure 7.31. A stimulus-response model for the memory of a single object. The model shows that the recognition of a single object can be achieved via either the non-hippocampal (non-HPC) system or the hippocampal (HPC) system. Arrows denote sequence of process(es); dotted lines denote access to informational process(es).

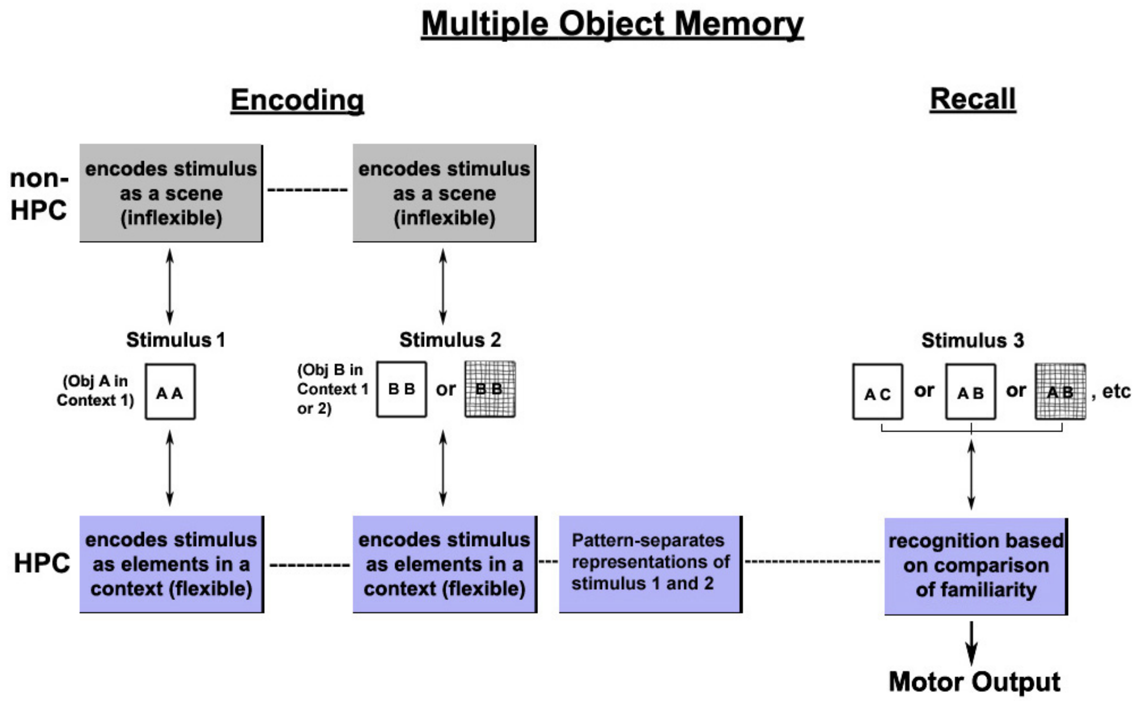


Figure 7.32. A stimulus-response model for the memory of multiple objects. The model shows that discrimination of multiple objects can only be achieved via the HPC system, which supports flexible representation of an event, pattern-separation of similar events, and comparison of familiarity at recall. Arrows denote sequence of process(es); dotted lines denote access to informational process(es).

7.4 Relation to Theories of the Hippocampal Function

Consistently with the proposed model, a common theme for contemporary theories of the hippocampal function is the functional dissociation between hippocampal and non-hippocampal systems. Many existing theories of hippocampal function have considerable overlap and are nonspecific in such a way that they are able to account for parts of the present results. In this section, I will briefly relate our results to the three theories reviewed in the introduction of this thesis: the cognitive map theory, the working / reference memory theory, and the configural association theory.

The cognitive map theory (O'Keefe and Nadel, 1978) states that the goal-directive hippocampal-independent taxon system is highly prone to interference since the system lacks the ability to form spatial / contextual associations. Indeed, interference was observed when rats with hippocampal damage were required to remember multiple objects. As well, these rats could not remember the recency order of objects, nor form any association of context and recency with objects due to the taxon system's lack of ability to form spatial / contextual associations. Thus, in agreement with the cognitive map theory, rats can only remember and recognize a single object after hippocampal lesion.

Another spatial-based theory of the hippocampus, Olton and colleagues' theory of working / reference memory (1979), which states that working memory is hippocampal-dependent, can also be seen as explaining the results. Working memory refers to a type of "online", short-term memory readily accessible for object discrimination. Thus, working memory would be utilized in tasks that require the discrimination of multiple objects and / or multiple contexts. This theory predicts that hippocampal lesions impair rats on object-recency discrimination and object context-recency association. The theory also predicts these rats are impaired in the object interference task. For rats to demonstrate intact novel object recognition following object interference, rats must remember both the interference object and the sample object in order to discriminate either one of them from the novel object. Thus, the object interference task, amongst other tasks that require the memory of multiple objects, can be thought of as a working memory problem that requires the hippocampus.

Lastly, Rudy and Sutherland's revised theory of configural association (1995) can be viewed as being consistent with the results. In brief, the theory states that the memory of elemental stimuli is stored elsewhere but the hippocampus remains important for enhancing the associative strengths between stimuli to form configurations. The theory is able to account for the atypical object preference in object context-recency task for rats without the hippocampus, as they cannot form proper associations between objects and contexts. The theory also accounts for hippocampal lesion impairing rats in object recency discrimination, as the configuration of objects and temporal order would be required for this discrimination. As well, the theory can be seen as explaining the hippocampal lesion rats' impairment following object interference. Since the theory contends that the hippocampus reduces interference amongst related memories via configural associations, without it, interference of similar memories would occur.

Interestingly, the proposed model of object memory can accurately predict some of the configural association findings that were not accounted for by Sutherland and Rudy's configural association theory (1989; Rudy and Sutherland, 1995). For instance, in McDonald and colleagues' (1997) assessment of the configural association theory, hippocampal lesion rats were found to be impaired in negative patterning and mildly impaired in biconditional discrimination (see section 2.4 for review), while unimpaired in conditional context discrimination (a problem very similar to the biconditional discrimination, in which in context 1: A+, B- and in context 2: A-, B+). The inconsistent pattern of impairment in some but not all configural problems cannot be predicted by the configural association theory; however, they can be predicted base on the concept of feature-overlap. Firstly, hippocampal lesion rats were unimpaired in conditional context discrimination as the two problems were learned and recalled in distinctly different contexts and these memories had little chance of interference with one another. Secondly, hippocampal lesion rats were mildly impaired in biconditional discrimination; although the two problems were held in the same contexts, they were made different due to the distinct features of the configurations (i.e. A+ when light is off, B+ when light is on) and thus interference is minimized. Thirdly, hippocampal lesion rats were impaired in negative patterning in which both rewarded stimuli were learned in the same context with considerable feature-overlap; only when the stimuli were seen together would they signal non-reward (i.e. A+, B+, AB-). Thus, the present theory of object memory can potentially be applied to studies of Pavlovian conditioning and non-object memory.

7.5 Conclusion

The role of the hippocampus in object memory has been controversial. From reviewing our results, it is clear that object memory can be considered as a hippocampal problem. While investigating the role of the hippocampus in object memory, it was apparent that the existing theories of hippocampal function, although able to make some general predictions, cannot adequately account for our observations. Certain theories can rationalize our results *a posteriori*, but the theories were unable to definitively predict our results. Hence, the present thesis proposed a two-part stimulus-response model object memories to interpret our findings.

The model incorporates the present findings on pattern separation and the existing theories of the hippocampus into an object memory framework. Instead of dichotomizing tasks into hippocampal versus non-hippocampal dependent, the present model proposes that hippocampal involvement in object memory depends on interference. In particular, when there is interference, the task can only be solved by the hippocampal system. Thus, the present model can provide specific predictions for a variety of object problems.

In conclusion, the present thesis suggests that the hippocampus is required for remembering multiple objects. When hippocampal lesion rats encounter another object shortly before or after seeing a target object, the memory of the target object is impaired. Alternatively, when hippocampal lesion rats encounter a novel context shortly before or after seeing a target object, the memory of the target object is intact. When hippocampal lesion rats encounter another object in a distinctly different context shortly after seeing the target object, the memory of the target object is intact. The results indicate that without the hippocampus, rats are impaired in discerning objects in trials that have significant feature-overlap. Thus, the novel contribution of this thesis is that rats use the hippocampus to pattern-separate the memories of multiple objects.

8. REFERENCES

Aggleton, J. P., Hunt, P. R., and Rawlins, J. N. (1986). The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. Behav Brain Res., 19(2): 133-146.

Alvarado, M. C., Rudy, J. W. (1995a). Rats with damage to the hippocampal-formation are impaired on the transverse-patterning problem but not on elemental discriminations. Behav Neurosci., 109(2): 204-211.

Alvarado, M. C., Rudy, J. W. (1995b). A comparison of kainic acid plus colchicine and ibotenic acid-induced hippocampal formation damage on four configural tasks in rats. Behav Neurosci., 109(6): 1052-1062.

Amaral, D. G. and Lavenex, P. (2007). Hippocampal Anatomy. In: P. Anderson, R. Morris, D. Amaral, T. Bliss, and J. O'Keefe (eds.), The Hippocampus Book (pp. 37-114). New York: Oxford University Press.

Amaral, D. G. and Witter, M. P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience, 31(3): 571-591.

Amaral, D. G. and Witter, M. P. (1995). Hippocampal formation. In: G. Paxinos (ed.), The Rat Nervous System (pp. 443-492). San Diego: Academic Press.

Andersen, P., Holmqvist, B., and Voorhoeve, P. E. (1966). Excitatory synapses on hippocampal apical dendrites activated by entorhinal stimulation. Acta Physiol Scand., 66(4): 461-472.

Anderson, P., Morris, R., Amaral, D., Bliss, T., and O'Keefe, J. (2004). Historical perspective: proposed functions, biological characteristics, and neurobiological models of the hippocampus. In: P. Anderson, R. Morris, D. Amaral, T. Bliss, and J. O'Keefe (eds.), The Hippocampus Book (pp. 9-36). New York: Oxford University Press.

Anderson, P. Sundberg, S. H., Sveen, O., and Wigström, H. (1977). Specific long-lasting potentiation of synaptic transmission in hippocampal slices. Nature, 266(5604): 736-737.

Bannerman, D. M., Good, M. A., Butcher, S. P., Ramsay, M., and Morris, R. G. (1995). Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. Nature, 378(6553): 182-186.

Barrionuevo, G. and Brown, T. (1983). Associative long term potentiation in hippocampal slices. Proc Natl Acad Sci USA., 70: 7347-7351.

Bartko, S. J., Winters, B. D., Cowell, R. A., Saksida, L. M., and Bussey, T. J. (2007). Perceptual functions of perirhinal cortex in rats: zero-delay object recognition and simultaneous oddity discriminations. J Neurosci., 27(10): 2548-2559.

Bliss, T., Collingridge, G., and Morris, R. (2007). In: P. Anderson, R. Morris, D. Amaral, T. Bliss, and J. O'Keefe (eds.), The Hippocampus Book (pp. 343-474). New York: Oxford University Press.

Bliss, T. and Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol (Lond)., 232: 331-356.

Broadbent, N. J., Squire, L. R., and Clark, R. E. (2004). Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A., 101(40): 14515-14520.

Buckley, M. J. and Gaffan, D. (1998). Perirhinal cortex ablation impairs visual object identification. J Neurosci., 18(6): 2268-2275.

Bullock, T. H., Buzsaki, G., and McClune, M. C. (1990). Coherence of compound field potentials reveals discontinuities in the CA1-subiculum of the hippocampus in freely-moving rats. Neuroscience, 38(3): 609-619.

Clark, R. E., West, A. N., Zola-Morgan, S., and Squire, L. R. (2001). Rats with lesions of the hippocampus are impaired on the delayed nonmatching-to-sample task. Hippocampus, 11(2): 176-186.

Cohen, N. J. and Eichenbaum, H. E. (1993). Memory, amnesia and the hippocampal system (1st ed.). Cambridge, MA: MIT Press.

Corkin, S., Amaral, D. G., Gonzalez, R. G., Johnson, K. A., and Hyman, B. T. (1997). H. M.'s medial temporal lobe lesions: findings from magnetic resonance imaging. J Neurosci., 17: 3964-3979.

Davidson, T. L., McKernan, M. G., and Jarrard, L W. (1993). Hippocampal lesions do not impair negative patterning: a challenge to configural association theory. Behav Neurosci., 107: 227-234.

Douglas, R. M. and Goddard, G. V. (1975). Long term potentiation of the perforant path-granule cell synapse in the rat hippocampus. Brain Res., 86: 205-215.

Dunwiddie, T. and Lynch, G. (1978). Long term potentiation and depression of synaptic responses in the rat hippocampus: localization and frequency dependency. J Physiol (Lond)., 276: 353-367.

Eichenbaum, H. (2004). Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron, 44: 109-120.

Eichenbaum, H., Stewart, C., and Morris, R. G. (1990). Hippocampal representation in place learning. J Neurosci., 10(11): 3531-3542.

Ennaceur, A. and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res., 31(1): 47-59.

Forwood, S. E., Winters, B. D., Bussey, T. J. (2005). Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. Hippocampus, 15(3): 347-355.

Frey, U., Huang, Y. Y., and Kandel, E. R. (1993). Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science, 260: 1661–1664.

Frey, U., Krug, M., Reymann, K. G. and Matthies, H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region *in vitro* . Brain Res., 452: 57–65.

Gallagher, M. and Holland, P. C. (1992). Preserved configural learning and spatial learning impairment in rats with hippocampal damage. Hippocampus, 2: 81-88.

Gallinat, J., Kunz, D., Senkowski, D., Kienast, T., Seifert, F., Schubert, F., and Heinz, A. (2006). Hippocampal glutamate concentration predicts cerebral theta oscillations during cognitive processing. Psychopharmacology (Berl)., 187(1): 103-111.

Gemma, C., Imeri, L., and Mancia, M. (1999). Hippocampal type 1 (movement-related) theta rhythm positively correlates with serotonergic activity. Arch Ital Biol., 137(2-3): 151-60.

Green, J. D. (1964). The Hippocampus. Physiol Rev., 44: 561-608.

Hafting, T., Fyhn, M., Molden, S., Moser, M. B., and Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. Nature, 436(7052): 801-806.

Hannesson, D. K., Vacca, G., Howland, J. G., and Phillips, A. G. (2004). Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats. Behav Brain Res., 153(1): 273-285.

Hasselmo, M. E. (2005). What is the function of hippocampal theta rhythm? -- Linking behavioral data to phasic properties of field potential and unit recording data. Hippocampus, 15(7): 936-949.

Hok, V., Lenck-Santini, P. P., Roux, S., Save, E., Muller, R. U., and Poucet, B. (2007). Goal-related activity in hippocampal place cells. J Neurosci., 27(3): 472-482.

Jarrard, L. E. (1978). Selective hippocampal lesions: differential effects on performance by rats of a spatial task with preoperative versus postoperative training. J Comp Physiol Psychol., 92: 1119-1127.

Jarrard, L. E. (1986). Selective hippocampal lesions and behavior: implications for current research and theorizing. In: R. L. Isaacson and K. H. Pribram (eds.), The Hippocampus (pp. 93-126). New York: Plenum.

Jarosiewicz, B., McNaughton, B. L., and Skaggs, W. E. (2002). Hippocampal population activity during the small-amplitude irregular activity state in the rat. J Neurosci., 22(4): 1373-1384.

Jensen, O. and Lisman, J. E. (2000). Position reconstruction from an ensemble of hippocampal place cells: contribution of theta phase coding. J Neurophysiol., 83(5): 2602-2609.

Kandel, E. R., Schwartz, J. H., and Jessell, T. M. (1995). Essentials of neural science and behavior (1st ed.). New York: McGraw-Hill.

Kesner, R. P. and Hopkins, R. O. (2006). Mnemonic functions of the hippocampus: a comparison between animals and humans. Biol Psychol., 73(1): 3-18.

Kramis, R., Vanderwolf, C. H., and Bland, B. H. (1975). Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital. Exp Neurol., 49(1 Pt 1): 58-85.

Leutgeb, S., Leutgeb, J. K., Moser, E. I., and Moser, M. B. (2006). Fast rate coding in hippocampal CA3 cell ensembles. Hippocampus. 2006;16(9): 765-74.

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B., Moser, E. I. (2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. Science, 305(5688): 1295-1298.

Levy, W. B. and Steward, O. (1979). Synapses as associative memory elements in the hippocampal formation. Brain Res., 175: 233-245.

Levy, W. B. and Steward, O. (1983). Temporal contiguity requirements for long term associative potentiation / depression in the hippocampus. Neuroscience, 8: 791-797.

Marr, D. (1971). Simple memory: A theory for archicortex. Philos Trans R Soc Lond B Biol Sci., 262(841): 23-81.

Martin, S. J. and Clark, R. E. (2007). The rodent hippocampus and spatial memory: from synapses to systems. Cell Mol Life Sci., 64(4): 401-431.

McDonald, R. J., Murphy, R. A., Guarraci, F. A., Gortler, J. R., White, N. M., and Baker, A. G. (1997). Systematic comparison of the effects of hippocampal and fornix-fimbria lesions on acquisition of three configural discriminations. Hippocampus, 7(4): 371-388.

McNaughton, B. L., Douglas, R. M., and Goddard, G. V. (1978). Synaptic enhancement in fascia dentate: cooperativity among coactive afferents. Brain Res., 157: 277-293.

Morris, R. G. (2007). Theories of Hippocampal Function. In: P. Anderson, R. Morris, D. Amaral, T. Bliss, and J. O'Keefe (eds.), The Hippocampus Book (pp. 581-713). New York: Oxford University Press.

Morris, R. G. and Frey, U. (1997). Hippocampal synaptic plasticity: role in spatial learning or the automatic recording of attended experience? Philos Trans R Soc Lond B Biol Sci., 352(1360): 1489-1503.

Morris, R. G., Garrud, P., Rawlins, J. N., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. Nature, 297(5868): 681-683.

Morris, R. G., Schenk, F., Tweedie, F., and Jarrard, L. E. (1990). Ibotenate Lesions of Hippocampus and/or Subiculum: Dissociating Components of Allocentric Spatial Learning. Eur J Neurosci., 2(12): 1016-1028.

Moses, S. N., Cole, C., Driscoll, I., and Ryan, J. D. (2005). Differential contributions of hippocampus, amygdala and perirhinal cortex to recognition of novel objects, contextual stimuli and stimulus relationships. Brain Res Bull., 67(1-2): 62-76.

Moses, S. N., Sutherland, R. J., and McDonald, R. J. (2002). Differential involvement of amygdala and hippocampus in responding to novel objects and contexts. Brain Res Bull., 58(5): 517-527.

Muller, R. U. and Kubie, J. L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J Neurosci., 7(7): 1951-1968.

Mumby, D. G. and Pinel, J. P. (1994). Rhinal cortex lesions and object recognition in rats. Behav Neurosci., 108(1): 11-18.

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., and Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learn Mem., 9(2): 49-57.

Murray, E. A., Bussey, T. J., and Saksida, L. M. (2007). Visual perception and memory: a new view of medial temporal lobe function in primates and rodents. Annu Rev Neurosci., 30: 99-122.

Murray, E. A. and Richmond, B. J. (2001). Role of perirhinal cortex in object perception, memory, and associations. Curr Opin Neurobiol., 11(2): 188-193.

Nguyen, P. V., Abel, T., and Kandel, E. R. (1994). Requirement of a critical period of transcription for induction of a late-phase of LTP. Science, 265: 1104–1107.

O'Brien, N., Lehmann, H., Lecluse, V., and Mumby, D. G. (2006). Enhanced context-dependency of object recognition in rats with hippocampal lesions. Behav Brain Res., 170(1): 156-162.

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. Exp Neurol., 51(1): 78-109.

O'Keefe J and Dostrovsky J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res., 34(1): 171-175.

O'Keefe, J. and Nadel, L. (1978). The hippocampus as a cognitive map. Oxford, London: Clarendon Press.

O'Keefe, J., Nadel, L., Keightley, S., and Kill, D. (1975). Fornix lesions selectively abolish place learning in the rat. Exp Neurol., 48(1): 152-166.

Olton, D. S. (1977). The function of septo-hippocampal connections in spatially organized behavior. Ciba Found Symp., 58: 327-349.

Olton, D. S., Becker, J. T. and Handelman, G. H. (1979). Hippocampus, space and memory. Brain Behav Sci., 2: 313-365.

Olton, D. S. and Samuelson, R. J. (1976). Remembrance of places passed: spatial memory in rats. J Exp Psychol Anim Behav Process, 2: 97-116.

O'Reilly, R. C. and Rudy, J. W. (2001). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. Psychol Rev., 108(2): 311-345.

Pastalkova, E., Serrano, P., Pinkhasova, D., Wallace, E., Fenton, A.A., Sacktor, T.C. (2006). Storage of Spatial Information by the Maintenance Mechanism of LTP. Science, 313(5790): 1141-1144.

Paxinos, G. and Watson, C. (1998). The Rat Brain in Stereotaxic Coordinates (4th ed.). Toronto: Academic Press.

Pothuizen, H. H., Zhang, W. N., Jongen-Relo, A. L., Feldon, J. and Yee, B. K. (2004). Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working spatial memory. Eur J Neurosci., 19(3): 705-712.

Prusky, G. T., Douglas, R. M., Nelson, L., Shabanpoor, A., and Sutherland, R. J. (2004). Visual memory task for rats reveals an essential role for hippocampus and perirhinal cortex. Proc Natl Acad Sci U S A., 101(14): 5064-5068.

Ranck, J. B. Jr. (1984). Head direction cells in the deep layer of dorsal presubiculum in freely moving rats. Soc. Neurosci Abstr., 10: 599.

Rasmussen, M., Barnes, C. A., and McNaughton, B. L. (1989). A systematic test of cognitive mapping, working-memory, and temporal discontiguity theories of hippocampal function. Psychobiology, 17(4): 335-348.

Redish, A. D. (1999). Beyond the cognitive map, from place cells to episodic memory, pp. 177-198. Cambridge, MA: MIT Press.

Rudy, J. W. and Sutherland, R. J. (1995). Configural association theory and the hippocampal formation: an appraisal and reconfiguration. Hippocampus, 5: 375-389.

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B., Witter, M. P., Moser, E. I., and Moser, M. B. (2006). Conjunctive representation of position, direction, and velocity in entorhinal cortex. Science, 312: 758-762.

Saucier, D. and Cain, D. P. (1995). Spatial learning without NMDA receptor-dependent long-term potentiation. Nature, 378(6553): 186-189.

Scoville, W. B. and Milner, B. (1957). Loss of Recent Memory After Bilateral Hippocampal Lesions. J Neurol Neurosurg Psychiatry., 20: 11-21.

Shapiro, M. L., and Olton, D. S. (1994). Hippocampal function and interference. In: D. L. Schacter and E. Tulving (eds.), Memory Systems (pp. 87-117). Cambridge, MA: MIT Press.

Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. Psychol Rev., 99: 195-231.

Stackman, R.W., Clark, A. S., Taube, J. S. (2002). Hippocampal spatial representations require vestibular input. Hippocampus, 12: 291–303.

Steele, R. J. and Morris, R. G. (1999). Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. Hippocampus, 9(2): 118-136.

Stouffer, E. M. and White, N. M. (2007). Roles of learning and motivation in preference behavior: Mediation by entorhinal cortex, dorsal and ventral hippocampus. Hippocampus, 17: 147-160.

Sutherland, R. J. and Palmer, M. (1992). Impairment in spatial and nonspatial configural tasks after hippocampal ibotenate or kainite + colchicines lesions. Proceed 5th Conf Neurobiol Learn Mem., 92.

Sutherland, R. J. and Rudy, J. W. (1989). Configural association theory: the role of hippocampal formation in learning, memory, and amnesia. Psychobiol., 17(2): 129-144.

Swanson, L.W. (1978). The anatomical organization of septo-hippocampal projections. In: S. A. Gray (ed.), Functions of the Septo-Hippocampal System (pp. 25-48). Amsterdam: Elsevier North Holland.

Taube, J. S. (2007). The Head Direction Signal: Origins and Sensory-Motor Integration. Annu. Rev Neurosci., 30: 181–207.

Taube, J. S., Muller, R. U., and Ranck, J. B. Jr. (1990). Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations. J Neurosci., 10(2): 436-447.

Vanderwolf., C. H. (1969). Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol., 26(4): 407-418.

Vanderwolf, C. H. (1971). Limbic-diencephalic mechanisms of voluntary movement. Psychol Rev., 78(2): 83-113.

Whishaw I. Q. and Tomie, J. A. (1991). Acquisition and retention by hippocampal rats of simple, conditional, and configural tasks using tactile and olfactory cues: implications for hippocampal function. Behav Neurosci., 105: 787-797.

Whishaw, I. Q. and Vanderwolf, C. H. (1971). Hippocampal EEG and behavior: effects of variation in body temperature and relation of EEG to vibrissae movement, swimming and shivering. Physiol Behav., 6: 391-397.

Whishaw, I. Q. and Vanderwolf, C. H. (1973). Hippocampal EEG and behavior: changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. Behav Biol., 8: 461-484.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., and Bear, M.F. (2006). Learning induces long term potentiation in the hippocampus. Science, 313(5790): 1093-1097.

Winters, B. D., Forwood, S. E., Cowell, R. A., Saksida, L. M., and Bussey, T. J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J Neurosci., 24(26): 5901-5908.

Witter, M. P. (1989). Connectivity of the rat hippocampus. In: C. Chan-Palay and C. Kohler (eds.), The Hippocampus - New Vistas, Neurology and Neurobiology Vol X V (pp. 53-69). New York: Alan R. Liss Inc.

Witter, M. P., Wouterlood, F. G., Naber, P. A., and Van Haeften, T. (2000). Anatomical organization of the parahippocampal-hippocampal network. Ann N Y Acad Sci., 911: 1-24.

Wood, E. R., Mumby, D. G., Pinel, J. P., and Phillips, A. G. (1993). Impaired object recognition memory in rats following ischemia-induced damage to the hippocampus. Behav Neurosci., 107(1): 51-62.

Zola-Morgan, S., Squire, L. R., and Amaral, D. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to the CA1 field of the hippocampus. J Neurosci., 6: 2950-2967.