Gidyk, Darryl C.

2019

Maintenance and Behavioural Expression of Long-term Memories Acquired in the Absence of the Hippocampus

Department of Neuroscience

MAINTENANCE AND BEHAVIOURAL EXPRESSION OF LONG-TERM MEMORIES ACQUIRED IN THE ABSENCE OF THE HIPPOCAMPUS

DARRYL CHRISTOPHER GIDYK
Master of Science, University of Lethbridge, 2012

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfilment of the
Requirements for the Degree

DOCTOR OF PHILOSOPHY

Department of Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

MAINTENANCE AND BEHAVIOURAL EXPRESSION OF LONG-TERM

MEMORIES ACQUIRED IN THE ABSENCE OF THE HIPPOCAMPUS

DARRYL CHRISTOPHER GIDYK

Date of defence: April 15, 2019

Dr. Robert J. Sutherland Co-Supervisor	Professor	Ph.D.
Dr. Robert J. McDonald Co-Supervisor	Professor	Ph.D.
Dr. Masami Tatsuno Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. Paul Vasey Thesis Examination Committee Member	Professor	Ph.D.
Dr. Athan Zovoilis Thesis Examination Committee Member	Assistant Professor	Ph.D.
Dr. Clayton Dickson External Examiner University of Alberta Edmonton, Alberta	Professor	Ph.D.
Dr. Ian Q. Whishaw Chair, Thesis Examination Committee	Professor	Ph.D.

Dedication

I dedicate this thesis to Lily and Daisy Gidyk.

My beloved daughters,

Let this thesis stand as a permanent reminder that life is effort-based. I hope my struggles and dedication inspire you in times of self-doubt. I want you to know that to achieve rare things in life you must go all-in. Discover yourself. Be who you truly are. Know your worth. Ask yourself and others the difficult questions. Do not fear failure and constantly re-evaluate. Evolve. Most importantly, be relentless in your most cherished endeavors and have fun. Understand that your achievements come from within and you must never leave your happiness to chance, or the will of another. Do, or do not – there is no try (that's for your Mom). I hope one day you will understand this achievement came from within me, but only because of you. Lily and Daisy, you are my sources of strength. When you need it, I will help you find yours. Never forget that we earned this together. I won't. Thank you.

Love always,

Daddy, Ph.D.

Abstract

We examine the maintenance and behavioural expression of long-term memories acquired in the absence of the hippocampus. The hypothesis that the hippocampus is necessary to form stable and detailed long-term memories is tested. We find rats with extensive hippocampal damage made before learning exhibit normal maintenance and behavioural expression of contextual fear memory, object discrimination, and context discrimination. The discovery that non-hippocampal networks can encode, maintain, and retrieve memories, widely-thought to be dependent on the hippocampus and its consolidation processes adds to a growing body of literature which draws into question most views of the hippocampus and memory consolidation. Our findings suggest: 1) hippocampal-dependent systems-level consolidation is not required for stable long-term memory in the rat; 2) non-hippocampal networks possess sufficient representational complexity to support normal discriminative memory-guided behaviours; 3) the broad distinction between hippocampal and non-hippocampal memories requires re-evaluation through rigorous experimentation, rather than adherence to modal views.

Acknowledgements

My family are my biggest supporters, my team. I thank my amazing wife Thea, who despite marrying down, now has another obscure thing to tell people about me. I love you Thea, sorry about the poverty and the perpetual stress-o-coaster. I'll have more free time during my postdoc (said no one ever). Thank you, Mom and Dad. Please enjoy your very own ridiculously-overpriced paperweight. I suggest you put this thesis next to the last one – on the bookshelf located in the dark part of the basement. I'm kidding, the UofL no longer forces thesis binding on students, so free to ignore the PDF version I email you. I owe a great debt of gratitude to my supervisors, Rob Sutherland and Rob McDonald. When the chips were down, you had my back. The training I received from you transcends neuroscience, thank you. Sorry for the obscenities, but I'm from Edmonton – what can I do? To my lab mates and colleagues – get back to work. If you choose to procrastinate for long enough to read this thesis, you may recognize the quotes scattered throughout. I owe special thanks to Scott D. and Rui P., my trusted colleagues, my friends, and two of my go-to sources of deliberate procrastination and judgement-free theoretical discussions. Don't let the thorns ruin a perfectly good rose garden if you catch my drift. Thank you to the animal care staff, support staff, and individuals who attempt to make the CCBN tolerable for myself and others during the never-ending 80-hour work weeks (or as it's known to graduate students – the perfect work/life ratio). Thank you to my new colleague and mentor, Dr. Clement Hamani for allowing me to begin a postdoc prior to earning a Ph.D. Finally, thank you Long-Evans hooded rats. You're my favorite strain. No hippocampus? No big deal.

Contents

Tables

Figures

Abbreviations

A/P	Anterior - posterior
AA	Anterograde amnesia
CCAC	Canadian Council on Animal Care
cf.	Compare
CR	Conditioned response
CS	Conditional stimulus
D/V	Dorsal – ventral
fMRI	Functional magnetic resonance imaging
i.p.	Intraperitoneal
M	Molar (concentration)
M/L	Medial – lateral
mA	Milliampere
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
RA	Retrograde amnesia
s.c.	Subcutaneous
US	Unconditioned stimulus
vHPC	Ventral hippocampus
$\bar{X}$	Subpopulation mean
μL	Microliter

Commonly-used units and abbreviations, and those defined inline within parentheses are omitted from the list of abbreviations.

The use of quotations and footnotes is intended to accentuate the writing style and reflect personality of the author. As is the overall editorial tone.

Chapter 1

General Introduction

Thesis Overview

Memory is a fundamental cognitive trait – one that enables the flexible use of previous experience to guide adaptive behaviour (Gruber & McDonald, 2012; McDonald & Hong, 2013). Memory is generative in nature and acts like scaffolding – the foundation on which consciousness is constructed. Memory is often described as an autobiographical record (Tulving, 2002), yet unconscious behaviours like habits and conditioned reflexes arise from the brain's capacity for memory as well (Pavlov, 1927; Thorndike, 1913). For memory to contribute to normal thought and behaviour, it must persist and reflect experience with some degree of detail. It is undeniable that the properties and content of our memories change as they age. Accordingly, many fundamental questions about the organization of memory in the brain revolve around what happens to established long-term memories over time. Some long-term memories last a lifetime, while others decay and become more difficult to discriminate between, and others are forgotten entirely.

One prominent view is that the hippocampus (HPC) is essential for the acquisition, initial maintenance and long-term stabilization of certain long-term memories. ***Specifically, it has been hypothesized that the longevity and precision of episodic memory both require hippocampal involvement. The goal of this thesis was to test these ideas by examining the acquisition and maintenance of long-term memory via its behavioural expression over time in rats with and without a functional HPC.*** This work speaks to extant views of hippocampal-dependent systems-level memory consolidation. Notably, the mnemonic capacities of non-hippocampal cortical networks and their contribution to memory-guided behaviours was investigated in the absence of the hippocampus. Each experiment was

1

designed to explore a specific research question within the common theoretical framework of long-term memory. This framework is largely modal and based on a few central ideas. Therefore, key concepts are repeatedly highlighted and presented redundantly throughout the thesis.

Volumes of experimental and theoretical work have been dedicated to exploring the neurobiology of learning and memory-guided behaviours. Accordingly, the reader is encouraged to regard this thesis as a modest set of related novel contributions to a very large field. Chapter One provides a general overview of the concepts that lead to the experimental work in this thesis. The long-standing view that long-term memories belong to one of two broad categories; hippocampal or non-hippocampal, is emphasized. Chapter Two introduces evidence that non-hippocampal networks can acquire, maintain, and support the behavioural expression of a contextual memory for up to 30 days. Chapter Three builds on findings from Chapter Two and illustrates that the flexible expression of explicit memory does not always require the HPC, as non-hippocampal networks support accurate context discrimination behaviour for up to 15 days. A separate experiment in Chapter Three verifies that the method used to permanently damage the HPC completely disrupted hippocampal function. Chapter Four demonstrates that an instrumental object-reward memory is maintained and expressed normally with and without a functional HPC, as evident by accurate discrimination behaviour over time. Each experimental chapter of this thesis (i.e., 2 – 4) was written to be self-consistent and include a dedicated discussion section. Accordingly, Chapter Five (Conclusion) revisits key findings and their implications from an alternative perspective on the organization of memory and is more narrative in nature than the empirical chapters.

Memory Systems

The idea that long-term memory exists in many forms is ancient (Aristotle, 350/1984) and has persisted through time (De Biran, 1804/1929; James, 1890). This concept remains central to how memory is understood, with different types of memory classified hierarchically by their characteristics and supporting neural systems. In this way, long-term memory is thought of as modular rather than unitary; phenomenologically, functionally, and anatomically (Squire, 1992a; Tulving, 1972; White & McDonald, 2002). Pioneering work involving patient H.M.[1] (Scoville & Milner, 1957) and others (reviewed in, Winocur & Moscovitch, 2011) established that damage to medial temporal lobe (MTL) structures, including the HPC, severely impairs long-term memory. Anterograde amnesia, the inability to form and retain new memories, and temporally-graded retrograde amnesia, the loss of established, recently-formed memories and the relative sparing of older ones, were described as typical consequences of hippocampal damage in human patients (Rempel-Clower, Zola, Squire, & Amaral, 1996; Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957). Despite massive impairments in the acquisition of new memories and recall of memories for events and facts, basic mnemonic abilities like procedural learning, priming, conditioned reflexes, and simple emotional associations appeared unaffected in some patients with hippocampal damage (Cohen & Squire, 1980; Milner et al., 1968). Amazingly and tragically, these types of learning were not accompanied by conscious memories for the learning experiences. Long-term memories are now defined as belonging to one of two broad categories: declarative and non-declarative (Figure 1.1.) (Squire, 1992a).

---

[1] Identified posthumously as Henry Gustav Molaison (Squire, 2009). A friend of mine once said: "if you write about long-term memory and don't mention H.M., the neuroscience police will come and take you away".

Long-Term Memory	
Declarative/Explicit Episodic Semantic	**Non-Declarative/Implicit** Procedural: Skills and Habits Conditioning and Priming Reflexes
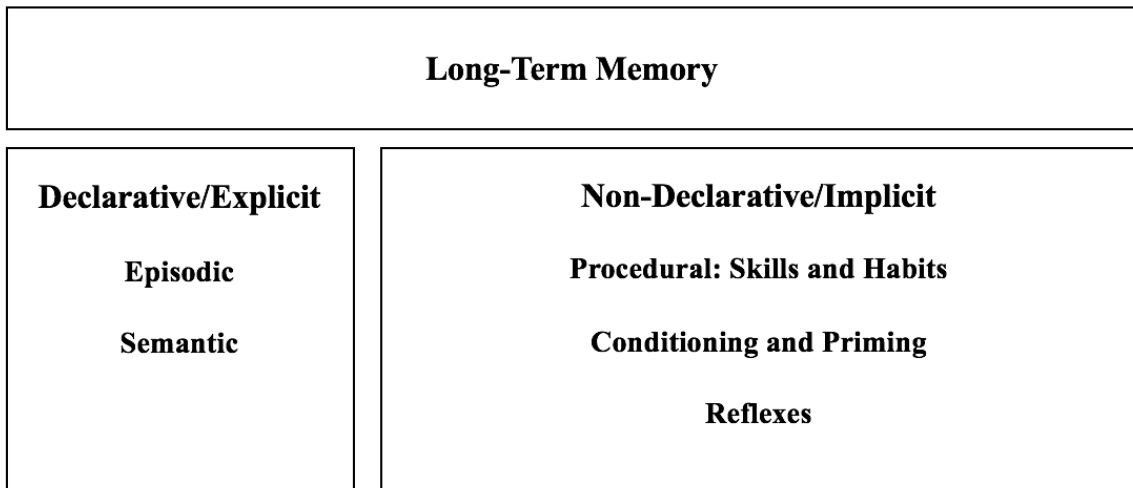
Figure 1.1. A simplified depiction of a standard taxonomy of long-term memory. This schematic represents a classification scheme of dual memory systems. Declarative memory is expressed consciously and is initially dependent on the MTL; specifically, the HPC. Declarative memory encompasses episodic memory (events, bound to context) and semantic memory (facts, context-free). Non-declarative memories like habits, motor skills, and reflexes are more basic forms of memory which are expressed unconsciously and independent of the HPC. Original figure, adapted from multiple sources (e.g., Squire, 2004).

The case study of H.M. is regarded as indisputable evidence that the HPC is critically-important for declarative memory, yet uninvolved in non-declarative memory. Shaped by this tradition, investigations into the organization of memory have focussed on explicit memory expression and its cognitive nature versus habit-based memory and the dependence of each on distinct brain networks in rats and non-human primates (Hirsh, 1974; Mishkin & Petri, 1984; O'Keefe & Nadel, 1978). In general, the HPC is thought to be important for memory that requires a high degree of spatial, temporal, and/or contextual detail (Dusek & Eichenbaum, 1997; Hirsh, 1974; O'Keefe & Nadel, 1978; Rudy, 2009; Sutherland & Rudy, 1989). Consistent with these ideas, rats with hippocampal damage are impaired on the hidden platform version of the Morris Water Task (MWT) (Morris et al., 1982; Sutherland, Kolb, & Whishaw, 1982), tests of temporal sequence (Fortin, Agster, &

Eichenbaum, 2002), and negative patterning tasks (Alvarado & Rudy, 1995; Sutherland, McDonald, Hill, & Rudy, 1989). The importance of the rat HPC to allocentric spatial cognition (*cognitive maps*; O'Keefe & Nadel, 1978) is particularly interesting because the ability to create and use a world-centered, ordered representation of co-occurring elements within an environment, context, or cognitive state space has been compared to the capacity for episodic memory in humans (Buzsáki & Links, 2017).

Along these lines, a key feature of hippocampal memory is thought to be the relationships between cues, stimuli, and/or elemental memoranda and the flexible use of these representations according to behavioural demands (Eichenbaum, Fagan, Mathews, & Cohen, 1988; Gruber & McDonald, 2012; McDonald & Hong, 2013; Sutherland & Rudy, 1989). However, rats with impaired hippocampal function can learn simple, more rigid associations. For example, an incrementally-learned association between an action (turning direction) and a food reward (Packard, Hirsh, & White, 1989). Dissociations between anatomically-distinct networks and their mnemonic functions and subsequent theoretical reviews illustrate the complexity of memory systems (Ferbinteanu, 2018; McDonald et al., 2017; McDonald & White, 1993), but the concept of memory systems is often reduced to a dual memory systems scenario (Squire, 2004). This approach can also be seen in computational and connectionist theories, which highlight unique and highly-specialized functions of the HPC in learning and memory and more elementary functions of other brain regions (Marr, 1971; McClelland, McNaughton, & O'Reilly, 1995). The HPC is regarded as the apex of the cortical associative processing hierarchy – performing rapid computations on complex polymodal (perhaps amodal) cognitive information from across the entire cortical mantle (McNaughton, 2010). Again, the perspective offered here is that contemporary views of long-term memory rely heavily on the distinction between two

broad categories of memory (and memory mechanisms) – hippocampal-dependent and non-hippocampal (Fanselow, 2009; Wiltgen & Tanaka, 2013; Winocur & Moscovitch, 2011; but see, Lee et al., 2016; Gruber & McDonald, 2012). Like other empirical work from our group (Lee, Sutherland, & McDonald, 2017; Lehmann et al., 2009; McDonald, Jones, Richards, & Hong, 2006), much of the present thesis investigates this dichotomy in the rat.

Memory Consolidation

A cornerstone concept in memory systems neuroscience is that experiences are encoded, and later retrieved in a systematic manner by distributed neural networks in the mammalian brain (Hebb, 1949; Marr, 1971; McClelland, McNaughton, & O'Reilly, 1995; McNaughton & Morris, 1987; Teyler & DiScenna, 1986). The reinstatement of similar neuronal activity patterns to those which occurred during an experience is widely-thought to be the neurobiological basis of memory retrieval (McDonald & Hong, 2013; McNaughton & Morris, 1987; McNaughton, 2010). For this to occur, experience must be maintained in some way (i.e., stabilized and stored) until memory retrieval. Psychologists have long recognized that new learning is unstable, labile, and prone to forgetting by decay (Thorndike, 1913), interference (Jenkins & Dallenbach, 1924; McGeoch, 1932), or perturbation of normal brain function (Ribot, 1881). In contrast, memory seems to stabilize as it ages, often becoming less susceptible to the forces of forgetting or interference over time (Ebbinghaus, 1885). A breakthrough insight into these properties of memory came with the concept of a memory consolidation period (Müller & Pilzecker, 1900; see also, Ribot, 1881). In general, memory consolidation is regarded as the time-dependent strengthening of new learning – and is described by neuroscientists as consisting of two

distinct but interdependent levels of neurobiological processes; cellular consolidation and systems-level consolidation (Dudai, Karni, & Born, 2015; Sutherland & Lehmann, 2011). It is widely-assumed that consolidation must occur at both levels for memory to be long-lasting (Genzel & Wixted, 2017). According to the dual account of long-term memory, it logically follows both consolidation processes must depend on the HPC for certain types of memory, but not others (Dudai, 2004; Dudai et al., 2015; Squire & Wixted, 2011).

Cellular Consolidation

The mechanism of cellular consolidation is thought to be essential for the generation of stable potentiated synaptic connections (Dudai, 2002), which are near-unanimously regarded as the neuronal substrates of memory (cf. Routtenberg, 2013). Theoretical and evidence-based connections between the persistence of activity-induced synaptic plasticity and the formation of long-term memory provide support for the neurobiological perspective (Bliss & Collingridge, 1993; Redondo & Morris, 2011; Rudy, 2014). On this view, the initial phase of memory consolidation consists of the synergistic biochemical cascades that form synaptic traces (memory engrams). Many influential reviews of cellular consolidation mechanisms exist (Korte & Schmitz, 2016; Redondo & Morris, 2011; Rudy, 2014; Takeuchi, Duszkiewicz, & Morris, 2014); therefore, the following overview is intended to illustrate two simple ideas: 1) synaptic potentiation in the HPC is impermanent and decays over time; 2) cellular consolidation in the HPC is regarded as the initial stage of episodic memory consolidation.

The gross properties of cellular consolidation were discovered in hippocampal long-term potentiation (LTP) experiments, both *in vivo* and *in vitro* (Bliss, Gardner-Medwin, & Lømo, 1973; Bliss & Lømo, 1973; McNaughton, Douglas, & Goddard, 1978). Typical LTP

protocols involve the repeated application of a plasticity-inducing electrical stimulus that is far more intense than any physiological stimulus endogenous to the brain (Whitlock, Heynen, Shuler, & Bear, 2006). Despite the robust nature of LTP-induced increases in synaptic efficacy, the enhancements decay to baseline in acute and chronic preparations when left unperturbed, albeit on different time scales (Bliss, Gardner-Medwin, & Lømo, 1973; Bliss & Lømo, 1973). The degradation of hippocampal LTP suggests that the mechanism of cellular consolidation is neither transient, nor constitutive. It remains unknown how (or if) the subcellular components of potentiated synapses survive molecular turnover and homeostatic processes to resist decay to baseline (Crick, 1984; Tononi & Cirelli, 2014)[2]. Despite the temporal limitations of LTP in the HPC, the persistence of cellular consolidation-induced synaptic modifications and memory longevity remain conceptually tied (Hardt, Nader, & Nadel, 2013; Redondo & Morris, 2011; Takeuchi et al., 2014).

Cellular consolidation requires overlapping cascades of biochemical processes, resulting in increases or decreases in synaptic efficacy through mechanisms similar to LTP and long-term depression (LTD), the opposing effects of spike timing-dependent plasticity (STDP) (Bi & Poo, 2001; Bliss & Collingridge, 1993; Korte et al., 2016). In this way, the increases/decreases in synaptic strength evolve in stages to generate and stabilize memory traces in a distributed set of synaptic weights (McNaughton, 2010). Post-translational modifications (cellular processes that do not require gene expression), *de novo* protein synthesis (gene transcription and translation), protein degradation, and constitutive cellular

---

[2] This is often referred to as the plasticity-stability dilemma, which is a major consideration of connectionist theories of the HPC and memory (see, Marr, 1971; McClelland et al., 1995). Systems-level consolidation was introduced to address the dilemma.

processes (genomic signaling, receptor trafficking, cytoskeletal changes) are crucial for synaptic strengthening and maintenance (Rudy, 2014). Cellular consolidation is thought to be completed in < 100 hours following a learning episode (Dudai, 2004; Sutherland & Lehmann, 2011; for a shorter estimate see, Rudy, 2014). In support of this idea, pharmacological antagonism of N-methyl-D-aspartate receptors (NMDARs) during this temporal window can disrupt the retention of learning-induced synaptic plasticity and memory-guided allocentric spatial behaviours (Bye, 2017; Kentros et al., 1998; R. J. McDonald et al., 2005; Morris, Anderson, Lynch, & Baudry, 1986; Tse et al., 2011).

Recent behavioural studies in rodents suggest α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptor (AMPAR) endocytosis is a key factor in trace decay, as memory persistence is enhanced when this process is blocked (Dong et al., 2015; Migues et al., 2016). A separate study found that chronic and combined administration NMDAR, calcineurin, and Ca^{2+} channel antagonists into the HPC after learning attenuates normal forgetting of object-location memory, possibly by inhibiting LTD-like reductions in synaptic strength (Sachser et al., 2016). Increasing or decreasing the persistence of memory by disrupting normal neurobiological processes in a timing-dependent manner suggests that synaptic plasticity and cellular consolidation mechanisms are bidirectionally-regulated. By extension, it also illustrates that hippocampal memory traces are prone to decay (Hardt et al., 2013; Tononi & Cirelli, 2014).

It must be noted that many pharmacological treatments have off-target and/or unintended effects on normal neurobiological processes and behaviour, ranging from gross motor impairments and reduced cognitive flexibility, to complete silencing of local neuronal activity (Cain, Saucier, Hall, Hargreaves, & Boon, 1996; LeBlancq, McKinney, & Dickson, 2016; Mills et al., 2014; Sharma, Nargang, & Dickson, 2012). Therefore,

reports of highly-selective pharmacological manipulations accompanied by specific behavioural effects should be interpreted with caution, yet certainly merit further investigation. The key point for the purpose of this thesis is that cellular consolidation in the HPC is hypothesized to be necessary for the initial formation of explicit memory (Martin, Grimwood, & Morris, 2000; McClelland et al., 1995; Takeuchi, Duszkiewicz, & Morris, 2014). For this prediction to be true, the perturbation of hippocampal function during cellular consolidation, or the complete removal of the HPC prior to hippocampal-dependent learning (e.g., an episodic experience), should result in anterograde amnesia.

Systems-Level Consolidation

Systems consolidation is the hypothetical process by which long-term memory reorganizes at the network level and becomes independent of the HPC (McClelland et al., 1995; Squire et al., 1984). During the process, changes in the longevity, detail, and neuroanatomical distribution of long-term memory are all thought to occur in a hippocampal-dependent manner (Alvarez & Squire, 1994; McGaugh, 2000; McNaughton, 2010; Wiltgen & Tanaka, 2013; Winocur & Moscovitch, 2011). Whereas interference (Underwood, 1957) and to a lesser degree trace decay (Thorndike, 1913) were once influential psychological accounts of non-pathological forgetting (reviewed in, Wixted, 2004), more recent neuroscientific accounts ascribe these types of normal forgetting to systems consolidation (Preston & Eichenbaum, 2013; Sekeres, Moscovitch, & Winocur, 2017; Squire & Wixted, 2011; Wiltgen & Tanaka, 2013). Moreover, according to contemporary theories, the removal of the HPC results in profound anterograde amnesia and temporally-graded retrograde amnesia for certain types of memory, but not others due to the interruption of hippocampal-dependent cellular and systems consolidation processes (Squire & Alvarez, 1995; Wiltgen

& Tanaka, 2013; Winocur & Moscovitch, 2011). The pattern of temporally-graded amnesia (i.e., spared remote memory) is widely-assumed to reflect the slow, network-level reorganization and strengthening of neocortical memory traces (Figure 1.2.)(McClelland, McNaughton, & O'Reilly, 1995; McNaughton, 2010; Sekeres, Moscovitch, & Winocur, 2017; Wiltgen & Tanaka, 2013).

The concept of systems consolidation wields significant explanatory power, as it offers an intuitive account for amnesia following hippocampal damage (Squire & Alvarez, 1995; Squire et al., 1984). Evidence for systems consolidation in humans and rodents is regarded as abundant (Axmacher & Rasch, 2017; Winocur & Moscovitch, 2011; Winocur, Moscovitch, & Sekeres, 2013) and early attempts to model temporally-graded retrograde amnesia in rats appeared promising. Several examples of impaired recent and intact remote memory after damage to the HPC appeared to support ideas that hippocampal involvement in certain memories was temporary (Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997; Winocur, 1990). However, a recent lack of corroborating evidence and the accumulation of contradictory findings raises questions about the temporal involvement of the HPC in long-term memory. For example, the duration of retrograde amnesia in human hippocampal patients is more variable (Amaral et al., 1996; Squire, Genzel, Wixted, & Morris, 2015), and the memory impairments less-specific than originally thought (Corkin, 2002; Winocur & Moscovitch, 2011). In at least one case, retrograde memory impairments extended to semantic concepts and knowledge (Verfaellie, Bousquet, & Keane, 2014; see also, Corkin, 2002). In fact, neuropsychological findings from hippocampal and MTL patients are highly-variable and difficult to interpret, rather than straight-forward and representative of textbook views.

In illustration of this point, systematic reevaluations of patient H.M.'s amnesia uncovered that his remote memory was far more impaired than described almost 50 years earlier (Steinvorth, Levine, & Corkin, 2005). Improved testing methods revealed that H.M. exhibited a flat gradient of retrograde amnesia – equivalent amnesia for recent and remote memory from specific episodes – rather than temporally-graded retrograde amnesia (Steinvorth et al., 2005; see also, Corkin, 2002; Sutherland & Lehmann, 2011; Sutherland, Sparks, & Lehmann, 2010)[3]. In addition to amnesia for past experiences, one study found that individuals with HPC damage cannot imagine new experiences (Hassabis, Kumaran, Vann, & Maguire, 2007). The deficit in mentally constructing a novel experience or narrative could reflect the inability to retrieve stored neocortical memory elements in a cohesive and flexible manner, like the impairments seen in flat-gradient retrograde amnesia. This raises the possibility that the human HPC is required to combine information into a usable cognitive representation, regardless of age, source, content, or nature of the information (Mayford, 2014). In line with this, fMRI studies involving healthy subjects strongly suggest the HPC is always involved in memory retrieval, regardless of the age and type of the memory (Hassabis & Maguire, 2009; Ritchey, Montchal, Yonelinas, & Ranganath, 2015; Ryan et al., 2001; Verfaellie et al., 2014). At minimum, these findings appear to contradict extant ideas about the reduction of hippocampal involvement in memory over time (Frankland & Bontempi, 2005 Squire & Alvarez, 1995; Winocur, Moscovitch, et al., 2013).

Perhaps most strikingly, the primary methodology used to investigate long-term memory and systems consolidation in rodents; contextual fear conditioning, has produced

---

[3] A wise man once said, "a paper that cites Scoville and Milner (1957), but not Steinvorth and colleagues (2005), exhibits poor scholarship and should not be taken too seriously".

a disproportionately large number of findings that are inconsistent with a temporally-circumscribed role of the HPC memory (Sutherland et al., 2010). Contextual fear conditioning is the gold standard for investigating systems consolidation and the properties of memory persistence due to the rapid acquisition of the conditioned fear response and robust longevity of the context-shock memory (Gale, 2004; Lehmann et al., 2009). By disrupting the HPC at various time points following fear conditioning (e.g., 1-day = recent; 30-days = remote), hippocampal involvement in the behavioural expression of context memory can be investigated. As previously mentioned, temporally-graded retrograde amnesia was originally reported by several researchers using this paradigm (Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992). Like the re-examination of patient H.M., the vast majority of experiments now find flat gradients of retrograde amnesia – that is, equal amnesia for recent and remote context memory after hippocampal damage (Broadbent & Clark, 2013; Sparks, Spanswick, Lehmann, & Sutherland, 2013; Sutherland & Lehmann, 2011; Sutherland et al., 2010). The preponderance of evidence indicates that the temporal involvement of the HPC in long-term memory remains unresolved, both in humans and rodents despite adherence to classic views which predict certain memories eventually become independent of the HPC. Rather, it seems more likely that neither the age, nor the putative type of long-term memory necessarily determines dependence on the HPC for retrieval.
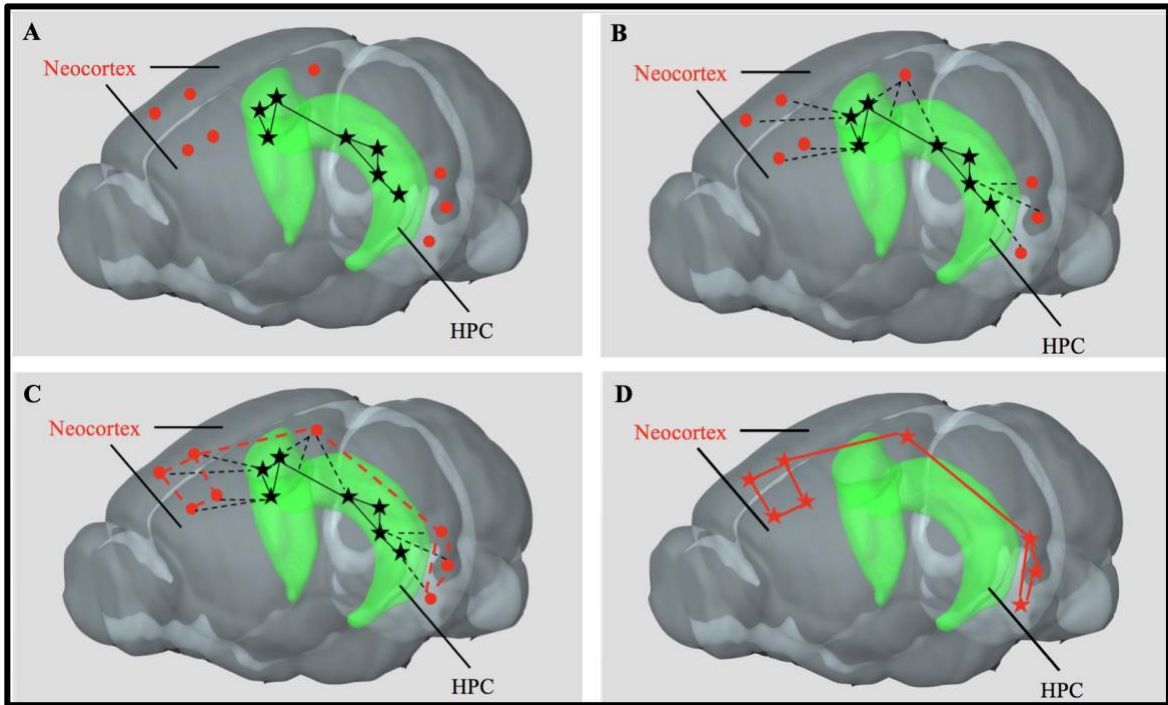
Figure 1.2. Graphic representation of hippocampal-dependent systems consolidation.
A) An experience is rapidly encoded in the HPC and to a lesser extent in neocortical
networks via cellular consolidation mechanisms. Red circles in neocortex depict elemental
representations contained in the experience. Black stars in the HPC connected with solid
lines represent a memory engram, or index of the recent experience. B) – C) Over some
undefined period, the HPC trace/index reactivates the neocortical memory elements,
strengthening their hetero-associative connectivity. D) The neocortical memory trace,
denoted by red stars + solid red lines, is consolidated and the memory can be expressed
independently of the HPC. Note that the HPC is always required for episodic memory
encoding, initial maintenance, and recall until the neocortical engram is consolidated and
the HPC engram no longer exists, or is no longer required. This general scheme is referred
to as the standard model of systems consolidation (SMSC), or standard consolidation theory
(SCT). Original figure designed with Brain Explorer 2 (Version 2.3.5.) © 2015 Allen
Institute for Brain Science.

Challenges to contemporary views of the HPC and memory arise when examined

from the anterograde direction as well. Recall that patients with damage to the HPC

typically exhibit anterograde amnesia, or the inability to form new episodic memories (e.g.,

Milner et al., 1968; Scoville & Milner, 1957), and that extensive research involving rodents

with hippocampal damage suggests the HPC is important for learning requiring allocentric

spatial, ordered temporal, and contextual information (Dusek & Eichenbaum, 1997; Fortin, Agster, & Eichenbaum, 2002; McDonald & White, 1993; Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland, McDonald, Hill, Rudy, 1989; Sutherland & Rudy, 1989; Sutherland & McDonald, 1990). This indeed suggests that the HPC is necessary for the acquisition and initial retention of episodic-like information (Genzel & Wixted, 2017; Squire & Wixted, 2011). This is also consistent with views on the organization of multiple (or dual) memory systems in the mammal – that memory networks are specialized for certain mnemonic functions and are relatively distinct anatomically (Sherry & Schacter, 1987; Squire, 1992a; Squire, 2004; White & McDonald, 2002). However, hippocampal damage before or after an experience does not typically produce equivalent memory impairments in rodents (Lee, Zelinski, McDonald, & Sutherland, 2016; McDonald & Hong, 2013).

Damage to the HPC after learning causes retrograde impairments for a wide range of memory-guided behaviours, whereas damage before learning causes anterograde impairments in a very limited number of learning and memory tasks (Lee et al., 2016). To illustrate this point, hippocampal damage after learning causes retrograde amnesia for: spatial memory, context fear, tone fear, simple visual discriminations, context discriminations and socially-transmitted food preference[4] (Epp et al., 2008; Korte et al., 2016; Lee et al., 2017; Mumby, Astur, Weisend, & Sutherland, 1999; Sutherland & McDonald, 1990; Winocur, 1990). Most commonly, precise allocentric spatial learning and memory has been reliably shown to be 100% dependent on the HPC. That is, complete hippocampal damage impairs precise allocentric mnemonic abilities – in both the

---

[4] List is representative, not exhaustive (see, Lee et al., 2016; Sutherland et al., 2010; cf. Winocur & Moscovitch, 2011).

anterograde and retrograde direction. However, other less-common tasks involving negative patterning or transitive inference are sensitive to hippocampal damage regardless of timing as well (Alvarado & Rudy, 1995; Driscoll, Howard, Prusky, Rudy, & Sutherland, 2005; Dusek & Eichenbaum, 1997).

Barring the addition of *ad hoc* hypotheses[5], the dissociation between anterograde and retrograde memory impairments in rodents with hippocampal damage is clearly at odds with consolidation-based accounts of the HPC and long-term memory. Hippocampal damage should impair any long-term memory (or memory mechanism) to which the HPC uniquely contributes, regardless of whether the damage occurs before or after learning (Lee et al., 2016). As outlined here, this is not the case in the rat, except for a very restricted number of learning and memory tasks. As hippocampal damage often causes a wider range of memory problems in the retrograde direction, this raises the somewhat paradoxical possibility that the rodent HPC plays a highly-specialized role in anterograde learning and memory processes and a more general role in memory recall (Driscoll, Howard, Prusky, Rudy, & Sutherland, 2005; Lee et al., 2017, 2016; Sutherland et al., 2001). By extension, the anterograde mnemonic capabilities of non-hippocampal networks may be far greater in the absence of the HPC than contemporary theories predict.

Theories of Hippocampal Memory and Systems Consolidation

As emphasized throughout this chapter, nearly all views of the HPC and long-term memory are based on the related concepts of multiple (or dual) memory systems, distinctions

---

[5] Hypotheses involving the concept of hippocampal overshadowing were introduced to account for unequal anterograde and retrograde amnestic effects of hippocampal damage (Driscoll et al., 2005; Fanselow, 2009; Maren et al., 1997).

between hippocampal and non-hippocampal memories, and the existence of hippocampal-dependent memory consolidation mechanisms. Among these theories, it can be argued that the most prevalent ones invoke systems consolidation in one form or another (McClelland et al., 1995; Nadel & Moscovitch, 1997; Squire et al., 1984; Winocur & Moscovitch, 2011). As depicted in Figure 1.2., the central tenet of the Standard Model of Systems Consolidation (SMSC) is that the HPC is only temporarily necessary for episodic memory. Within the model, systems consolidation is uniquely hippocampal-dependent and necessary to facilitate the assimilation of new experience into existing neocortical networks (see, Marr, 1971; McClelland et al., 1995). Importantly, memories are not handed off from the HPC to non-hippocampal networks *per se*; rather, the distributed non-hippocampal memory elements which are established during a learning episode and are gradually linked together and strengthened by the HPC (Squire et al., 2015). The underlying neurobiology of systems consolidation is empirically elusive, however it is often stated that the critical mechanistic features occur during sleep or offline periods via hippocampal "replay" (Jadhav & Frank, 2014; McNaughton, 2010; Ólafsdóttir, Bush, & Barry, 2018).

Numerous studies in rats suggest that spatial information is replayed during sharp-wave ripple events (SWRs) in periods of slow-wave sleep (Ji & Wilson, 2007; Lee & Wilson, 2002; Wilson & McNaughton, 1994). The repeated reactivations are brief (< 300 ms) and can occur an order of magnitude faster than the original activity patterns recorded during behaviour (Ji & Wilson, 2007; Lee & Wilson, 2002). Due to these qualities, hippocampal replay during offline periods is hypothesized to be the key mechanism of

systems consolidation[6] (Genzel et al., 2017; Jadhav & Frank, 2014). Replay in the HPC during SWRs is thought to drive coordinated excitatory responses in neocortex and other non-hippocampal networks and promote long-term memory storage (Jadhav & Frank, 2014; Skelin, Kilianski, & McNaughton, 2018). In support of this idea, interfering with SWRs can impair learning and memory performance (Ego-Stengel & Wilson, 2010; Jadhav, Kemere, German, & Frank, 2012). These findings collectively suggest that the HPC may indeed strengthen memory representations via replay during offline periods. Among studies that blocked SWRs, only transient impairments were observed on spatial memory tasks (Skelin et al., 2018). If offline replay is a critical component process of systems-level consolidation, repeated replay events for a single experience should be apparent over the same time course as memory consolidation (e.g., months, years, decades). This has yet to be established, as to my knowledge, replay of a novel spatial experience has not been recorded repeatedly across multiple days (Ólafsdóttir et al., 2018). Regardless, the SMSC assumes that episodic memory requires the HPC for cellular and systems-level consolidation; therefore, it necessarily predicts that rats with hippocampal damage are unable to form lasting contextual memories.

Whereas the SMSC is the original version within the group of theories that I refer to generally as contemporary views, Multiple Trace Theory (MTT) was introduced to account for experimental results which could not be explained by the standard model. Namely, the heterogeneous effects of partial versus complete hippocampal damage on

---

[6] If the HPC houses an index of neocortical memory elements for a given recent experience (Teyler & DiScenna, 1986), reinstatement of a hippocampal activity pattern which occurred [6] during a learning episode should in principle strengthen the entire cortico-hippocampal memory engram.

long-term memory (i.e., temporal gradients) were ascribed to multiple related traces being created within the HPC, leading to a more permanent role for the HPC in memory that retains episodic quality (Nadel & Moscovitch, 1997). Stated another way, every time an episodic memory is recalled it will invariably be in a new context and because of the HPC's obligatory role in memory encoding, a new trace will be laid down and consolidated. It is this recall/reencode/consolidate process that leads to the extraction of factual semantic information from multiple episodes (see bullets 1-9, p. 233; Nadel & Moscovitch, 1997)[7]. Therefore, MTT predicts the HPC is always involved in memory requiring episodic detail and partial damage to the HPC might disproportionately impair recent episodic memory due to the existence of fewer cortico-hippocampal traces. By extension and most relevant to this thesis, MTT necessarily predicts that non-hippocampal networks cannot support detailed memories for episodes or contexts independently of the HPC.

The last theory of memory and systems consolidation I will introduce is Trace Transformation Theory (TTT), also known as the transformation hypothesis (Sekeres et al., 2017; Winocur, Moscovitch, & Bontempi, 2010). Except for the hypothetical mechanisms involved, the central tenet of TTT is identical to MTT. Namely, that highly-detailed episodic memories always require the HPC (e.g., context memory in rodents; Moscovitch, Cabeza, Winocur, & Nadel, 2016). Like the name implies, the transformation hypothesis involves a time and experience-dependent post-encoding shift from highly-detailed context-specific memory (hippocampal), to a less detailed version which only retains the gist of an experience (non-hippocampal) (Winocur & Moscovitch, 2011). Therefore, the transformation hypothesis asserts there are two broad types of memory which are classified

---

[7] It should be noted that the authors explicitly rejected the idea of systems consolidation multiple times in the article. In my view, MTT represents a version of the same theme.

by content, or level of detail, and dependence of hippocampal and non-hippocampal substrates. Finally, TTT proposes that both types of memory representations can exist simultaneously and interact, such that a transformed gist-like memory which can be expressed independently of the HPC can once again become hippocampal-dependent and highly-detailed through a brief reminder that reinstates contextual details (Wiltgen & Tanaka, 2013; Winocur & Moscovitch, 2011).

Each of these theories of the HPC and long-term memory make similar predictions about anterograde memory processes and the nature of hippocampal versus non-hippocampal memories – memories that can and cannot be supported after damage to the HPC. Collectively, they are part of the dual memory zeitgeist that attributes the pattern of spared and impaired mnemonic abilities after hippocampal damage to a single cause – hippocampal-dependent, systems-level consolidation[8]. This thesis tests the general prediction that the longevity and precision of long-term memory both require the HPC.

---

[8] "The various classifications (boxes) that were developed to explain the observable behavioural (sic) properties and content of memory and its many dysfunctions were established when the brain was still a box itself. Neuroscience is now tasked with the job of retrofitting modern data into boxes that are fifty, upwards to one hundred years old" – Jerry Rudy (2008).

Chapter 2

Remote Contextual Fear Memory Without the Hippocampus

Introduction

Contextual fear memory exhibits a fundamental property of long-term memory, extended longevity. In one conditioning episode, rats form a context-shock associative memory for the experience that survives extended train-to-test intervals (e.g., 1.5 years, Fanselow & Gale, 2003; 180 days, Lehmann, Lacanilao, & Sutherland, 2007). Demonstrations of retrograde amnesia caused by damage to the HPC indicate normal expression of contextual fear memory requires hippocampal function, at least for some period of time after learning (Anagnostaras, Maren, & Fanselow, 1999; Broadbent & Clark, 2013; Kim & Fanselow, 1992; Lehmann et al., 2007; Lehmann, Rourke, Booker, & Glenn, 2013; Maren, Aharonov, & Fanselow, 1997; Sparks, Spanswick, Lehmann, & Sutherland, 2013). In contrast, when the HPC is damaged before a conditioning episode there is often no anterograde amnesia (Frankland, Filipkowski, Cestari, McDonald, & Silva, 1998; Maren et al., 1997; Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006). The absence of anterograde amnesia with hippocampal damage means that non-hippocampal networks can acquire and express contextual fear memory in the absence of the HPC (Lee, Zelinski, McDonald, & Sutherland, 2016; Rudy, 2009; Wiltgen & Tanaka, 2013). However, the hallmark longevity of the memory is thought to require the HPC (Zelikowsky et al., 2013; Zelikowsky, Bissiere, & Fanselow, 2012). This idea is based on hippocampal function *between* learning and memory expression, rather than *during* learning, or memory expression (cf. Lee et al. 2016).

The role of the HPC in memory longevity centers on systems-level consolidation; the hypothetical process by which new memories gradually transition from a state of

fragility to a more permanent, consolidated form (Dudai, 2004; McClelland, McNaughton, & O'Reilly, 1995; McGaugh, 2000; Squire, 1992; Squire & Alvarez, 1995). Views on systems consolidation vary (e.g., *complementary learning systems*, McClelland et al., 1995; SMSC, Squire & Alvarez, 1995; *hippocampal memory indexing theory,* Teyler & DiScenna, 1986; TTT, Winocur & Moscovitch, 2011), yet certain general principles are shared: 1) learning episodes are rapidly encoded, primarily by the HPC (Marr, 1971; McClelland et al., 1995), via cellular consolidation mechanisms (Dudai, 2002; Martin et al., 2000; McNaughton & Morris, 1987); 2) Memory is established more slowly and incrementally in non-hippocampal networks (Marr, 1971; McClelland et al., 1995; Teyler & DiScenna, 1986); 3) This requires an extended reorganization and strengthening of memory, which is uniquely dependent on hippocampal function (Alvarez & Squire, 1994; McClelland et al., 1995); 4) Once complete, memory is consolidated and less vulnerable to amnestic agents and normal forgetting (Axmacher & Rasch, 2017; Squire & Alvarez, 1995; Squire et al., 1984).

At the core of this framework is the prediction that the HPC is necessary to create stable explicit memories (Squire & Wixted, 2011). Stated another way, memory acquired after hippocampal damage, by definition, cannot undergo hippocampal-dependent systems consolidation – the process that confers memory longevity. Therefore, if non-hippocampal networks acquire a memory, like a context representation and its association with foot shock, the memory will be "fragile" (Squire & Wixted, 2011), exhibit rapid decay, and ultimately be lost to anterograde amnesia. This prediction was recently tested (Zelikowsky et al., 2012). Briefly, rats received neurotoxic lesions of the dorsal hippocampus (dHPC), or a SHAM (no lesion) procedure prior to a single contextual fear conditioning episode (8 min, 4 foot shocks). Rats were tested for memory retention 1, 3, 10, or 30 days later in the

same context. Rats without dHPC lesions exhibited intact memory for the conditioning episode at all retention intervals. In contrast, rats with dHPC damage exhibited memory for the experience 1 d, but not 30 d later. Moreover, dHPC damaged rats exhibited memory decay beginning at the 3 d test interval. The findings suggest intact acquisition, but rapid decay of contextual fear memory in the absence of the dHPC. The authors attributed the rapid decay to the loss of hippocampal-dependent systems consolidation (Zelikowsky et al., 2012).

The report from Zelikowsky and colleagues (2012) is the first known demonstration of rapid decay of contextual fear memory in HPC damaged rats. The findings and interpretation are consistent with systems consolidation accounts of the HPC and memory. Specifically, their results confirm the prediction that the HPC is essential for longevity of memory. However, only the dHPC was damaged, leaving the entire ventral hippocampus (vHPC) intact; therefore, attributing memory decay to the loss of a hippocampal-dependent process is problematic – conflating dHPC damage with the complete loss of hippocampal mnemonic function. The preponderance of evidence strongly suggests that dHPC and extensive HPC damage do not always produce equivalent memory dysfunction. For example, variability in the magnitude and temporal extent of RA for contextual fear memory may be related to the extent of HPC damage, especially when only dHPC is damaged (Lee, Zelinski, McDonald, & Sutherland, 2016; Sutherland & Lehmann, 2011; Sutherland, Sparks, & Lehmann, 2010). In fact, when damage to the HPC is extensive (e.g., > 70%), RA is more consistent between studies (Broadbent & Clark, 2013; Sutherland & Lehmann, 2011; Sutherland et al., 2010; but see, Winocur, Sekeres, Binns, & Moscovitch, 2013). When damage is incomplete (e.g., ≤ 50%), typically when dHPC is targeted and vHPC is spared, memory can remain partially intact (Scott, Saucier, & Lehmann, 2016),

but not in a reliable pattern (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Sutherland, O'Brien, & Lehmann, 2008). Moreover, the vHPC is reciprocally-connected to many regions required for memory, including, medial prefrontal cortex, nucleus accumbens, and amygdala (Preston & Eichenbaum, 2013; Skelin et al., 2018; Strange, Witter, Lein, & Moser, 2014). These regions contribute to normal acquisition and/or expression of contextual fear memory (Kim & Jung, 2006; Kitamura et al., 2017; McDonald & Hong, 2013; Zelinski, Hong, Tyndall, Halsall, & McDonald, 2010). It has also been shown that damage to the vHPC can result in RA for contextual fear memory, even when dHPC is fully intact (Sutherland, O'Brien, & Lehmann, 2008). Because the vHPC is within the neuroanatomical network involved in long-term memory and contributes to memory in often overlooked ways (Gruber & McDonald, 2012; Kitamura et al., 2017; McDonald & Hong, 2013; McDonald, King, Wasiak, Zelinski, & Hong, 2007), results obtained under conditions of moderate HPC damage should be interpreted with caution (Lee, Sutherland, & McDonald, 2017; Scott et al., 2016; Sutherland & Lehmann, 2011; Sutherland et al., 2010).

With this information in hand, it is unclear whether the rapid memory decay observed by Zelikowsky et al. (2012) can be attributed to memory instability in non-hippocampal networks, or if incomplete hippocampal damage resulted in the acquisition of dysfunctional memory, which could not be maintained by the compromised HPC. The goal of this study was to revisit the experiment by Zelikowsky et al. (2012), by attempting to reproduce the experimental methods for contextual fear conditioning, memory retention tests, and dHPC lesion surgeries. A sixteen-site lesion condition targeting the entire septo-temporal axis of the HPC (dHPC + vHPC) was included to test the hypothesis that spared HPC could function to support the acquisition, retention, or expression of contextual fear

memory. In general, standard views of the HPC and memory make two predictions regarding contextual fear memory without the HPC. First, contextual fear will be acquired more slowly by rats with hippocampal damage due to slower learning rates in non-hippocampal networks. Second, the memory will be unstable and exhibit rapid decay due to the absence of hippocampal-dependent systems consolidation.

Methods

Subjects

Ninety-six male Long–Evans hooded rats (Charles River, NC, USA), weighing 400 – 500 g at the time of surgery were housed in pairs in standard shoe-box acrylic cages (Allentown, Inc., NJ, USA) in the University of Lethbridge rodent vivarium (20°C, 50% relative humidity). Access to food and water was *ad libitum* upon arrival until experimental endpoint. The housing room light / dark cycle was 12 h, with lights on at 7:30 am daily. Rats were handled for several minutes daily during the week prior to surgery. All experimental procedures adhered to CCAC policy and were approved by the University of Lethbridge Animal Welfare Committee.

dHPC Lesions

Surgical procedures followed those employed by Zelikowsky and colleagues (2012). Rats were anesthetized with sodium pentobarbital (65 mg / kg, i.p.) and received atropine sulfate (0.4 mg / kg, i.p.) and Metacam® (1mg / kg, s.c.). The head was shaved, eye ointment was applied, and the scalp cleaned with chlorohexidine and 70% alcohol. Once fixed in the stereotaxic frame (David Kopf, Germany), a midline incision was made and the scalp retracted. Four small holes were drilled into the skull (5 mm diameter). Thirty-gauge

stainless steel injection cannulae, soldered into 23-gauge cannulae, were connected to 10 µL micro-syringes (Hamilton Co., NV, USA) via polyethylene tubing (PE50) and attached to the arms of the stereotax. Syringes were mounted on an infusion pump (Harvard Apparatus, MA, USA) and N-methyl-D-aspartic acid (NMDA; 20 mg / ml; Sigma-Aldrich, MO, USA), dissolved in 0.01 M PBS was drawn up into the cannulae and tubing. Infusions of NMDA were delivered sequentially, two at a time at corresponding bilateral sites (0.1 µL / min; coordinates and volumes, Table 2.1.). Cannulae were left in place for 2 min following each bilateral infusion. Once all infusions were delivered, the scalp incision was sutured and animals were placed in cages with soft paper bedding until fully recovered from anesthesia. Identical procedures were employed for SHAM surgeries, except the cannulae were not lowered into the brain and infusions omitted. The home cage surgical recovery period was 12 days.

HPC Lesions

Rats were medicated with phenobarbital (30 mg / kg, i.p.) and Metacam® (1 mg / kg, s.c.), then anesthetized via isoflurane inhalation (4% in 4 L / min oxygen for induction, then 1-2% in 1 L / min oxygen to maintain a surgical plane). Following surgical site preparation and scalp incision (see above), 14 holes (0.5 mm diameter) were drilled in the skull and infusions were delivered in sequence (7.5 mg / mL NMDA; 0.15 µL / min; coordinates and volumes listed in Table 2.1.). The infusion cannulae were left in place for 3 mins following delivery of NMDA. After all infusions, the scalp was sutured and diazepam (5 mg / kg, i.p., repeated as needed) was administered for seizure prophylaxis. SHAM surgeries followed the same procedures, except the cannulae were not lowered, NMDA infusions were omitted, and diazepam was not administered. The surgical recovery period was 12 d.

Table 2.1. NMDA infusion volumes and site coordinates. dHPC coordinates and volumes (Zelikowsky et al., 2012); HPC coordinates and volumes adapted from Sparks et al. (2013); (all coordinates; mm relative to bregma).

	Site	A/P	M/L	D/V	Volume
dHPC	1	-2.8	±1.6	-3.5	0.4μL
	2	-4.2	±2.6	-3.5	0.4μL
HPC	1	-2.6	±1.5	-3.4	0.3 μL
	2	-3.1	±1.5	-3.5	0.4 μL
	3	-4.1	±3.0	-3.5	0.4 μL
	4	-5.0	±3.0	-3.5	0.4 μL
	5	-5.0	±5.2	-7.3	0.4 μL
	6	-5.0	±4.4	-4.4	0.4 μL
	7	-5.8	±5.1	-7.5	0.5 μL
	8	-5.8	±5.1	-6.0	0.5 μL
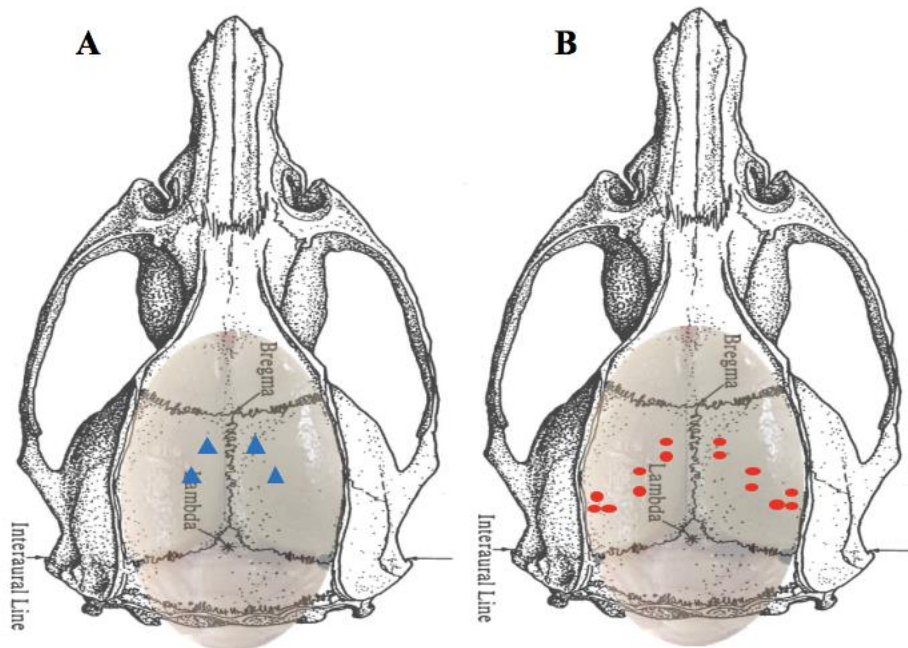
Figure 2.1. Graphic representation of stereotaxic drill sites for hippocampal lesion surgeries. A) Drill sites for dHPC lesions involving four infusions; B) Drill sites for HPC lesions involving sixteen infusions.

Contextual Fear Apparatus

Contextual fear conditioning and memory retention test sessions took place in chambers with aluminum side walls and transparent acrylic rear wall, ceiling, and door (MED-Associates, VT, USA; outer dimensions: 33 x 25 x 27 cm). The floor consisted of 19 stainless steel rods (4 mm diameter) spaced 1.3 cm apart, wired to a shock source and grid scrambler (MED-Associates) for the delivery of foot shock US. The chambers were mounted in cabinets located in a standard behaviour room. Ventilation fans within the cabinet supplied background noise (~ 65 dB). The chambers were cleaned thoroughly with dilute, germicidal quat-sanitizer (4 mL / L H_2O; Quatsyl-D Plus®, Pfizer Canada Inc., QC, Canada) between every session. The same chamber was used for conditioning and testing for each rat (Figure 2.2.).

Figure 2.2. Contextual fear conditioning chamber. Identical chambers within a common testing room served as the fear conditioning and memory retention testing contexts. Each rat was conditioned and tested in the same chamber.

Conditioning and Memory Retention Test Procedures

The contextual fear conditioning episode was carried out as a standard procedure for all experimental groups. Following the 12 d surgical recovery period, rats were transported individually from their home cage to the testing room in an opaque transport box and placed in the conditioning context. Following 180 s of exposure to the context, four US trials (foot shocks; 4 x 0.9 mA, 2 s each) separated by an 88 s inter-trial interval were delivered (Zelikowsky et al., 2012). Following the final shock, rats remained in the context for 60 s before being returned to their home cage. Rats were brought back to the context 1, 3, 10, or 30 d later for a single 8 min contextual fear memory test. Each rat was conditioned once and tested once in the same context.

Behavioural Data Analysis

Freezing was the measure of contextual fear memory. All sessions were recorded through a high-definition USB camera connected to a PC laptop computer running FreezeFrame™ 4.0 software (Actimetrics, Coulbourn Instruments, IL, USA), which captured session video and freezing data. For conditioning sessions, freezing during the 30 s preceding each US delivery was analyzed (Zelikowsky et al., 2012; [time immobile (s) $\div$ 30 (s) x 100 = Freezing %]). For testing sessions, the entire 8 min was analyzed ([time immobile (s) $\div$ 480 (s) x 100 = Freezing %]). Frame rate and motion detection thresholds were identical for all conditioning and testing sessions and employed FreezeFrame™ optimal system performance settings. Conditioning data were analyzed with two-way analysis of variance (ANOVA), with repeated measures for each US delivery (Group x Trial) and *post hoc* comparisons. Memory test data were analyzed with two-way ANOVA (Group x Retention

interval). All analyses were performed in Prism $6^{©}$ (GraphPad Software, CA, USA). Data from rats that did not reach the experimental endpoint due to postoperative health concerns, like severe scratching, were excluded from all analyses (n = 4). Three rats died during, or shortly after surgical procedures.

Histology and Lesion Volume Estimates

Animals were sacrificed with an overdose of sodium pentobarbital, then perfused transcardially with 0.9% PBS, followed by 4% PFA (in 0.9% 0.01 M PBS). The brains were extracted and post-fixed for at least 24 h in PFA-PBS solution, then transferred to a cryoprotecting solution (30% sucrose in 0.9% 0.01 M PBS with 0.02% sodium azide) for at least 48 h. The cryoprotected brains were frozen and sectioned in the coronal plane at 40 μm on a cryostat (Leica Biosystems, IL, USA), with every fourth section throughout the entire HPC mounted on microscope slides. Sections were stained with cresyl violet and cover-slipped for quantification of HPC volumes.

The volume of intact HPC principle cell fields was quantified stereologically (Sparks et al., 2013) via the Cavalieri method (Schmitz & Hof, 2005) using a brightfield microscope equipped with a motorized stage and StereoInvestigator® software (MBF Bioscience, VT, USA). The measured volume of intact principle cell fields in each dHPC group ($n = 24$) and HPC group ($n = 29$) brain was then divided by the mean HPC volume calculated from a subset of SHAM group animals ($n = 6$) and multiplied by 100 to yield intact HPC %. Intact HPC% was then subtracted from 100 to yield HPC% damage (100 – [(HPC mm^3 / $\overline{X}$ $_{(SHAM)}$ mm^3) x 100] = HPC% damage). Pearson correlation was performed with HPC% damage (x) and Freezing% (y) as variables. Rats with HPC damage deemed to be insufficient; < 40 % (dHPC group, $n = 6$) and < 65 % (HPC group, $n = 4$), were excluded

from all data analyses. These values were determined *a priori* and not in response to behavioural data.
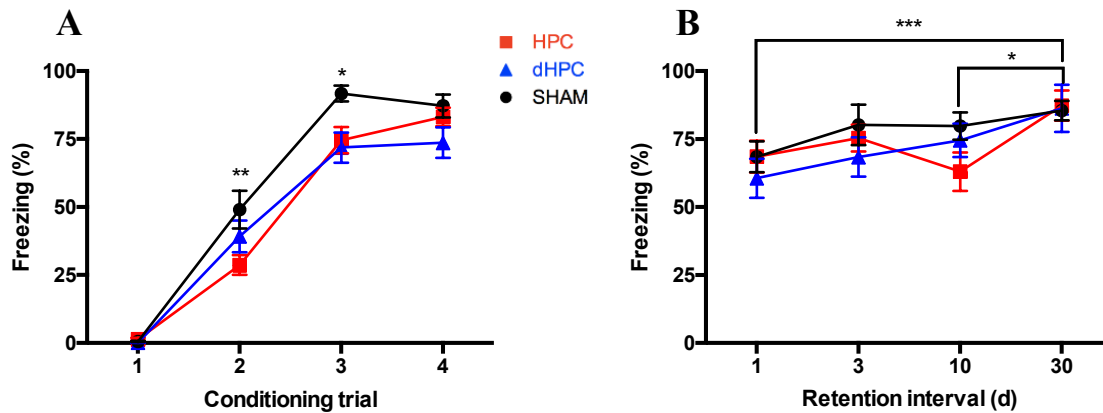
Results

Contextual Fear Conditioning and Memory Retention

During the conditioning episode, SHAM, dHPC, and HPC group rats exhibited similar levels of freezing prior to the first footshock and after the third footshock. Between-group differences were apparent after Trial 1 and Trial 2, with dHPC and HPC groups freezing less than the SHAM group (Trial 2; SHAM vs. HPC, $p = 0.0016$; and Trial 3, SHAM vs. dHPC, $p = 0.0043$ and SHAM vs. HPC, $p = 0.01$; Figure 2.3.). For the memory retention test, all groups exhibited conditioned freezing at all retention intervals, with no between-group differences, nor an interaction (Group, $F(2, 67) = 0.9744$, $p = 0.38$; Interaction, $F(6, 67) = 0.7617$, $p = 0.6026$). However, there was a main effect of retention interval ($F(3, 67) = 5.34$, $p = 0.0019$). Post hoc analyses indicated differences in conditioned freezing between Retention intervals (1 vs. 30 d, $p = 0.0009$; 10 vs. 30 d, $p = 0.031$). In summary, all rats learned to fear the context during the conditioning episode and demonstrated normal behavioural expression of memory for the experience at all retention intervals.

Hippocampal Lesion Volumes

Lesion volume estimates for dHPC group rats confirmed extensive and consistent damage across groups of animals tested at 1, 3, 10, or 30 d, as indicated by statistically similar means ($\overline{X}$) ($F(3, 20) = 0.5954$, $p = 0.6254$, ANOVA) and standard deviations (Table 2.2., Figure 2.4.). A conservative proportion of 50% volume of the entire HPC was assigned to dHPC (e.g., Broadbent, Squire, & Clark, 2004). Lesion estimates for HPC group rats

indicated HPC damage ranged from substantial to extensive (65.11% - 89.34%; Table 2.3., Figure 2.5.). Analysis of lesion volumes grouped by retention interval (1, 3, 10, and 30 d) revealed a main effect of group; $F(3, 27) = 6.03$, $p = 0.028$ (ANOVA).

Figure 2.3. Contextual fear conditioning acquisition and retention test. All data points expressed as mean ± SEM. A) Acquisition. SHAM rats exhibited more freezing behaviour than HPC rats on trial 2, and HPC and dHPC rats on Trial 3. All groups displayed similar levels of freezing by the end of the conditioning episode. B) Retention. SHAM, dHPC, and HPC rats exhibited similar levels of freezing behaviour at all retention intervals. On average, rats froze more during the 30 d test than the 1 d test and the 30 d test vs. 10 d test.

Correlation Between HPC Damage and Contextual Fear Memory Retention

Due to differences in mean HPC damage between retention intervals, HPC damage and freezing scores were analyzed with Pearson correlation. The results indicated a non-significant positive correlation coefficient (r) between HPC damage and Freezing during the contextual fear retention test; $r = 0.16$, $p = 0.26$, $n = 55$ (Figure 2.6.). The coefficient of determination ($r^2 = 0.026$) indicated that 2.6% of the variance in Freezing was attributable to variation in extent of hippocampal damage. Freezing in the SHAM group ($\overline{X} = 78.5$ %), was within the 95 % confidence interval (-0.15 to 0.55) of the line of best fit (y = 0.2016x + 61.63) when y = 0 (i.e., 0 % HPC damage in SHAM rats).

Table 2.2. dHPC lesion volumes. Damage expressed as % of the entire HPC for each retention interval (SD; standard deviation). Irrespective of retention interval, mean dHPC group lesion volume was 45.09% of the entire HPC.

	dHPC 1 Day	dHPC 3 Day	dHPC 10 Day	dHPC 30 Day
Smallest:	40.86%	41.67%	41.00%	40.01%
Mean:	44.60%	46.62%	43.98%	45.16%
Largest:	47.79%	49.50%	48.20%	50.00%
SD:	3.94%	2.84%	2.33%	3.70%
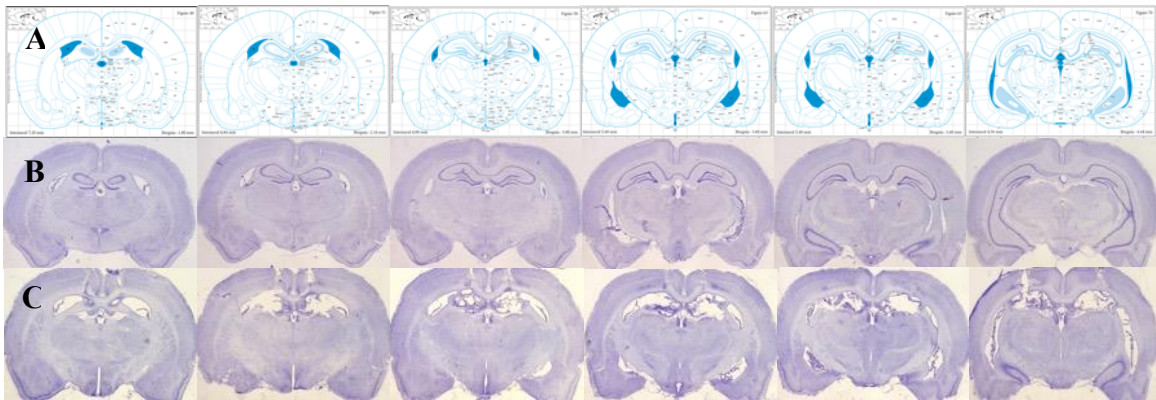
Figure 2.4. Photomicrographs of a SHAM brain and a typical dHPC lesion. A) Stereotaxic atlas -1.80 mm to -4.44 mm relative to bregma (left to right). B) SHAM brain with intact HPC. C) dHPC lesion brain, 45.1% total HPC damage (dHPC group; $\bar{X}$ = 45.01%).

Table 2.3. HPC lesion volumes. Damage expressed as % of the entire HPC ($\bar{X}$ = 73.31% for all HPC rats).

	HPC 1 Day	HPC 3 Day	HPC 10 Day	HPC 30 Day
Smallest:	65.11%	65.12%	65.51%	69.63%
Mean:	70.90%	75.84%	68.77%	77.74%
Largest:	76.64%	89.34%	74.96%	84.33%
SD:	4.21%	7.90%	3.06%	4.25%
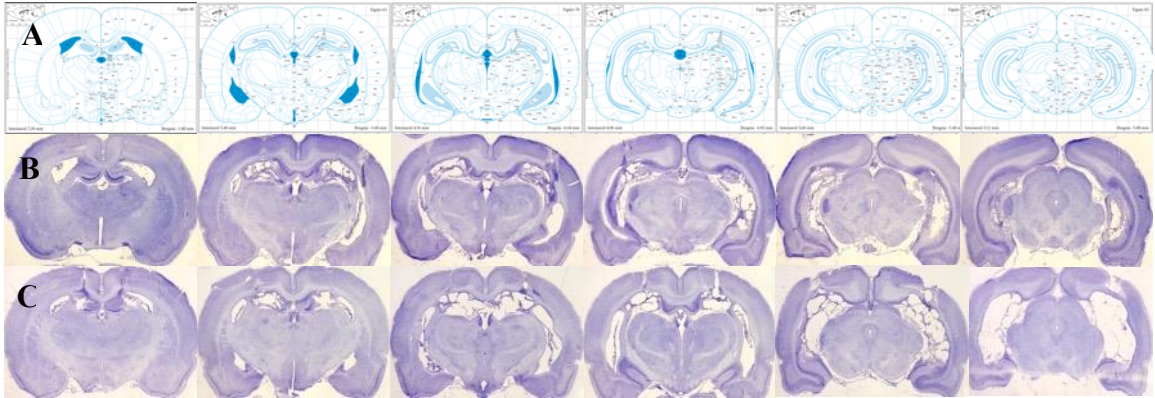
Figure 2.5. Photomicrographs of two HPC group brains illustrating different extents of HPC damage. A) Stereotaxic atlas -1.80 mm to -5.88 mm relative to bregma (left to right). B) Brain with 73.1% HPC damage (HPC group; $\overline{X}$ = 73.31%). C) Brain with 84.33% HPC damage (HPC group; max = 89.34%).
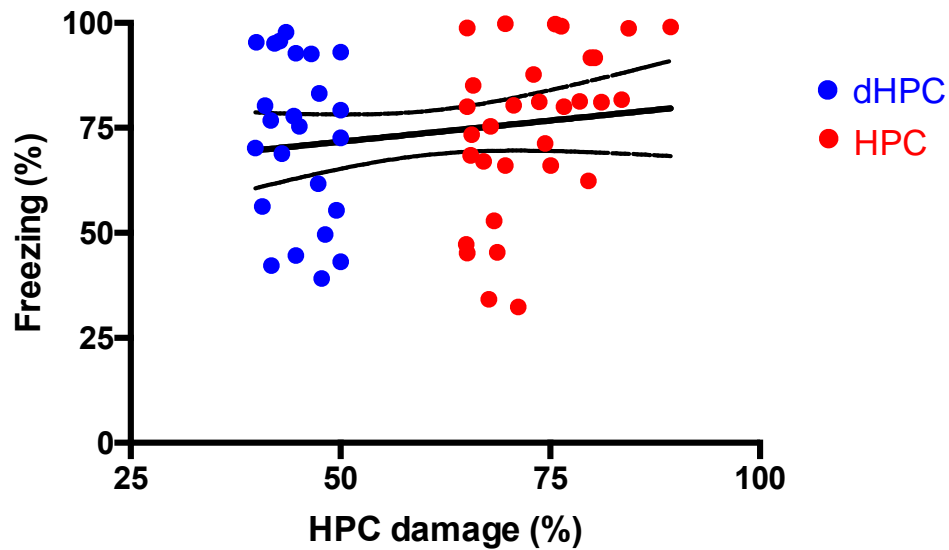
Figure 2.6. Correlation between extent of HPC damage and behavioural expression of contextual fear memory. Blue icons (dHPC); Red icons (HPC). Solid black line denotes line of best fit and curved dashed lines correspond to the upper and lower 95% confidence intervals. Freezing behaviour during context memory tests did not correlate strongly with extent of HPC damage (r = 0.16).

Discussion

The goal of the present experiment was to investigate one aspect of the systems-level consolidation framework; we tested the core prediction that memory acquired by non-hippocampal networks is fragile and lacks longevity (Squire & Wixted, 2011; Zelikowsky et al., 2012). The present data do not confirm this prediction. Like others, this experiment found that damage to the HPC before learning has little effect on the acquisition, and no detectable effect on recent contextual fear memory expression (Frankland et al., 1998; Maren et al., 1997; Wiltgen et al., 2006; Zelikowsky et al., 2012). The discovery that memory remains intact for 30 d is novel. Only one other study tested memory without the HPC over this time course (Zelikowsky et al., 2012) and found rapid memory decay, whereas our findings build on the finding of normal memory expression after 14 d (Wiltgen et al., 2006). Two clear conclusions can be drawn from the present study, Wiltgen et al. (2006), and Zelikowsky et al. (2012): 1) non-hippocampal networks acquire, maintain, and express contextual fear memory in the absence of hippocampal function and the memory exhibits normal longevity; 2) partial hippocampal damage may produce less reliable effects on contextual fear memory than extensive, or complete damage.

Despite duplicating the dHPC lesion procedures, conditioning session parameters, and retention tests as described (Zelikowsky et al., 2012), no evidence of memory decay, rapid or otherwise, was observed. Rather, the magnitude of freezing exhibited by separate groups of dHPC and HPC lesion rats increased between recent and remote testing intervals (1 vs. 30 d, and 10 vs. 30 d), indicating either stronger remote memory, or fear incubation (Poulos et al., 2016). Memory strength and magnitude of fear are difficult to dissociate, but reports of time-dependent increases in freezing typically involve 4 or more foot shocks, while lower numbers of shocks typically yield stable levels of freezing over time

35

(Fanselow, 1980; Poulos et al., 2016). The recent-to-remote increase in conditioned fear was also observed in SHAM rats, which supports the fear incubation account, yet does not preclude the possibility of stronger remote memory.

Conditioning parameters such as number of foot shocks, shock intensity, and distributed conditioning episodes among others, are known to modulate both the behavioural expression of remote contextual fear memory and the magnitude of fear (Fanselow, 1980; Hugo Lehmann et al., 2009; Pickens, Golden, Adams-Deutsch, Nair, & Shaham, 2009; Poulos et al., 2016; Winocur, Moscovitch, et al., 2013). However, the conditioning parameters used in the present experiment, Zelikowsky et al. (2012), and Wiltgen et al. (2006), were very similar – the former two were as similar as we could make them. Small differences in general procedures, such as disinfectant used between conditioning sessions (quat-sanitizer vs. 70% alcohol) or transporting rats singly versus in squads to the testing room, are unlikely to influence the expression of remote memory, provided they are held constant within-experiment. Notably, Zelikowsky et al. (2012) included trimethoprim sulfa (an antibiotic/protein synthesis inhibitor) in rats' drinking water for a week during the surgical recovery period. Anterograde memory impairments secondary to trimethoprim sulfa drug regimens have been reported by human patients (Sternbach, 1997). It is unknown if this side effect extends to rodents, or if this antibiotic could cause profound, evolving AA for contextual fear memory selectively in rats with HPC damage as observed by Zelikowsky et al. (2012). However, antibiotics like anisomycin are known to impair memory and silence neural transmission when infused directly into brain parenchyma (Nader, Schafe, & Le Doux, 2000; Sharma, Nargang, & Dickson, 2012). It is possible that the uncontrolled use of trimethoprim sulfa is an extraneous variable, or a confound (see, Sharma et al., 2012). Because the prolonged use

of antibiotic and the observation of rapid memory decay in dHPC damaged rats are unique to Zelikowsky et al. (2012), drawing conclusions about either may not be possible.

The present findings are less ambiguous: dHPC and HPC damaged rats exhibit robust freezing behaviour by the end of the single conditioning episode and during all retention tests. These data demonstrate intact acquisition, retention, and normal expression of contextual fear memory in the absence of the HPC. The idea that incomplete damage to the HPC might have resulted in the memory decay observed by Zelikowsky et al. (2012) was neither supported by the present experiment. Memory expression was not correlated with extent of hippocampal damage, despite considerable variability between rats (minimum, 41%; maximum, 89%; including dHPC and HPC lesion conditions). Whereas the present findings and those from the Zelikowsky study provide a prime example of how very similar experiments can find diametrically opposite effects of partial HPC damage on contextual fear memory, this study and Wiltgen et al. (2006) found similar effects of extensive-to-complete HPC damage. This further validates concerns that incomplete HPC damage can have unpredictable, often incoherent effects on memory due to unknown extent of hippocampal dysfunction (Lee et al., 2017, 2016; Scott et al., 2016; Sutherland & Lehmann, 2011; Sutherland et al., 2010).

The finding of strong remote fear memory in rats with extensive hippocampal damage ($\overline{X} = 73.31\%$) is statistically valid, yet an interesting coincidence exists in a subset of the retention and lesion volume data. Figure 2.3B shows that rats assigned to the HPC lesion condition and 10 d retention test exhibited less contextual fear compared to every other retention interval + extensive HPC lesion condition subgroup. Table 2.3 indicates the lowest mean (68.77%) and maximum (74.96%) HPC damage values, along with the lowest standard deviation (3.06%) belongs to the same subgroup of rats. It must be considered that

moderate damage to the entire HPC (~ 68%) before conditioning, like dHPC damage (< 50%), disrupts hippocampal function incompletely. Views on what extent of damage constitutes complete hippocampal disruption are few, but current estimates range from > 70% to > 80% (Lee et al., 2017, 2016; Scott et al., 2016; Sutherland & Lehmann, 2011; Sutherland et al., 2010). As previously described, incomplete hippocampal damage after a conditioning episode yields less reliable RA than extensive damage, which suggests spared hippocampal tissue retains some mnemonic function. This is likely the case in the anterograde direction as well, yet it cannot be known from the present data. Conceivably, learning and memory in dHPC and dHPC + vHPC damaged rats might be supported by the remaining HPC, or solely by non-hippocampal networks, be dysfunctional, or appear normal. The recurrent spared-impaired function confound is unlikely to be resolved through behavioural analysis alone (Lee et al., 2016; Scott et al., 2016; Sutherland & Lehmann, 2011; Sutherland et al., 2010). The discovery that rats with extensive hippocampal damage exhibit normal behavioural expression of remote (30 d) contextual fear memory and interpretation of this finding are less problematic for this reason. Extensive anterograde HPC damage does not cause rapid decay of contextual fear memory and this is also the case with complete HPC damage (14 d; Wiltgen et al., 2006).

Interestingly, one subtle effect of hippocampal damage on contextual fear conditioning was observed by Zelikowsky et al. (2012), this study, and others (Maren et al., 1997; Wiltgen et al., 2006)(Chapter 3., this thesis). Rats with HPC damage regularly exhibit less conditioned fear responding during acquisition compared to intact rats. However, the deficit is transient and is no longer apparent after the second or third foot shock (Figure 2.3A). One idea is that non-hippocampal networks possess a slower learning rate compared to hippocampal networks and acquire memories like contextual fear less

efficiently (Fanselow, 2009; Wiltgen et al., 2006; Zelikowsky et al., 2013, 2012). Proponents of this view often cite the concept of *complementary learning systems* (McClelland et al., 1995) and Marr's *Simple Memory: A Theory for Archicortex* (Marr, 1971), which highlight the computational advantages of coexisting fast and slow memory networks. Specifically, rapid learning by hippocampal networks that is highly-detailed and specific to a relevent environment or context, then slow generation of semantic memory and knowledge by non-hippocampal (neocortical) networks from overlapping elements of many experiences in order to flexibly guide behaviour in novel environments. Indeed, attenuated conditioned responding in HPC damaged animals that disappears after $2-3$ foot shocks might reflect slower learning by non-hippocampal networks. However, this idea should be addressed in more detailed terms than fast versus slow (Wiltgen et al., 2006; Zelikowsky et al., 2012)[9] – an oversimplification of the ideas proposed by Marr (1971) and McClelland et al. (1995).

Finally, the strong aversive nature of fear condtioning must be considered. Damage to the HPC is likely to affect a myriad of processes involved in fear learning, both directly and indirectly. For example, the strength and specificity of memory are known to be modulated by arousal during learning, and this is dependent on norepinephrine and gluccocorticoid-mediated effects in the amygdala and HPC (McGaugh, 2018). Rats with HPC damage might initially exhibit less conditioned fear due in part to pertubation of the processes involved in the emotional modulation of learning, but as the number of shocks increase, so does the fear response (Fanselow, 1980; Poulos et al., 2016). The fact that the

---

[9] If the transient deficit in conditioned fear responding during contextual conditioning is due to a difference between hippocampal (fast) and non-hippocampal (slow) learning rates, the difference might be analgous to "*comparing Ferraris to Lamborghinis*" (S.H. Deibel, personal communication).

primary (often only) measure of contextual fear learning is freezing makes it difficult to dissociate context-shock associative learning from the emotional-behavioural effects of an inescapable aversive stimulus, like a foot shock US (but see, Antoniadis & McDonald, 1999; Antoniadis & McDonald, 2001).

To summarize, the present findings do not support a core prediction of the systems-consolidation framework, that the HPC is required to create stable long-term context memory. Rats with hippocampal damage demonstated learning during a single contextual fear conditioning episode and expressed normal memory for the expereince up to thirty days later. There was no evidence of memory decay and extent of hippocampal damage was not predictive of memory performance. It must be concluded that contextual fear memory acquired in the absence of the HPC has the same hallmark longevity of normal memory. Present findings therefore suggest that hippocampal function is not always an essential feature of memory longevity. This discovery adds to a growing body of empirical evidence that draws into question our fundamental views on memory and the HPC, views derived from dichotomous taxomomies of memory types, memory systems, and memory processes (Roediger et al., 2017; Squire, 2004)[10].

---

[10] Squire (2004) briefly reviews and summarizes the history of memory systems and memory taxonomies.

Chapter 3

Context Discrimination Without the Hippocampus

Introduction

Prominent theoretical views have advanced the idea that the HPC and non-hippocampal networks support different types of memories (Squire, 1992a; Squire, 2004; Squire et al., 1984), while other views focus more on unique mnemonic processes of networks and their interactions (Gruber & McDonald, 2012; McDonald et al., 2017; Lee et al., 2016; Sutherland & Rudy, 1989; White & McDonald, 2002). In line with the former framework, one proposition states the precision of episodic memory, not the longevity depends critically on the HPC (Winocur & Moscovitch, 2011; Winocur et al., 2013; see also, Fanselow, 2009; Wiltgen & Tanaka, 2013). In contrast to highly-contextualized hippocampal memories, non-hippocampal networks are purported to support a more general, *contextually impoverished* version of memory (Sekeres, Moscovitch, & Winocur, 2017; Winocur et al., 2013). Moreover, a transformation is hypothesized to take place – from detail-rich, hippocampal-dependent memory, to a gist-like, schematic version which can be supported by non-hippocampal networks and expressed independently of the HPC (Sekeres, Moscovitch, & Winocur, 2017; Winocur & Moscovitch, 2011; Winocur et al., 2013). One difference between these ideas and earlier systems consolidation-based theories of memory (e.g., McClelland et al., 1995; Squire et al., 1984) is the transformation view asserts two versions of one memory – detailed (hippocampal), and schematic (non-hippocampal) – can "co-exist, complement each other or compete with one another" (Winocur et al., 2013; see also, Sekeres et al., 2017). According to these ideas, non-hippocampal networks do not possess the representational complexity to support precise

context memories, like those required for accurate context discrimination, regardless of whether the HPC is functional or non-functional.

Past reports of impaired context discrimination in hippocampal-disrupted rats support the idea that the HPC is required for detailed contextual memories (Antoniadis & McDonald, 2000; Frankland et al., 1998). In addition, intact rodents exhibit a context-specific fear response shortly after a conditioning episode, but memory for the experience generalizes over time to novel, unconditioned contexts (Poulos et al., 2016; Riccio & Joynes, 2007; Wiltgen & Silva, 2007). Thus, context fear generalization is thought to reflect a loss of memory detail. Context discriminability can be extended by repeated context pre-exposures prior to a fear conditioning episode, which suggests stronger or more detailed context memories are less-prone to generalization (Biedenkapp & Rudy, 2007). A brief reminder, in the form of pretest re-exposure to the conditioning context (without shock), is also thought to re-establish details in hippocampal-dependent context memory, as this treatment temporarily attenuates generalization (de Oliveira Alvares et al., 2012; Zhou & Riccio, 1994). Some evidence suggests that reversibly disrupting dHPC function selectively impairs detailed context memory, both at retention intervals normally associated with accurate discrimination, and in mice that exhibited accurate discrimination on previous memory tests (de Oliveira Alvares et al., 2012; Wiltgen et al., 2010) (cf. Goshen et al., 2011; Wang, Teixeira, Wheeler, & Frankland, 2009). Collectively, these reports have been interpreted to support ideas on systems consolidation and memory transformation as follows: 1) fear generalization reflects a contextual-to-schematic shift in memory detail over time; 2) the degree of detail in long-term memory determines its dependence on the HPC for retrieval; 3) the HPC is uniquely involved in precise contextual memories that enable context discrimination; 4) different versions of a memory might co-exist in distinct

memory systems (Fanselow, 2009; Rudy, 2009; Sekeres et al., 2017; Wiltgen & Tanaka, 2013; Winocur et al., 2013).

Forgetting, or memory decay, is one parsimonious explanation for the time-dependent reduction in context discriminability (Hardt, Nader, & Nadel, 2013; Rudy, Biedenkapp, & O'Reilly, 2005). As emphasized here, current views typically reject this possibility and describe generalization in terms of the HPC and systems memory consolidation processes (Jasnow, Lynch, Gilman, & Riccio, 2017; Sekeres et al., 2017; Squire, 1992a; Wiltgen & Tanaka, 2013). Like extant views on the HPC and systems-level consolidation, which have become empirically tenuous (Chapter 2; Gidyk, McDonald, & Sutherland, 2016; Lee et al., 2016; McDonald & Hong, 2013; Sutherland & Lehmann, 2011; Sutherland et al., 2008), recent work found that non-hippocampal networks can support accurate context discriminations (Lee et al., 2017). Employing our discriminative fear conditioning to context procedure (McDonald, Koerner, & Sutherland, 1995; see also, Frankland et al., 1998) Lee and colleagues (2017) discovered that rats with extensive hippocampal damage (> 80%) can discriminate between contexts for up to three days, even under weak conditioning parameters. A separate experiment in the same study found that rats with extensive post-training hippocampal damage exhibited profound RA for the conditioning episodes and impaired context discrimination (Lee et al., 2017). The findings suggest that non-hippocampal networks can acquire, maintain, and express context memories with sufficient detail to support normal context discrimination behaviour. Moreover, the observation of RA suggests that gist-like versions of the conditioning and unpaired context episodes did not persist after hippocampal damage. However, it could be argued that the memories had not undergone systems consolidation/transformation before the HPC was damaged. It is unclear if contemporary consolidation-based theories, or

transformation views of memory and the HPC can accommodate data from Lee and colleagues (2017). Further investigation is required to resolve uncertainties about the HPC, non-hippocampal networks, and context discrimination. More specifically, the putative timeline and boundary conditions for the hypothesized consolidation-based loss of detail in long-term memory needs to be elucidated.

The present experiments were designed to explore systems consolidation and one aspect of the memory transformation framework, which in our view is based heavily on systems-level consolidation theories (e.g., McClelland et al., 1995). The following predictions were tested: 1) rats with extensive hippocampal damage cannot acquire memory that facilitates accurate context discrimination; 2) in intact rats, a precise hippocampal context representation co-exists with a gist-like non-hippocampal version during memory transformation and detail can be reinstated with a pre-test reminder at a time point when generalization normally occurs; 3) a low ambiguity context discrimination involving little overlap between environmental cues should delay generalization in intact rats, because less detail from the conditioning episode is required to distinguish the shock and novel context.[11] In addition to the primary experiment which tested the predictions 1 – 3, a behavioural lesion verification in the form of a hippocampal-dependent, precise allocentric learning and memory task (MWT) was performed to confirm that extensive damage to the HPC substantially disrupted hippocampal function, and to ensure that key outcomes from the primary experiment were reproducible.

---

[11] To my knowledge, prediction three is not explicitly stated by the authors of TTT, or its proponents. It is my own logical extension of predictions [(1 - 6), p. 532] stated by Winocur and colleagues (2013), and subsequent interpretations of the transformation framework by the authors and others (e.g., Sekeres et al., 2017; Wiltgen & Tanaka, 2013).

Methods

Subjects

Fifty male Long–Evans hooded rats (Charles River, NC, USA), weighing 330 – 420 g at the time of surgery were housed in pairs in the University of Lethbridge rodent vivarium (20 °C, 50% relative humidity). A 12 h light – dark schedule was maintained, with lights on at 7:30 am daily. Access to food and water was *ad libitum* upon arrival until endpoint. Rats were handled for several minutes each day for 5 d prior to surgery. All experimental procedures adhered to CCAC policy and were approved by the University of Lethbridge Animal Welfare Committee. Eleven male Long–Evans hooded rats (300 – 360 g) were subjects in the behavioural lesion verification study and were cared for as described above.

Surgery

Procedures for the 16-site HPC lesions ($n = 27$) and SHAM ($n = 25$) surgeries followed those described in Chapter 2. The coordinates for the most rostral bilateral drill site (Chapter 2.; Table 2.1., HPC site 1) were modified to A/P: - 2.2 mm, M/L: ± 1.2 mm (relative to bregma). All rats recovered from surgery in home cages for 10 d prior to contextual fear conditioning. For the behavioural lesion verification study, HPC ($n = 5$) and SHAM rats ($n = 6$) recovered from surgery for 7 d prior to Morris Water Task (MWT) training.

Conditioning and Novel Contexts

The conditioning and testing contexts were identical to that described in Chapter 2. (Figure 2.1.). A second chamber in a different testing room served as the novel context for the discrimination test (Figure 3.1.). The chamber was triangular with non-transparent black

acrylic walls (64 cm x 64 cm x 64 cm) and a removable white acrylic lid (floor-to-ceiling height: 30 cm). The standard grid floor made of stainless steel bars was covered with a smooth transparent acrylic insert. The novel context was cleaned before and between sessions with a dilute persulfate solution (Virkon®; Vetoquinol N.A. Inc., Quebec, Canada).
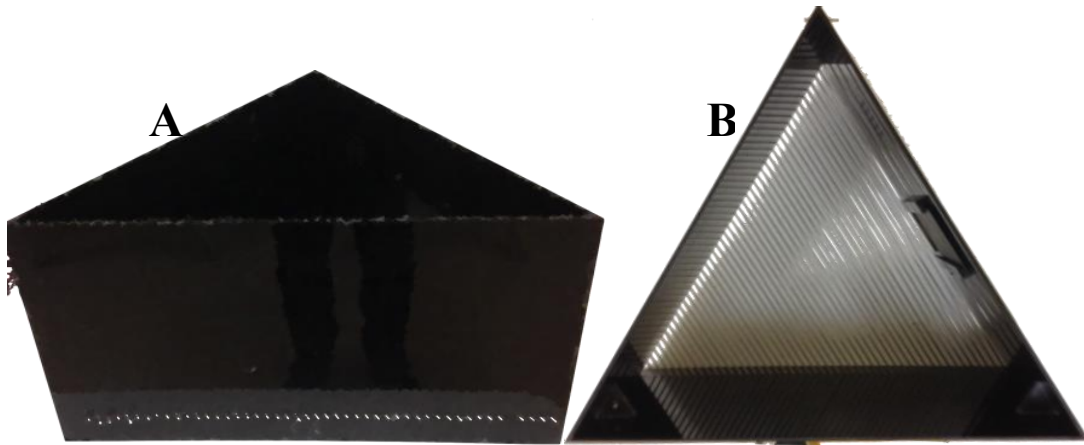
Figure 3.1. Photographs of the novel context used for discrimination testing. A) Oblique view of the novel context without lid. B) Top view of the novel context without lid showing the steel rod floor.

MWT Apparatus

All sessions were carried out in a circular fiberglass pool (~ 125 cm diameter) situated in a testing room equipped with a ceiling-mounted video camera connected to a laptop PC running HVS 2100 tracking software (HVS Image Ltd, UK). The pool was filled with water (21°C) and made opaque with non-toxic white paint prior to each session. The escape platform (13 cm diameter) was located approximately 2 cm below the surface of the water in the center of the North-West quadrant for all training sessions. Large posters of geometric shapes fixed to the walls of the testing room provided extra-maze visual cues that rats could use to navigate and learn the location of the platform. The pool was drained and thoroughly cleaned following completion of each session.

Contextual Fear Conditioning, Retention, and Context Discrimination

The single conditioning session was carried out as a standard procedure for all rats. The procedures and parameters were identical to those described in Chapter 2. The contextual fear memory retention test was also identical to Chapter 2. The context discrimination test consisted of one 5 min exposure in the novel context. The sequence of the tests was counter-balanced; half of the subjects in each group (HPC, SHAM) received the contextual fear memory test first, and half received the context discrimination test first (novel context exposure). The tests were separated by 6 h, regardless of which occurred first. Testing took place 1, 7, and 15-d after conditioning. For the behavioural lesion verification study, only the 15-d train-to-test interval was used and all rats received the context discrimination test first, 6 h before the contextual fear memory test.

MWT: Behavioural Lesion Verification

Rats received 5 consecutive days of MWT training, with 8 swim trials each day for a total of 40 swim trials. The starting position for the first trial was randomly assigned to one of four cardinal points labelled: N, S, E, and W. The sequence of start points for the 8 swim trials varied each day. A trial began with placing a rat in the pool facing the pool wall at the assigned start position and ended either with the rat locating the platform and escaping, or until 60 s had elapsed without escape. For trials with unsuccessful escapes, rats were led to the platform by the experimenter. Rats remained on the platform for 10 s after every trial before being placed back in the transport cage. Twenty-four hours following completion of MWT training, rats received one 60 s spatial memory probe trial. The probe followed the same general procedures as MWT training, except the escape platform was removed and all rats began the trial from a novel starting position (SE).

Histological and Behavioural Data Analyses

Rats were sacrificed 24 – 72 h following completion of behavioural testing. All endpoint and histological procedures followed those described in Chapter 2. Lesion volumes were calculated and analyzed as described in Chapter 2. Freezing data for contextual fear conditioning, memory retention, and context discrimination were also captured and analyzed as described in Chapter 2. Additionally, a context discrimination index was calculated; (Freezing% $_{(shock\ context)}$) ÷ (Freezing% $_{(shock\ context)}$ + Freezing% $_{(novel\ context)}$) x 100 = Discrimination(%). For the behavioural lesion verification MWT sessions, data were captured as described above. For training, mean escape latency was analyzed by Group (HPC, SHAM) x Trial (1 – 8) within training day, and Group x Day (1 – 5) for the training period. For the probe trial, dwell time in the correct quadrant (quadrant 4; the NW location of the platform in all training trials) was compared with dwell time in all incorrect quadrants [(t quad 4) / ($\sum t$ quad 1,2,3,4 ÷ 4)] *100 = % in correct quadrant] for each rat then analyzed by group. All analyses performed in Prism $6^{©}$.

Results

HPC Lesion Volumes

As illustrated by Table 3.1. and Figure 3.2., extent of hippocampal damage ranged from extensive (> 70%) to near-complete (> 95%), with similar means between subgroups of HPC rats tested at 1, 7, and 15-d retention intervals; $p = 0.56$ (ANOVA). Two HPC group rats were excluded from all data sets due to insufficient hippocampal damage.

Table 3.1. HPC lesion volumes by retention interval. Extent of damage to the HPC expressed as % of the entire HPC ($\bar{X} = 90.99\%$ for all HPC rats).

	HPC 1 Day	HPC 7 Day	HPC 15 Day
Smallest:	83.01%	78.17%	70.22%
Mean:	90.09%	89.96%	92.93%
Largest:	96.75%	97.69%	98.21%
SD:	4.00%	5.80%	8.89%
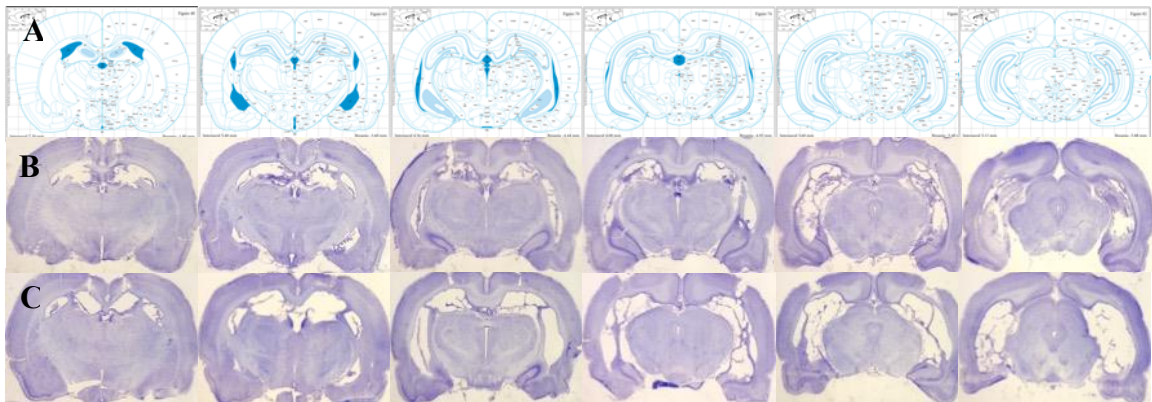
Figure 3.2. Photomicrographs of two HPC brains with varying extents of extensive hippocampal damage. A) Stereotaxic atlas -1.80 mm to -5.88 mm relative to bregma (left to right). B) 89.27% damage indicative of an average HPC lesion. C) 98.21% damage indicative of a near-complete HPC lesion.

Contextual Fear Conditioning and Memory Test

On average, HPC rats exhibited less fear responding compared to SHAM rats on Conditioning trials 2 and 3 and both groups froze similarly by the end of conditioning. Analysis of Freezing(%) by Group x Trial (1, 2, 3, 4) revealed main effects of: Group, $F(1, 51) = 8.89$, $p = 0.0033$; Trial, $F(3, 153) = 234.50$, $p < 0.0001$; and a Group x Trial interaction, $F(3, 153) = 3.57$, $p = 0.016$ (ANOVA). Sidak *post hoc* comparisons confirmed

between-group differences on Trial 2; SHAM vs. HPC, $p = 0.0021$, and Trial 3; SHAM vs. HPC, $p = 0.0066$. Magnitude of freezing behaviour during retention tests was similar for subgroups of HPC and SHAM rats tested at different retention intervals. There was no effect of Group; $F(1, 47) = 0.87$, $p = 0.35$, nor Retention interval; $F(2, 47) = 1.02$, $p = 0.37$. There was a statistically significant Group x Retention interval interaction; $F(2, 47) = 3.25$, $p = 0.048$. However, Sidak *post hoc* comparisons did not indicate differences between HPC and SHAM subgroups at any Retention interval. Conditioning and retention data are displayed in Figure 3.3. (A, B).

Context Discrimination

Groups of HPC and SHAM rats tested at 1, 7, or 15 d intervals froze more in the conditioning context compared to the novel context. Analysis of Freezing(%) in the novel context confirmed a main effect of Retention interval; $F(2, 47) = 8.61$, $p = 0.0007$, and Group x Retention interval interaction; $F(2, 47) = 3.39$, $p = 0.042$ (ANOVA). Sidak *post hoc* comparisons revealed a difference the 15 d test, with SHAM rats freezing more than HPC rats; $p = 0.01$ (see Figure 3.4. A, B, C). Context discrimination(%) calculations and subsequent ANOVA yielded a main effect of Retention interval; $F(2, 47) = 6.00$, $p = 0.0048$. Analysis with one-sample t tests indicated above chance (50%) discrimination performance by HPC rats on 1, 7, and 15 d tests ($p = 0.0002$; $p = 0.002$; $p = 0.01$, respectively). The SHAM group discrimination performance was above chance on 1, 15, but not 7 d tests ($p = 0.003$; $p = 0.009$; $p = 0.10$, respectively) (Figure 3.4. D). Additional planned analyses (Sidak and one-sample t tests) uncovered effects of testing sequence in subgroups of SHAM and HPC rats at each retention interval (Figure 3.5. A-F; Figure 3.6.).
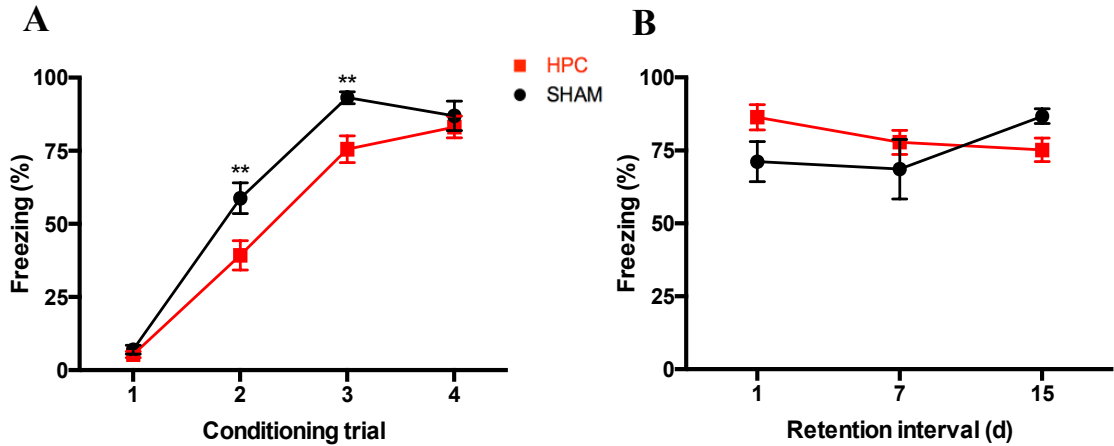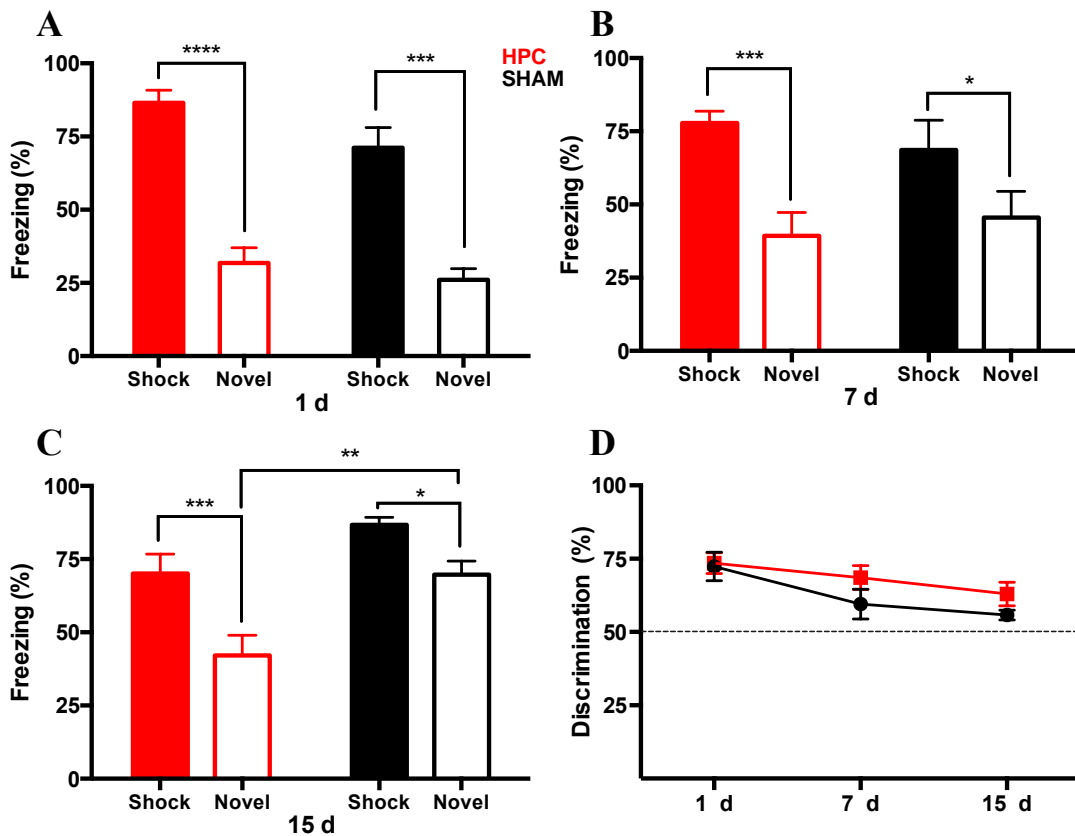
Figure 3.3. Contextual fear conditioning and memory test. A) Conditioning: HPC rats exhibited less freezing than SHAM rats on trial 2 and 3. B) Retention: HPC and SHAM rats performed similarly on the context memory tests at 1, 7, and 15-d retention intervals. Note that all statistically significant between-group differences are indicated (**).

Figure 3.4. Context discrimination by HPC and SHAM group rats. SHAM and HPC rats tested 1 d (A), 7 d (B), or 15 d (C) after contextual fear conditioning exhibited context discrimination. Compared to HPC rats, SHAM rats froze more in the novel context during the 15 d test. D) HPC and SHAM rats demonstrated similar context discrimination ability at all train-to-test intervals (dashed line denotes chance level discrimination).
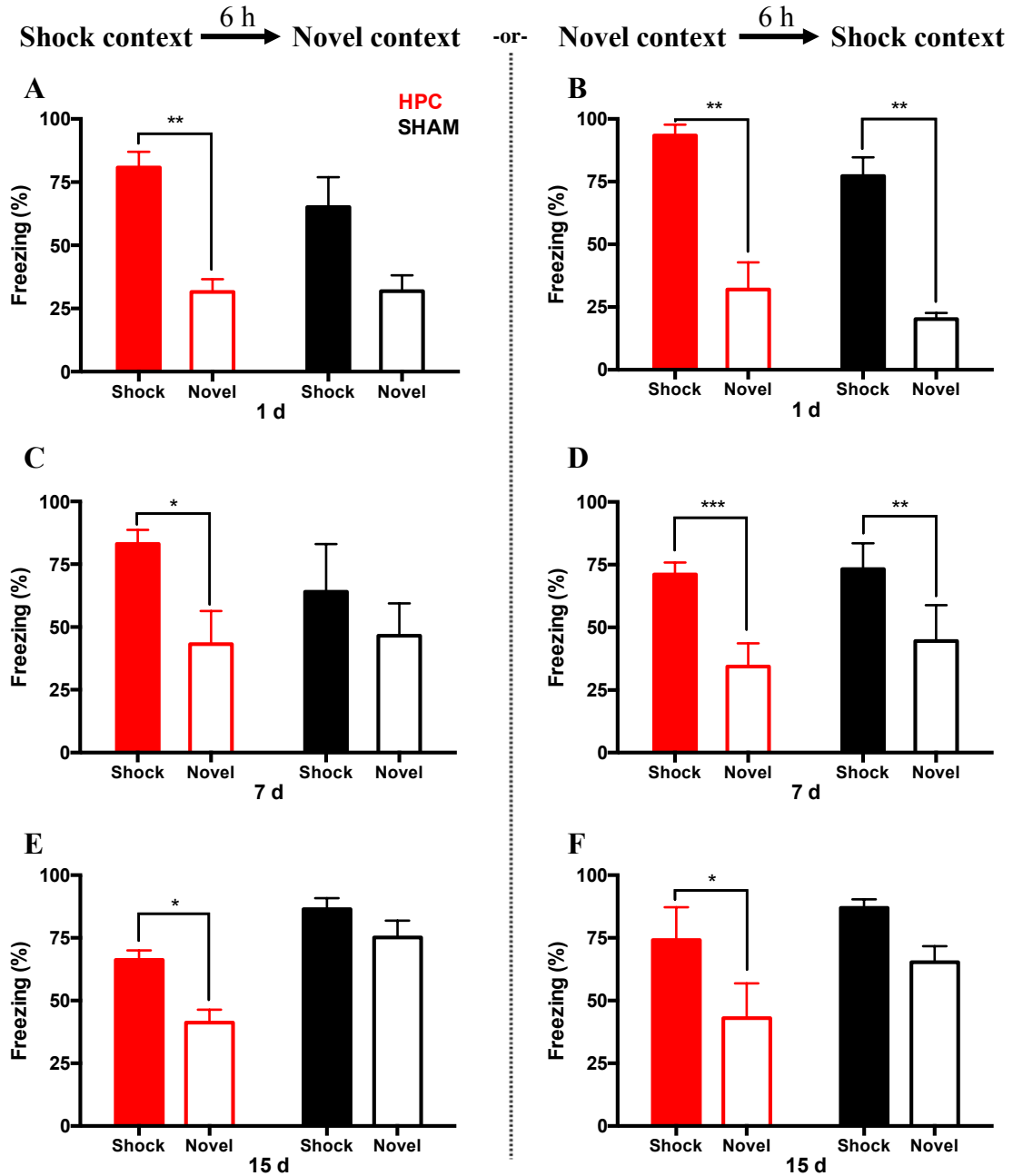
Figure 3.5. Context discrimination performance displayed by test sequence at 1, 7, and 15-day retention intervals. (A, B) 1 d discrimination: SHAM rats discriminated more accurately when tested in the novel context first ($p = 0.0076$). (C, D) 7 d discrimination: SHAM rats discriminated between contexts in the novel context-first sequence ($p = 0.0022$), but as not accurately in the shock context-first test sequence ($p = 0.48$). (E, F) 15 d discrimination: on average, SHAM rats did not exhibit accurate context discrimination in either test sequence ($p = 0.35$, $p = 0.16$). HPC rats discriminated between shock and novel contexts at all retention intervals, regardless of test sequence.
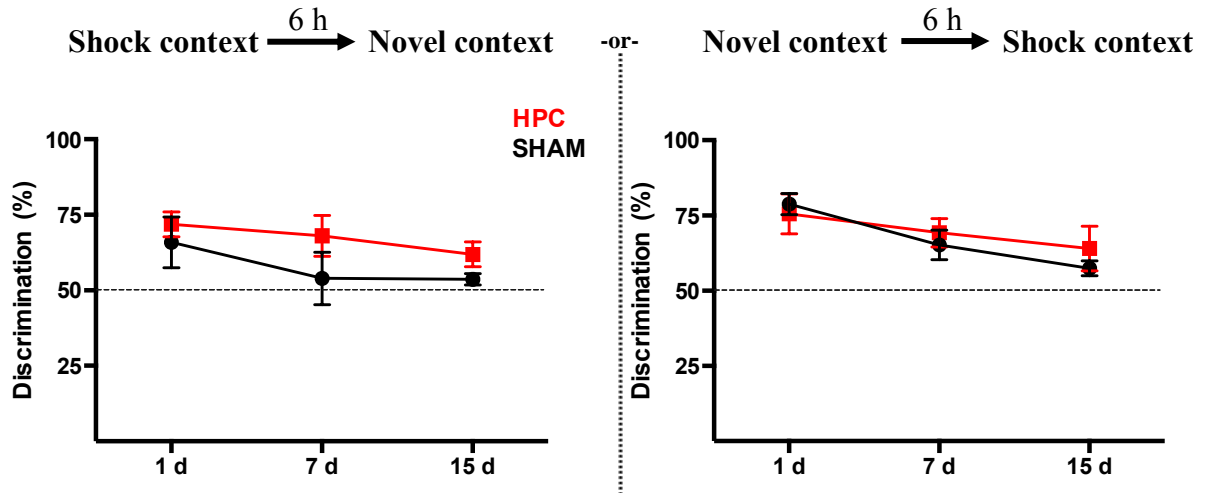
Figure 3.6. Context discrimination across retention intervals displayed by test sequence. Left panel: SHAM discrimination was not statistically above chance (hypothetical mean, 50 %) at any retention interval in the shock-to-novel context test sequence. Mean HPC discrimination was significantly greater than chance at all retention intervals (1 d, $p < 0.0001$; 7 d, $p = 0.006$; 15 d, $p = 0.001$). Right panel: in the novel-to-shock context test sequence SHAM discrimination was above chance at all retention intervals (1 d, $p = 0.0037$; 7 d, $p = 0.047$; 15 d, $p = 0.041$), as was HPC discrimination (1 d, $p = 0.00015$; 7 d, $p = 0.0007$; 15 d, $p = 0.001$).

Results: Behavioural Lesion Verification

HPC Lesion Volumes

As shown in Table 3.2., damage to the HPC ranged from extensive (79.41%) to near-complete (92.72%), and was consistent between rats ($n = 5$; $\overline{X} = 87.04\%$; $SD = 5.62\%$).

MWT

Over five days of MWT training, escape latencies decreased. Statistical analysis of mean escape latency by Group (SHAM, HPC) x Training day (1, 2, 3, 4, 5) revealed main effects of Group; $F(1, 9) = 11.36$, $p = 0.008$, and Training day; $F(4, 36) = 52.47$, $p < 0.0001$. Between-group *post hoc* comparisons indicated HPC escape latencies were longer than

SHAM latencies on Training day 2 and 3; $p = 0.0021$; $p = 0.045$, respectively (Sidak; Figure 3.7.A). For the probe, dwell time in the correct quadrant (location of the escape platform during training) was greater than in incorrect quadrants for SHAM rats; $p = 0.001$, but not HPC rats; $p > 0.99$, which performed at chance-level (Figure 3.7.B).

Contextual Fear Conditioning and Context Discrimination

By the end of the conditioning episode, SHAM and HPC rats exhibited similar magnitude of fear. ANOVA of Freezing(%) with Group x Trial as factors revealed main effects of: Group; $F(1, 36) = 71.73$, $p < 0.0001$ and Trial; $F(1, 36) = 4.61$, $p = 0.039$. Sidak *post hoc* indicated a between-group difference on Trial 2; SHAM vs. HPC, $p = 0.037$, with HPC rats exhibiting less freezing (Figure 3.7.C). Freezing behaviour in the conditioning context during the 15 d memory test was similar between-groups ($p = 0.46$), as was Freezing% in the novel context ($p = 0.34$). Both groups froze more in the conditioning context than novel context (HPC, $p < 0.0001$; SHAM, $p = 0.0002$), and exhibited above chance context discrimination as confirmed by alpha values from one-sample t tests; HPC, $p = 0.0002$; SHAM, $p < 0.0001$.

Table 3.2. Hippocampal lesion volumes.
HPC damage as % of the entire HPC.

Smallest:	79.41%
Mean:	87.04%
Largest:	92.72%
SD:	5.62%
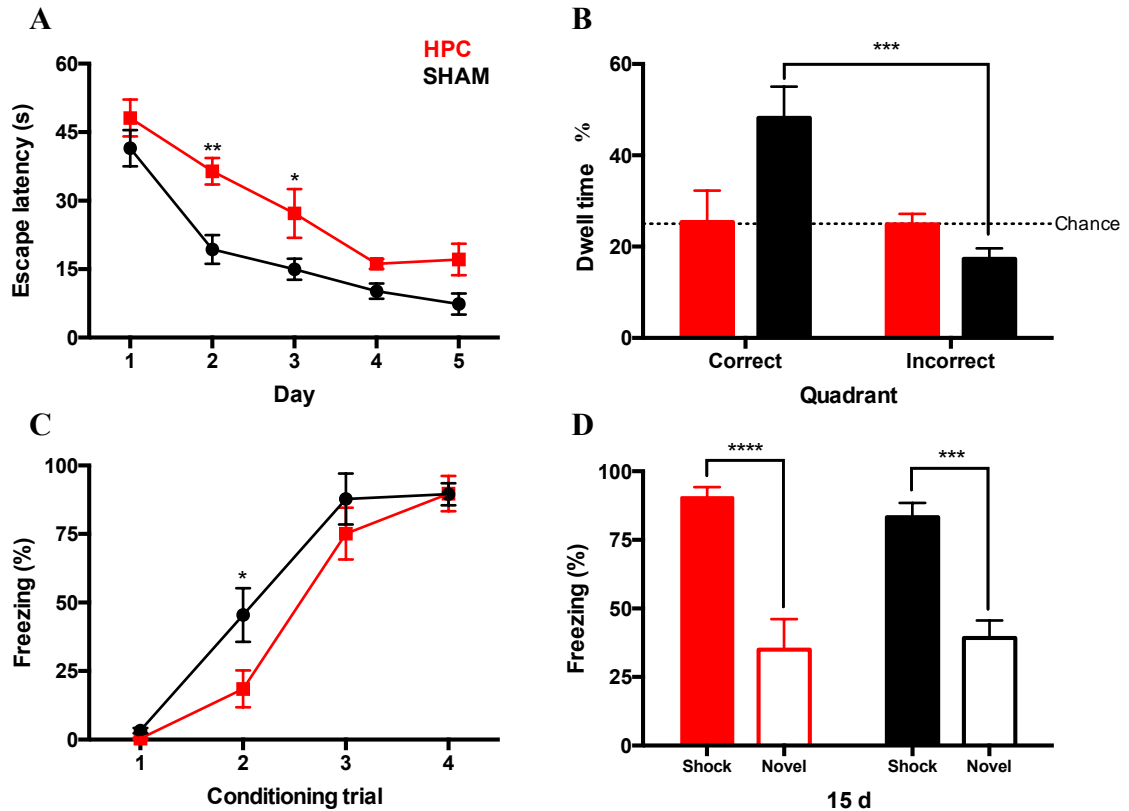
Figure 3.7. MWT, contextual fear, and context discrimination data from the lesion verification experiment. A) MWT training: SHAM and HPC rats decreased escape latencies over 5 training days. Between-group differences denoted (** and *). B) MWT probe: SHAM rats demonstrated memory for the platform location, but HPC rats did not as indicated by equal dwell time across quadrants. C) Contextual fear conditioning: HPC rats froze less than SHAM rats on Conditioning trial 2 (*). D) 15-day context discrimination test: Both groups expressed accurate context discrimination, freezing more in paired (shock) than novel contexts.

Discussion

Contemporary views of the HPC assert that precise memories, like those required for accurate context discrimination in rats and episodic memory in humans, always depend on the HPC (Sekeres et al., 2017; Squire et al., 2015). Over time, highly-detailed memory (i.e., context-specific and hippocampal-dependent) is consolidated into semantic memory – a gist-like, schematic version supported by non-hippocampal networks (e.g., neocortex). On

this view, a schematic version of contextual fear memory can be expressed independently of the HPC, but it lacks the precision necessary to support accurate context discrimination behaviour. In contrast to traditional consolidation-based theories, a key prediction of the transformation hypothesis asserts both versions of memory for a contextual fear conditioning episode can be represented in the brain simultaneously by the HPC and non-hippocampal networks (Sekeres et al., 2017; Winocur, Frankland, Sekeres, Fogel, & Moscovitch, 2009; Winocur et al., 2013). It logically follows that when the HPC is damaged before learning, only the schematic, less-detailed version of contextual fear memory can be acquired and accurate context discriminations should not be possible. The present experiments tested these ideas which are common to systems consolidation-based theories of memory.

We found that near-complete anterograde hippocampal damage did not impair the acquisition, maintenance, or behavioural expression of contextual fear memory, nor context discrimination at any retention interval (1, 7, or 15 d). Additionally, HPC group rats' discrimination accuracy was unaffected by test sequence. These findings add to work that suggests non-hippocampal networks can support context memory with a substantial degree of detail and express appropriate memory-guided discriminative behaviours without a functional HPC (Lee et al., 2017; Lehmann et al., 2009; Wiltgen et al., 2006). The idea that the precision of contextual fear memory and subsequent accuracy of context discrimination always depends on the HPC is not supported by these experimental outcomes. Results from the behavioural lesion verification experiment confirm that damage to the HPC severely disrupted normal function, as profound impairments in the spatial cue version of the MWT were evident. We consider the MWT to be a good indicator of complete HPC dysfunction, because lesioned rats with as little as 26% residual HPC learn and remember the task

normally (Moser, Moser, Forrest, Andersen, & Morris, 1995). It is unlikely the ability to discriminate between the paired and novel context exhibited by groups of HPC rats in separate experiments was due to spared hippocampal function (means of 90.99% and 87.04%). Key data from the primary experiment were also replicated, with the HPC group demonstrating accurate context discrimination 15 d after the conditioning episode. Taken together, the absence of fear generalization in HPC rats must mean that non-hippocampal networks acquired, maintained, and expressed memory for the conditioning episode with sufficient detail to generate appropriate context-specific behaviours.

As confirmed by statistically different mean freezing values, the SHAM subgroups discriminated between the shock and novel contexts at 1, 7, and 15 d retention intervals. An additional experimental factor was the counterbalanced test sequence at every retention interval, as this procedure tested a prediction of the transformation hypothesis. Namely, the precision and hippocampal-dependence of context memory can be reestablished with a brief reminder at time points associated with generalization and predominance of the gist-like, non-hippocampal version (Winocur et al., 2009; see also, Jasnow et al. 2016). Indeed, the temporal order of the memory tests may have affected context discrimination, but not in a manner consistent with a reinstatement of detail in the memory for the shock context. Subgroups of SHAM rats tested on the shock-to-novel sequence appeared to exhibit less-accurate discrimination behaviour, especially at 7 and 15 d retention intervals. At minimum, reexposure to the paired context 6 h prior to novel context exposure did not improve discrimination performance. This despite minimal cue overlap between the shock and novel contexts, thus the putative ease (i.e., low-to-medium ambiguity) of the discrimination. In contrast, SHAM rats tested in the novel context first exhibited accurate discrimination in both experiments. This pattern of memory-guided behaviour seems

incompatible with consolidation theories and the transformation hypothesis (Table 3.3.). Winocur and colleagues' (2013) prediction that a reminder skews subsequent context memory retrievals to the detailed representation of the conditioning episode is not supported by these data. The opposite trend, quite possibly a confluence of factors or different effect, was observed. Notably, systems consolidation-based theories focus solely on mnemonic aspects of contextually-conditioned fear, however emotional and neuromodulatory mechanisms also contribute to generalized fear (Maren, Phan, & Liberzon, 2013).

In contrast to the present findings, a few studies in rodents have provided indirect support for systems consolidation. In one such study, Wiltgen and colleagues (2010) infused an AMPAR antagonist into the dHPC of fear conditioned mice before a memory retention test 1 d, or 28 d after learning and observed a reduction of freezing on the recent, but not remote tests. In a separate experiment in the same study, mice were implanted with chronic dHPC cannulae, then received fear conditioning (context A). Mice were tested for conditioned freezing in a novel context (context B) fourteen days later, then separated into two groups based on freezing scores in each context (e.g., a discrimination ratio) (Wiltgen et al., 2010). One day later mice were tested for conditioned fear in context A after bilateral dHPC infusions of AMPAR antagonist or saline. Pharmacological disruption of dHPC function reduced freezing in mice that previously discriminated between contexts, whereas mice that generalized were unaffected and exhibited high-levels of freezing (Wiltgen et al., 2010). The findings suggest a selective role of the HPC in detailed contextual memory retrieval. In addition, generalized context memories, presumably containing less detail, were retrieved without the HPC (Wiltgen et al., 2010).

Table 3.3. Predictions of contemporary views of the HPC and present context discrimination outcomes in SHAM and HPC rats. Rats with extensive damage to the HPC exhibited accurate context discrimination ability at all retention intervals and were unaffected by test sequence. On average, SHAM rats exhibited context discrimination ability, but accuracy may have been affected by test sequence – the shock-to-novel test sequence resulted in context discrimination that was not statistically above chance at all retention interval. In contrast, SHAM rats tested with the novel-to-shock sequence exhibited accurate discrimination at all retention intervals. Notably, the low-to-medium ambiguity of the context discrimination did not result in superior performance by SHAM rats.

	Prediction	Experimental outcome
HPC Memory	-Precise ✅	-Intact rats exhibit context discrimination.
	-Needed for accurate context discrimination ✖	-Falsified. Rats with HPC damage exhibit accurate context discrimination for up to 15 days.
	-Detail restored with reminder ✖	-Intact rats discriminated less accurately when tested in conditioning context first.
	-Transformation ─?─	-Unclear. If detailed memory was transformed, the schematic version was expressed even after reminder.
	-Generalization due to transformation ─?─	-Rats with HPC damage did not generalize despite only having access to schematic memory.
Non-HPC Memory	-Schematic ─?─	-Unclear, but non-HPC context memory is not always devoid of detail, as it flexibly guides appropriate context-specific behaviour.
	-Cannot support accurate context discrimination ✖	-Falsified.

As discussed at length in Chapter Two, incomplete disruption of hippocampal function can result in heterogeneous and unpredictable effects on memory-guided behaviours. Rather than invoking a covert systems consolidation process, a more parsimonious explanation could involve the decay of learning-induced synaptic potentiation in the HPC leading to generalization at 14 d after conditioning, and incomplete (dHPC only) inactivation leading to heterogeneous effects on memory tests one day later. Previous work in our laboratory illustrates that if the HPC is functional during a contextual fear conditioning episode, temporary pharmacological inactivation before retention testing can cause unpredictable effects, even on non-discriminative tests (Sparks, Lehmann, & Sutherland, 2011)[12].

Wang and colleagues (2009) conditioned groups of mice using a contextual fear discrimination procedure, then performed HPC lesions 1-day, or 42-days later. The authors reported no evidence of retrograde amnesia for the conditioned context and intact context discrimination at recent and remote time points. It was concluded that the HPC is not always required for the expression of detailed context memories. Again, the authors did not explicitly describe any lesion quantification procedures, only that the HPC damage was near complete (Wang et al., 2009). In this case, the discriminative training procedure; repeated experience in the paired and unpaired contexts before HPC damage, may have yielded sufficiently-detailed memories for each context in non-hippocampal networks. Interestingly, a similar procedure resulted in retrograde amnesia for context discrimination in a study by Lee and colleagues (2017) and the opposite result was found using a non-discriminative learning procedure involving repeated exposures to a paired context, then

---

[12] Notably, Sparks and colleagues (2011) found little evidence of RA when the HPC was pharmacologically inactivated 24 h following conditioning.

HPC lesions, then a fear generalization test (Lehmann et al., 2009). Lastly, Lee and colleagues (2017) also found that rats with extensive anterograde hippocampal damage could resolve context discriminations as well as controls under weak conditioning parameters. This suggests that non-hippocampal networks encode and retrieve context memories more efficiently than purported by others (Fanselow, 2009; Wiltgen et al., 2006).

In summary, present findings indicate that neither the longevity, nor the precision of contextual memories always depend on the HPC in the anterograde direction. Notably, the discrimination used here is considered low-to-medium ambiguity with regard to cue overlap. As such, it is unclear why SHAM rats exhibited a trend toward less-accurate discrimination over time when compared to HPC rats. One possibility is that the time-degraded memory trace coupled with hippocampal pattern completion processes resulted in an increase in freezing to the novel context over time. Regardless, the novelty of these findings rests with the disconfirmed prediction that non-hippocampal networks only support memories that are contextually-impoverished.

Chapter 4

Remote Object Discrimination Memory Without the Hippocampus

Introduction

The involvement of the HPC in visual discriminations is unresolved despite considerable empirical study. Rats with hippocampal damage can resolve two-choice visual discriminations in a variety of behavioural tasks (Alvarado & Rudy, 1995; Driscoll, Howard, Prusky, Rudy, & Sutherland, 2005; Epp et al., 2008; McDonald & White, 1993; Mumby, Astur, Weisend, & Sutherland, 1999; Sutherland, McDonald, Hill, Rudy, 1989). When the absence of learning impairments is considered alone, one might conclude the HPC is not required for visual discriminations. In contrast, when hippocampal damage follows learning, both varying degrees of RA and intact memory have been reported (Broadbent, Squire, & Clark, 2007; Driscoll et al., 2005; Epp et al., 2008; Lehmann, Glenn, & Mumby, 2007; Mumby et al., 1999; Sara, 1981). Even though rats with and without hippocampal damage appear to learn visual discriminations equally, some data suggest the memory exhibits rapid decay when supported by non-hippocampal networks (Broadbent et al., 2007; Gulbrandsen, Zelinski, Gidyk, McDonald, & Sutherland, 2012; Vnek & Rothblat, 1996; Wiig et al., 1996). The reliable pattern of spared learning and sporadic occurrence of impaired retention for visual discriminations is reminiscent of the dissociable anterograde and retrograde effects of hippocampal damage on contextual fear memory (Lee et al., 2016). This despite fundamental differences in: 1) the learning requirements for each task (Gruber & McDonald, 2012; McDonald & Hong, 2013; McDonald et al., 2007); 2) differences between the type and underlying neuroanatomy of each memory (Devan, Hong, & McDonald, 2011; Mishkin & Petri, 1984; Squire, Knowlton, & Musen, 1993; Squire, 2004); 3) different views on whether the HPC is required for consolidation and longevity

of the memory (Lehmann et al., 2007; Sutherland & Lehmann, 2011; Wiltgen & Tanaka, 2013).

In rats, the ability to resolve visual discriminations can be acquired incrementally through instrumental stimulus-response (S-R) associative learning (Thorndike, 1912, 1932). In its basic form, S-R learning requires an association between a specific sensory stimulus and an overt behaviour, which is strengthened through repeated reinforcement (Devan et al., 2011; Gruber & McDonald, 2012; McDonald & White, 1993; White, 2009; Yin & Knowlton, 2006). A well-learned S-R association is defined as procedural (habit) memory[13] - a type of memory characterized as inflexible, habitual, anoetic, consolidated independently of the HPC (Squire et al., 1993; Squire, 1992a; Tulving, 1985), and dependent on the dorsolateral striatum (DLS; Devan et al., 2011; Gruber & McDonald, 2012; McDonald et al., 2017). In contrast, contextual memory requires conjunctive stimulus-stimulus (S-S) associations, is defined as explicitly expressed, flexible, and dependent on the HPC for consolidation, thus longevity (Hirsh, 1974; O'Reilly & Rudy, 2001; Rudy, 2009; Squire & Wixted, 2011; Zelikowsky et al., 2012)(see also, Lee et al., 2016; Sutherland & Lehmann, 2011). Contemporary views assert that memory for visual discriminations (S-R habit) and contexts (explicit memory) are fundamentally distinct forms of memory supported by different neural networks (Mishkin & Petri, 1984; Squire, Knowlton, & Musen, 1993a; Squire, 1992a). Despite its importance, this framework cannot explain observations of retrograde amnesia for visual discriminations after hippocampal

---

[13] Procedural memory is non-declarative and is acquired incrementally as function of reinforcement. Unlike declarative memory, procedural memory is expressed implicitly, habitual, or reflexive, rather than cognitive and flexible in nature. Textbook accounts of memory belie the complexity of S-R behaviours (Devan et al., 2011; Gruber & McDonald, 2012; McDonald et al., 2017).

damage (Broadbent et al., 2007; Driscoll et al., 2005; Epp et al., 2008). Despite strong theoretical consideration to the contrary, the weight of empirical evidence collectively indicates that this form of S-R learning may not be independent of the hippocampus.

A central feature of instrumental S-R learning is that it proceeds incrementally – often requiring 60 – 200 (or more) trials distributed over several days (or weeks) to achieve accurate memory-guided performance (Broadbent et al., 2007; Driscoll et al., 2005; Epp et al., 2008; McDonald, King, & Hong, 2001; Mumby et al., 1999). The iterative and distributed learning does not result in memory that invariably survives HPC damage (Broadbent et al., 2007; Driscoll et al., 2005; Epp et al., 2008). Perhaps counterintuitively, if contextual fear conditioning episodes are distributed in a similar manner (e.g., 11 episodes over 6 days), or an established memory for one conditioning episode is repeatedly recalled (10 brief reminders over 5 days), both cases result in preserved behavioural expression of memory after hippocampal damage (i.e., no RA) (Lehmann & McNamara, 2011; Lehmann et al., 2009). The general idea here is that memory strength is modulated by repetition/rehearsal, and increased memory strength is correlated with increased longevity of memory (Ebbinghaus, 1885). Whether memory is consolidated and strengthened via putative systems-level mechanisms involving the HPC (McClelland et al., 1995; Skelin et al., 2018), or an S-R habit is strengthened by distributed learning over many trials like in visual discrimination tasks, contemporary views predict either case will result in memory that is less-susceptible to forgetting and hippocampal damage (Axmacher & Rasch, 2017). Again, this is not always the case and no consensus on the role of the HPC in visual discriminations exists.

Among studies that investigated hippocampal involvement in visual discriminations, nearly all employed substantially different experimental designs and

procedures. For example, some studies utilized concurrent, serial, and/or interleaved training, testing, and retraining procedures on 3 − 5 pairwise object discriminations (Lehmann et al., 2007; Mumby et al., 1999). These procedures might preclude conclusions about what was learned, remembered, forgotten, or relearned (Lehmann et al., 2007). Some studies did not systematically quantify or report extent of hippocampal damage (Mumby et al., 1999; Wible, Shiber, & Olton, 1992), or only damaged the dHPC (Vnek & Rothblat, 1996) (see also, Chapter 2.) A few studies did not adequately control for the possibility of multiple solutions to object discrimination problems (i.e., olfaction, behavioural strategy) (Broadbent et al., 2007; Mumby et al., 1999; Vnek & Rothblat, 1996), or employed low learning criterion (e.g., 80%, Broadbent et al. 2007; Lehmann et al., 2007). Some reports contain self-inconsistent data that obscures any one conclusion[14] (Broadbent et al., 2007; Mumby et al., 1999). Procedural and interpretive issues aside, one trend has emerged from visual discrimination studies in the rat: picture discriminations and object discriminations do not exhibit identical properties in the presence and absence of the HPC. Whereas most reported cases of RA involve pictures or other simple visual stimuli (Driscoll et al., 2005; Epp et al., 2008; Sara, 1981), cases of intact memory, variable RA, and memory decay, involve objects as the discriminative memoranda (Broadbent et al., 2007; Lehmann, et al., 2007; Mumby et al., 1999; Vnek & Rothblat, 1996). To summarize, uncertainty about the HPC and visual discriminations might be due to findings from object discrimination studies, rather than visual discrimination studies involving more simple stimuli like pictures.

---

[14] Data from Broadbent (2007) are more representative of a minor retrograde memory impairment than RA for a preoperative object discrimination. Data from Mumby (1999) illustrate varying degrees of retrograde impairments, ranging from complete RA to intact memory, and substantial relearning on various object discrimination problems.

Following these ideas, it is prudent to revisit the question of whether the longevity of memory for an object discrimination differs when the HPC is intact versus non-functional during learning. To test this question rats with and without extensive hippocampal damage were trained on an instrumental two-choice object discrimination task, then tested for memory retention 3, 10, or 30 d later. The retention intervals were chosen based on previous demonstrations of minor memory decay ~ 14 d after learning (Broadbent et al., 2007) and profound memory decay after ~ 20 d (Vnek & Rothblat, 1996) in rats with damage to the HPC. To my knowledge, the 30 d retention interval used here was among the longest tested in the rat. Procedures from previous object discrimination studies which might obscure experimental outcomes were addressed: 1) the extent of HPC damage was maximized to lessen the chance of intact hippocampal function at the time of learning; 2) equivalent amounts of training on one object discrimination problem was given to all rats to minimize individual effects of under-training, over-training, and concurrent/sequential training on multiple discriminations; 3) a stringent learning criterion was set to eliminate the use of behavioural strategy (e.g., win-shift, etc.); 4) a single-session memory test was used at each retention interval, including a first-trial success measure to eliminate potential relearning confounds; 5) olfactory cues were eliminated before every trial. These adaptations to commonly-used procedures were implemented to reduce the possibility of alternative solutions to the discrimination and facilitate unambiguous conclusions about the longevity of object discriminations in rats with and without the HPC.

Methods

Subjects

Forty-eight male Long–Evans hooded rats (Charles River, NC, USA), weighing 350 - 500 g at the time of surgery were housed in pairs in standard shoe-box acrylic cages (Allentown, Inc., NJ, USA) in the University of Lethbridge rodent vivarium (20°C, 50% relative humidity). A 12 h light/dark schedule was maintained, with lights on at 7:30 am daily. All experimental work occurred during the 12 h light period. Access to water was *ad libitum* upon arrival until endpoint. Access to food was *ad libitum* until after the surgical recovery period, at which time food was controlled by the experimenter. Rats were handled for several minutes each day for 5 d prior to surgery. All experimental procedures adhered to CCAC policy and were approved by the University of Lethbridge Animal Welfare Committee.

Surgeries

All procedures for 16-site HPC lesions and SHAM surgeries were identical to those employed in Chapter 2., except the A/P coordinate for the most rostral bilateral drill site (HPC site 1) was changed from -2.6 mm to -2.4 mm relative to bregma (Chapter 2., Methods section; Table 2.1. and Figure 2.1.B.). All animals recovered from surgery in home cages for 10 d prior to commencement of food restriction and visual discrimination training.

Food Restriction

Following the surgical recovery period, rats received food once a day (~ 4.0 g rat chow / 100 g bodyweight) until weights reached ~ 95% of free-feeding values. The food reward

(sweetened cereal; President's Choice® Toasted Oat Os, Loblaw Companies Ltd, Canada) was introduced to rats in their home cages during the same 3 d period. Food restriction continued throughout the experiment until endpoint, with bodyweights monitored daily and allowed to gradually increase by approximately 5 g each week during the various intervals between training and testing. No sweetened cereal was given in home cages once training began. Rats received their daily allotment of food between 5:30 pm and 7:30 pm, which corresponded to 12 to 14 h before daily visual discrimination training sessions. Food restriction procedures were carefully designed to ensure motivation at the time of training/testing rather than to drastically reduce bodyweight.

Dry Land Visual Discrimination Task

The instrumental object discrimination task could be solved independent of the spatial location of the objects, other sensory cues (e.g., olfaction), and information about the training context itself. Simply, rats had to discriminate between two objects in a rectangular arena. Each object was set on a reward dish and a reward was delivered when the correct object was moved from its position. Displacement of one object was always rewarded (S+), while displacement of the other was never rewarded (S-), regardless of which dish the objects were covering.

Apparatus

Pre-training, training, and testing took place in a custom-designed arena constructed from corrugated white plastic, with inner dimensions measuring; 90 cm x 60 cm x 45 cm. The two-layered floor consisted of a removable insert resting atop the arena ultrastructure. The removable insert had two circular depressions cut into it, which secured reward dishes (2.54

cm diameter) in place during trials, and allowed the dishes to be removed and cleaned between trials. The dishes were designed so objects could be placed on them by the experimenter before trials and displaced by rats during trials. The back wall of the box housed plastic tubes (2 cm diameter) positioned for manual delivery of reward to the dishes. A divider (15 cm) protruded from the center point of the back wall at a 90° angle to deter rats from displacing both objects simultaneously (Figure 4.1.). The arena and its features were adapted from various sources (Broadbent et al., 2007; Epp et al., 2008; Mumby et al., 1999).
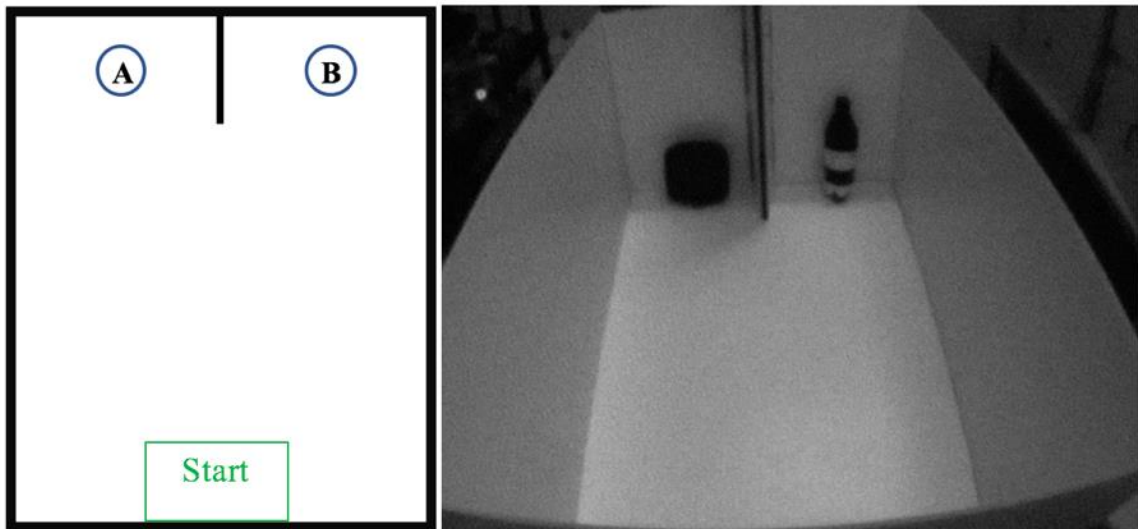
Figure 4.1. Simple 2-D schematic and still-frame capture of the dry land visual discrimination task arena. (Left) Line drawing of the dry land visual discrimination arena, with reward dishes "A" and "B" and start position labelled. (Right) Picture of the arena with the objects balanced on dishes before a trial (rectangle on A; cylinder on B).

Phase I (pre-training/shaping)

Each rat was exposed to the arena prior to commencement of visual discrimination training. Daily pre-training sessions lasted 10 min and were designed to gradually shape rats' behaviour in a uniform manner. On day one rats were free to explore the arena, objects and

reward dishes. Food reward was available in the dishes and throughout the box. The objects were not covering the food dishes. All rats explored the arena, reward dishes, objects, and consumed food reward. On day two the reward was only available in the dishes and the objects were not covering the dishes. All rats consumed reward from both dishes. On the final day of pre-training the reward was in the dishes (one piece in each dish) with the objects set on the dishes. When rats moved an object to access and consume a reward, the reward and object were replaced by the experimenter. All rats moved objects off the dishes repeatedly to access reward. All rats learned the general requirements of the task by the end of Phase I (i.e., remove an object to receive a reward).

Phase II (training)

Rats received 10 trials a day until 2 consecutive days of 90% correct were achieved. Pairs of rats were brought to the testing room in a shoe box cage and received trials sequentially, in an alternating manner. Correct and incorrect choices were recorded manually by the experimenter for each trial. The correct object (S+) and incorrect object (S-) were counterbalanced, so half of the HPC and SHAM rats learned each possible contingency (i.e., cylinder S+, rectangle S-; rectangle S+, cylinder S-). The position (dish A, or B) was varied pseudo-randomly, such that win-stay, or lose-switch behavioural strategies could not facilitate the 90% correct criterion. When employing either strategy the maximum correct% using an ABBABAABAB, or BAABABBABA design is 80% if a correct first choice is made and 70% if an incorrect first choice is made. Unlike in pre-training, the reward was not delivered until the correct object (S+) was displaced; therefore, rats could not simply locate the reward via olfaction and remove the object to access it. When a correct choice was made, the rat consumed the reward before being removed from the arena. When an

incorrect choice was made, no reward was delivered and the rat was removed from the arena. Thus, a trial began with the rat being placed in the start position within the arena and ended once the correct object had been displaced and the reward was delivered and consumed, or the incorrect object had been displaced and no reward was delivered (Figure 4.2.). Critically, the removable floor, walls, reward dishes, and objects were thoroughly cleaned with dilute quat-sanitizer between every trial to further ensure rats could not use olfaction to solve the discrimination. The inter-trial interval was variable, but estimated to be 2 or 3 minutes due to between-trial cleaning procedures. To my knowledge, the thorough cleaning of the arena, objects, and reward dishes before every trial is unique to this study, as all others referenced here reported cleaning between sessions, or did not specify cleaning procedures.

<center>Phase III (retention test)</center>

Rats were tested 3, 10, or 30 d after the final training session. The procedures for retention testing were identical to those in Phase II, with each rat receiving 10 trials on its previously-learned object discrimination. All rats remained on the food restriction schedule (one food allotment each day, 12 – 14 h before normal testing time) during their respective train-to-test intervals, with bodyweights and adjusted food allotments recorded twice daily to ensure uniformity of experimental methods and health of animals.
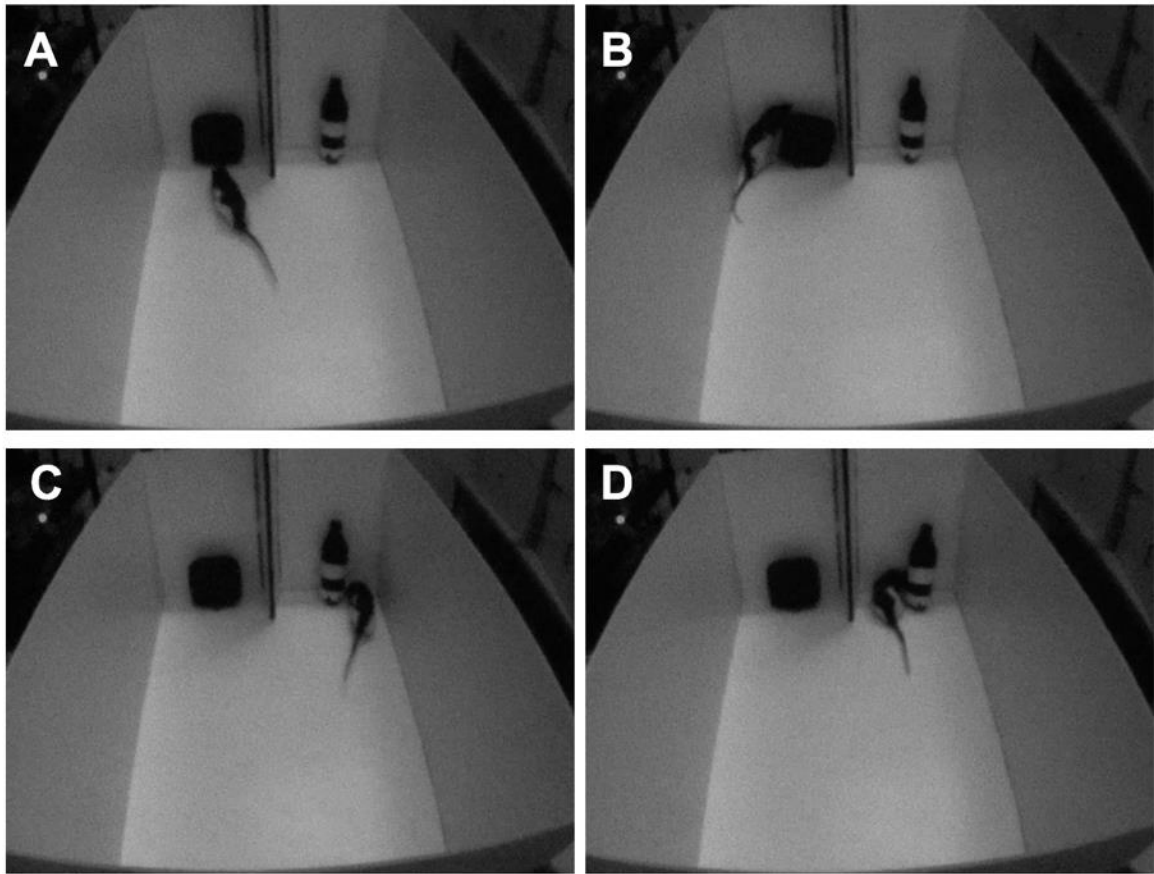
Figure 4.2. Still-frame video captures of a rat performing two object discrimination training trials (Phase II). A) Rat approaching incorrect object (rectangle S-). B) Rat displacing incorrect object (incorrect trial). C) Rat approaching correct object (cylinder S+); D) Rat displacing correct object (correct trial).

Endpoint, Histology, and Data Analysis

Animals were sacrificed via overdose of sodium pentobarbital (~ 300 mg/kg, i.p.) and perfused transcardially with 0.9% PBS, then 4% PFA in 0.9% 0.01 M PBS for tissue fixation. Brains were extracted and post-fixed for at least 24 h in fixative, then transferred to a cryoprotecting solution for at least 48 h (30% sucrose in 0.9% 0.01 M PBS and 0.02% sodium azide). Cryoprotected brains were frozen and sectioned in the coronal plane (40 μm) with every fourth section throughout the entire HPC mounted on microscope slides.

Sections were stained with cresyl violet and slides cover-slipped for quantification of HPC volumes.

Behavioural Data and HPC Lesion Volumes

Manually-recorded training trial data were expressed as Correct% for each session (#correct trials / #total trials x 100 = Correct%). Phase II training scores were analyzed using two-way ANOVA (Group; SHAM, HPC) with repeated measures (Training day). Phase III retention test scores were analyzed with two-way ANOVA (Group x Retention interval). All analyses performed in Prism 6®. Statistical analysis was not performed on the Phase III first trial success measure. The volume of intact HPC principle cell fields was quantified stereologically as described in Chapter 2. The same equipment and methods were used. All calculations that were performed to yield HPC% damage values were also identical to Chapter 2., except fewer SHAM brains ($n = 5$) were quantified to calculate the mean intact HPC volume.

Results

Object Discrimination Training and Memory Retention

As shown in Figure 4.3.A, SHAM and HPC rats learned the object discrimination equally. This was confirmed though analysis of Group (SHAM, HPC) by Training day (1, 2, 3, 4, 5, 6, 7), which revealed a main effect of Training day; $F(6, 276) = 364.20$, $p < 0.0001$. Neither a main effect of Group ($F(1, 46) = 0.1706$, $p = 0.6815$), nor an interaction was present ($F(6, 276) = 1.406$, $p = 0.2123$). For retention tests, a main effect of Retention interval (3, 10, 30 d) was found ($F(2, 42) = 4.054$, $p = 0.025$). Like the discrimination learning data, neither an effect of Group, nor an interaction was present ($F(1, 42) = 0.1386$,

$p = 0.7115$; $F(2, 42) = 0.6584$, $p = 0.5229$, respectively). Analysis of Retention interval data (*post-hoc*; Tukey) indicated a difference in mean Correct% between the 3 d and 30 d Retention intervals, $p = 0.024$; Figure 3.3.B. First trial success for individual SHAM and HPC rats indicated that mean Correct% values obtained during 10-trial retention sessions were an accurate measure of memory rather than relearning (Table 3.1.).
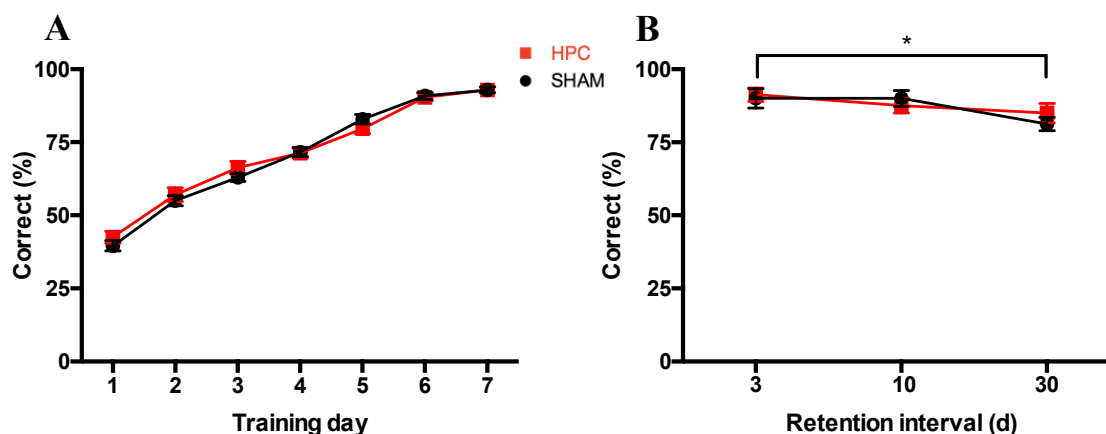
Figure 4.3. Object discrimination training and memory retention tests. A) Training: HPC and SHAM groups learned to discriminate S+ from S- to > 90 % accuracy by day 7. B) Retention tests: performance of HPC and SHAM groups was similar at each train-to-test interval. There was a small, but statistically significant decrease in memory performance exhibited by both groups between 3 and 30 d retention tests (HPC, 91.25 % - 85.00 %; SHAM, 90.00 % - 81.25 %).

HPC lesion volumes

As shown in Table 4.2., damage to the entire HPC ranged from extensive to near-complete (72.53% - 93.75%). Analysis of HPC % damage indicated similar means between retention intervals; $p = 0.36$ (ANOVA). Figure 4.4. (B - C) below shows two examples of extensive and selective HPC damage which are representative of typical (i.e., mean) and near-complete (largest) HPC lesion volumes corresponding to values in Table 4.2.

Table 4.1. First-trial memory probe: discrete measure of object discrimination retention for SHAM and HPC rats. Each green check denotes a correct first-trial choice for an individual rat. Each red X denotes an incorrect first-trial choice for an individual rat. Each retention interval; 3 d, 10 d, and 30 d contained 8 HPC and 8 SHAM rats. For example: all 8 SHAM rats chose the correct object on the first trial on the 3 d retention test and 7 of 8 HPC rats chose correctly.

	3 d	10 d	30 d
HPC	✓✓✓✓✓✓✓✗	✓✓✓✓✓✓✓✗	✓✓✓✓✓✓✗✗
SHAM	✓✓✓✓✓✓✓✓	✓✓✓✓✓✓✗✗	✓✓✓✓✓✓✗✗

Table 4.2. HPC lesion volumes by retention interval. Extent of damage to the HPC expressed as % of the entire HPC (83.92% mean for all HPC rats).

	HPC 3 Day	HPC 10 Day	HPC 30 Day
Smallest:	73.67%	72.53%	80.18%
Mean:	83.25%	82.35%	86.10%
Largest:	93.75%	91.74%	90.59%
SD:	7.50%	6.20%	3.22%
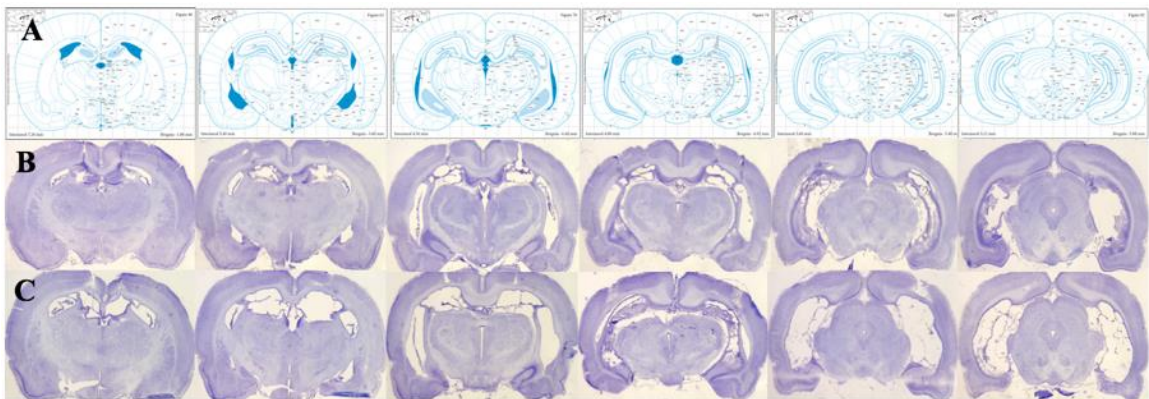
Figure 4.4. Photomicrographs of two HPC lesion brains with extensive damage. A) Stereotaxic atlas -1.80 mm to -5.88 mm relative to bregma (left to right). B) 83.25% damage indicative of a typical extensive HPC lesion. C) 91.74% damage indicative of an near-maximum HPC lesion.

Discussion

The present study found no detectable differences between intact and HPC damaged rats in visual discrimination learning (Driscoll et al., 2005; Epp et al., 2008; Lehmann et al., 2007; Mumby et al., 1999; but see, Mumby, Pinel, Kornecook, Shen, & Redila, 1995). Collectively, these data indicate that if the HPC is not functional during learning, non-hippocampal networks support visual discriminations, apparently normally. Although other networks support learning when the HPC is damaged, this does not preclude the possibility that hippocampal function is required for certain properties of the memory. Our study investigated this possibility. Namely, whether the longevity of memory for an object discrimination is affected when the HPC is nonfunctional at the time of learning. Rats exhibited intact behavioural expression of memory at short and intermediate retention intervals (3 and 10 d), and minor memory decay at 30 d (Figure 4.2.B). Importantly, HPC rats performed similarly to SHAM rats on all object discrimination tests, including the first trial success measure (Table 4.1.), a highly-sensitive measure that eliminates the possibility of conflating relearning with memory retention. The intact behavioural expression of memory exhibited by HPC rats, even after a long delay, unambiguously demonstrates that non-hippocampal networks encode, maintain, and retrieve memory for object discriminations when the HPC is nonfunctional at the time of learning. Taken at face value, the findings support fundamental ideas on the organization of learning and memory in the mammalian brain (Squire et al., 1993a; Squire, 1992a; White & McDonald, 2002; Zola-Morgan & Squire, 1993) – the key structures and networks that support instrumental visual discrimination learning and resultant S-R habits are anatomically and functionally independent of those required for explicit memory expression. However, this conclusion

becomes less coherent when present findings are discussed within the larger context of the visual discrimination experimental literature.

Reconciling examples of RA for picture discriminations after HPC damage (Driscoll et al., 2005; Epp et al., 2008) with varying degrees of intact object discriminations (this study, Lehmann et al., 2007; Mumby et al. 1999) is challenging because the findings belie a single explanation that is consistent with contemporary views of memory. As described earlier, the crux of the problem lies in the *consistent* lack of anterograde impairments and *sporadic* occurrence and variable extent of RA for visual discriminations – this is also the case for other memory-guided behaviours (McDonald et al., 2017; Lee et al., 2016; Sutherland & Lehmann, 2011; Sutherland et al., 2010). To reiterate, memory impairments caused by perturbations of normal brain function are typically attributed to *a priori* differences in: memory type, age/consolidation, or strength (Axmacher & Rasch, 2017; McGaugh, 1999; Müller & Pilzecker, 1900; Ribot, 1881; Squire & Wixted, 2011), and memory content/detail (Rudy, 2009; Wiltgen & Tanaka, 2013; Winocur, Moscovitch, et al., 2013). Just as normal object discrimination behaviour following pre- and post-learning hippocampal damage suggests the memory can be maintained, and expressed independent of the HPC (present study; Lehmann et al., 2007; Mumby et al., 1999), demonstrations of RA for 2-D picture discriminations after HPC damage suggest the opposite (Driscoll et al., 2005; Epp et al., 2008).

Consider two known cases of intact object discrimination after HPC damage (Lehmann, et al., 2007; Mumby et al., 1999). Mumby and colleagues (1999) trained rats to solve five pairwise object discriminations, one problem at a time sequentially. Learning to criterion (85 %) for each problem varied from 60 – 140 trials and each discrimination problem was trained at a planned time point prior to HPC or SHAM surgery (1 – 13 weeks,

Mumby et al. 1999). Despite extensive pre-surgical training, it was found that both HPC and control rats required many trials (35 to > 40) over more than one day to regain pre-surgical performance on the 13-week object discrimination (Mumby et al., 1999). In fact, performance on the first five trials was chance-level (50%), indicating that the memory had decayed significantly (Mumby et al., 1999). Performance on the other four object discriminations was better-than-chance during the first five trials. The extensive pre-surgical training, coupled with many post-surgical trials on several memory tests likely revealed intact memory, but also memory decay, re-learning, and possibly memory interference (Lehmann et al., 2007). Most importantly, HPC rats performed similarly to controls on all memory tests, which implies hippocampal damage did not differentially affect performance (Mumby et al., 1999).

Lehmann and colleagues (2007) employed a more constrained learning paradigm involving two concurrent pairwise object discrimination problems acquired in 65 -79 trials (each) in one day 72 h prior to extensive hippocampal damage (~ 75%) or SHAM surgery. The same rats learned another object discrimination and a reversal of one of the 72 h discriminations 1 h prior to surgery. Despite receiving massed, concurrent, and considerably less training compared to the Mumby (1999) study, HPC rats performed similarly to controls on all preoperative discrimination problems, averaging ~ 80 % correct choices on 20 trials over 2 days under a non-rewarded testing procedure that was interleaved with rewarded trials on a new discrimination (Lehmann, et al., 2007). Taken together with Mumby and colleagues (1999), these findings suggest the following learning features did not differentially affect object discrimination performance after HPC damage: massed versus distributed learning, number of learning trials, learning-to-surgery interval

(1 h – 13 weeks)[15], or concurrent versus sequential learning of multiple object discriminations.

Now consider cases of RA for picture discriminations after damage to the HPC (Driscoll et al., 2005; Epp et al., 2008). Both studies required rats to resolve pairwise picture discriminations in a trapezoidal pool by swimming toward the correct picture and escape via a submerged platform. Intact rats learned to discriminate between 2-D images in a range of 90 – 200 trials and were assigned to HPC lesion or SHAM conditions. During retention testing HPC rats exhibited profound RA for the preoperative picture discriminations (Driscoll et al., 2005; Epp et al., 2008). Importantly, these studies revealed that nethier the number of learning trials, nor the learning-to-surgery interval affected memory retention, as RA was profound[16] (Driscoll et al. 2005; Epp et al., 2008). The findings suggest the HPC is required for recall of picture discriminations if present during learning – the opposite of object discriminations (Lehmann et al., 2007; Mumby et al., 1999). Barring the addition of *ad hoc* hypotheses, it is unclear if any view on memory and the HPC can account for findings from object and picture discrimination studies (but see, Lee et al., 2016; McDonald et al., 2017) – findings that might be better explained in terms of the mnemonic requirements and features of each task.

Indeed, one explanation for the different effects of hippocampal damage on object and picture discriminations involves the tasks themselves. It is unclear why visual discriminations involving substantially different memoranda (S) and overt behavioural

---

[15] Mumby and colleagues (1999) found significant deficits for the object discrimination learned 13 weeks prior to surgery in HPC rats. This was likely due to memory decay rather than RA, as SHAM rats exhibited the same decrease in performance.

[16] Driscoll and colleagues (2005) observed intact memory performance for an *over-trained* picture discrimination problem after HPC damage. Rats were trained for ~ 200 trials over nine days and the memory survived HPC damage.

responses (R) would be expected to result in learned associations and memories that exhibit identical properties under the umbrella terms; *visual discriminations* or *S-R habits*. For example, brightness-shock avoidance associations (S (black/white) – R (run, escape)) (Sara, 1981), picture-escape associations (S (2-D pictures) – R (swim/approach, escape)) (Driscoll et al., 2005; Epp et al., 2008; Gulbrandsen, et al. 2012), and object-reward associations (S (3-D objects) – R (approach/displace, reward)) (Broadbent et al., 2007; Lehmann, et al., 2007; Mumby et al., 1999; Vnek & Rothblat, 1996), likely involve memory representations of varying complexity (Devan et al., 2011; Gruber & McDonald, 2012; McDonald et al., 2017). Following this idea, a possible relationship exists between cases of RA for simple visual memoranda and instrumental responses (e.g., picture-approach/escape) – and the inverse (no RA, or minor retention deficits) for more complex memoranda and responses (3-D objects-approach/displace/reward). The empirical evidence suggests not all S-R associations exhibit the same susceptibility to hippocampal damage and the difference might be related to the available compliment of sensory cues (e.g., polymodal vs. unimodal), the complexity of the behavioural response, and the associative structure that results (e.g., simple S-R, Thorndike, 1912; complex S-S, Tolman, 1948; and "higher-order" (S-S)-R, Devan et al. 2011; McDonald et al. 2017). At minimum, it seems that visual discrimination memories involving pictures and objects exhibit different properties in the presence and absence of the HPC. The differences appear less-related to discrete learning parameters, like number of learning trials or massed/distributed training sessions, than to the task itself. Contrary to contemporary views, as the complexity of the associations increase, the retrograde amnestic effects of HPC damage appear less severe.

The general perspective offered here is in the absence of hippocampal function, the properties of learning and memory are not necessarily identical for all tasks, including those

assumed to involve a defined type of learning and memory. Stated another way, greater recruitment of distributed sensory and associative regions during learning – i.e., greater depth of information processing requiring greater representational complexity – depending on the task, might result in memory after few iterations in the anterograde direction (HPC damage before learning). This appears to be the case for object discriminations as compared to picture discriminations, and contextual fear memory (Chapter 2), which require the integration of polymodal stimuli during learning. To my knowledge, no peer-reviewed study to date has employed an exclusively anterograde design involving picture discriminations. All known studies to investigate picture discrimination learning in the absence of the HPC involved pre-surgical learning, HPC damage, post-surgical testing, then relearning (Driscoll et al., 2005; Epp et al., 2008). However, some findings from picture discrimination studies are consistent with the ideas presented here. For example, the number of trials required for picture discriminations to become resistant to HPC damage and RA is much larger (~ 200 trials over 9 days) (Driscoll et al., 2005) than for object discriminations (~ 80 in one day) (Lehmann et al., 2007). Whereas preoperative object discriminations require few trials to be relearned in cases of incomplete RA or memory decay (Mumby et al., 1999), some data suggest picture discriminations are relearned more slowly (Epp et al., 2008).

In summary, rats with extensive to near-complete hippocampal damage learned object discriminations and maintained the memory for 30 d. When interpreted in the larger context of the visual discrimination literature the findings suggest: 1) visual discriminations involving objects and pictures exhibit different properties in rats with HPC damage; 2) task-related representational complexity might predict the properties of memory for visual discriminations better than taxonomic (S-R habit), or neuroanatomical classifications (HPC

dependent/independent); 3) the HPC is not always required for the behavioural expression of memory-guided discriminations involving polymodal stimuli, even after long train-to-test intervals. Further study is clearly needed to determine the boundary conditions of learning and memory for visual discriminations across tasks, in both the anterograde and retrograde direction, in the presence and absence of hippocampal function. Moreover, continued investigation into the mnemonic capacities of non-hippocampal networks may help resolve recurrent theoretical questions about memory consolidation, its dependence on the HPC, and its importance to the longevity of memory.

Chapter 5

"Depending on who you ask, either the hippocampus does everything, or it does nothing."

–Roger Jardin (personal communication)

Conclusions

The goal of my thesis was to examine the properties of long-term memory in rats with and without a functional HPC. The present experiments demonstrate that rats with extensive hippocampal damage acquired long-term memories with sufficient detail and stability to facilitate appropriate memory-guided behaviours over time. These findings are difficult to reconcile with certain views of memory and hippocampus, and systems-level consolidation in particular, which predict that the longevity and precision of long-term memory both require the HPC. My intent is not to ignore, or minimize the many known hippocampal contributions to learning and memory, including within allocentric spatial, temporal, and relational domains (Lee et al., 2016). Rather, I offer the perspective that observations of anterograde amnesia after hippocampal damage are best explained by the absence of unique hippocampal information processing functions, not dysfunctional systems consolidation mechanisms. Similarly, when a memory can be acquired and expressed by rats with hippocampal damage, it does not mean the HPC is uninvolved in the memory when it is present, nor does it mean memories are equivalent in the presence and absence of the HPC. Instead, it reveals that cortical networks can acquire and maintain similar information about experiences and generate task-appropriate memory-guided behaviours under a variety of conditions not predicted by systems consolidation-based theories of memory. The remainder of this chapter focuses on what I believe are the main conclusions to be gleaned from this work when interpreted through an emerging view on the organization of learning and memory.

Heterarchic Reinstatement Theory

Present findings provide indirect support for an emerging view of memory organization. Members of our group recently developed a theory – Heterarchic Reinstatement Theory (HR), which posits a dynamic hierarchical organization of long-term memory networks to account for the anterograde/retrograde dissociation across tasks following hippocampal damage (Lee et al., 2016; see also, McDonald et al., 2017). The basic assumption of HR is that high-order associative regions which receive convergent afferents and send distributed efferents to many regions (often reciprocally) strongly influence memory network dynamics and behavioural output (Lee et al., 2016). Such central non-hippocampal (neocortical) regions likely include: retrosplenial cortex (Cowansage et al., 2014; Gulbrandsen, 2015), the rhinal cortices (Eacott, 1998; Eacott & Norman, 2004) and parahippocampal regions in general (McDonald et al., 2017).

Like dual-system hierarchical views in which the HPC is situated conceptually at the apex of a pyramid (Marr, 1971; McClelland et al., 1995; McNaughton, 2010), HR too assumes global memory network activity is strongly-influence by top-down cortico-hippocampal output. When the HPC is functional during a learning episode, it acquires a code for the later reinstatement of the cortical activity state which corresponds to the experience (e.g., Teyler & DiScenna, 1986). Importantly, with very limited experience, top-down reinstatement of the entire distributed cortical representation requires the hippocampal code, regardless of the content of the memory (Lee et al., 2016). However, as experiential exposure increases in one or more dimension (e.g., Lewandowsky, Ecker, Farrell, & Brown, 2012), a greater proportion of the representational heterarchy necessarily becomes engaged. This may serve to increase the availability of multiple cognitive representations and their viability with regard to producing task-appropriate behaviours.

84

Subsequently and depending on task demands, the hippocampal code may no longer be required for top-down reinstatement of the relevant activity state and the generation of appropriate behaviours (Lee et al., 2016).

This model may account for the presence or absence of amnesia after hippocampal damage across learning and memory tasks better than poorly-understood systems consolidation mechanisms. For example, HR can explain the occurrence of retrograde amnesia for incrementally-acquired memory-guided behaviours involving unimodal sensory features (Driscoll et al., 2005; Epp et al., 2008); and similarly distributed experience supporting rich contextual memories and polymodal object discriminations that survive hippocampal damage (Lehmann et al., 2009; Lehmann et al., 2007; Lehmann & McNamara, 2011). Additionally, HR may explain anomalous cases of spared remote contextual fear memory after hippocampal damage better than systems consolidation-based accounts – as most of these studies involved a mixed conditioning paradigm (15 tone + shock pairings followed by context tests) and therefore greater engagement of non-hippocampal networks (e.g., Anagnostaras et al., 1999; Kim & Fanselow, 1992; Winocur, Sekeres, et al., 2013; but see, Sparks et al., 2013). For the heterarchic model to be valid, it must be applicable to cases of hippocampal damage before learning as well, thus the experimental outcomes of this thesis. I uncovered few if any differences in the maintenance and expression of memory-guided behaviours in the presence or absence of the HPC, which aligns well with the central framework of HR.

Non-Hippocampal Networks Support Stable Memories

Rats with hippocampal damage demonstated normal memory longevity in all present experiments. There was no evidence of rapid memory decay, nor a progressive impairment

in discriminative behaviour that could be attributed to lesion condition. In fact, hippocampal damage was not predictive of performance on any test; except on the MWT probe, which was planned and expected in order to verify hippocampal dysfunction. This leads me to two general conclusions: 1) hippocampal function is not always an essential feature of memory longevity; 2) anterograde damage to the rat HPC does not interrupt memory consolidation in other structures. It is clear from my experiments that asssociative structures outside the HPC possess intrinsic memory consolidation mechanisms (see, McDonald & Hong, 2013). Moreover, when rats without a HPC acquire memories, they appear equally persistent when compared to the version expressed by intact rats. This is incompatable with commonly-purported claims that memory exhibits rapid decay when supported by non-hippocampal networks due to impaired systems consolidation mechanisms (Squire & Wixted, 2011; Zelikowsky et al., 2012). It is more likely that anterograde amnesia after hippocampal damage in rats is due to the absence of information processing capabilities unique to the HPC. That is, there are a subset of cognitive processes that non-hippocampal networks may be incapable of supporting, even with extended training (see, Lee et al., 2016).

Why Do Rats With Hippocampal Damage Condition More Slowly?

Rats with damage to the HPC reliably exhibit less fear very early in conditioning compared to intact rats. I find this to be one of the most interesting results from this thesis. The deficit is transient and is no longer apparent after the second or third shock, but it occurred in every conditioning cohort in the present experiments (Figure 2.3.A., Figure 3.2.A., Figure 3.7.C.) and amazingly many others (Fanselow, 2009; Wiltgen et al., 2006; Zelikowsky et al., 2013, 2012). As discussed in Chapter 2, the reduction in conditioned responding is

commonly attributed to a difference in associative learning rates between the HPC and non-hippocampal networks. Alternatively, I propose that the difference in the magnitude of freezing reflects the time it takes lower levels of the heterarchy to form a contextual representation of the conditioning chamber in the absence of the HPC, rather than the time it takes to learn the chamber predicts foot-shocks (the context-US association). This subtle difference in interpretation may be supported by certain observations. First, there is evidence that non-hippocampal networks acquire contextual representations of environments incidentally in the absence of the HPC, albeit less-efficiently[17]. As elegantly demonstrated by the Fanselow laboratory, rats require a minimum amount of exploration time prior to a shock and immediate removal to overcome the immediate-shock deficit (Fanselow, 1990; Wiltgen et al., 2006). The deficit refers to the failure of a foot shock US to become associated with a contextual representation when presented simultaneously upon introduction to the conditioning context. Stated another way, rats need time to form a contextual representation and this must occur prior to the US for conditioning to occur (Matus-Amat, Higgins, Barrientos, & Rudy, 2004; Wiltgen et al., 2006). Interestingly, rats with hippocampal damage exhibit less absolute freezing than controls 24 h after a conditioning episode involving a 48 s placement-to-shock and immediate removal procedure, but like controls benefit from increased preexposure time prior to a single shock (Wiltgen et al., 2006). This demonstrates that some version of a contextual representation

---

[17] Incidental in the current discussion means prior to presentation of salient punctate stimuli (foot-shock US). There are several reports that suggest the HPC engages in incidental encoding of task-irrelevant contextual information (McDonald et al., 2001; McDonald, Ko, & Hong, 2002) and context-based disambiguation functions (Honey & Good, 1993; McDonald et al., 1997). The required conditions for non-hippocampal networks to engage in incidental memory encoding are relatively unexplored.

is formed incidentally by non-hippocampal networks which is then associated with the shock (Wiltgen et al., 2006).

A second example was discussed briefly in Chapter 3. Rats with extensive hippocampal damage can form contextual memories that are detailed enough to support equivalent discriminative place preference behaviour as compared to controls, even under extremely weak conditioning parameters (1 unsignaled shock) (Lee et al., 2017). In my view, these results altogether suggest that the minor, transient deficit in conditioning throughout the present experiments is not indicative of a slower learning rate *per se*, but rather a slightly slower developing representation of context by lower, non-hippocampal levels of the heterarchy. In fact, one line of evidence indicates that under standard contextual conditioning parameters, learning-tagged retrosplenial neuronal populations can reinstate a learned fear response when optogenetically stimulated, even when the HPC is non-functional (Cowansage et al., 2014). There are other examples of very similar learning rates in rats with and without a functional HPC, including Chapter 3 (Figure 3.1.A) and several others (Broadbent et al., 2007; Epp et al., 2008; Lehmann et al., 2007; but see, Packard, Hirsh, & White, 1989). At minimum, these examples suggest that non-hippocampal networks share similar fundamental associative functions with the HPC and that only challenging variants of learning and memory tasks are likely to reveal differences in anterograde memory processes in the absence of the HPC. However, I caution against following a more-of-the-same approach. Stated another way, it is unlikely that the number of elemental memoranda within an environmental context (i.e., memory "detail"), or mere task repetition (i.e., memory strength) are parametric variables, nor are they sufficient to explain how and when the HPC is required for learning and the behavioural expression of memory. There is considerable evidence regarding which tasks can and cannot be learned

and remembered by rats with hippocampal damage (see, Lee et al., 2016; McDonald et al. 2017), but no comprehensive account exists to explain all of the data.

Non-Hippocampal Networks Support Memory-Guided Discriminations

Contemporary views of the HPC posit that non-hippocampal networks cannot support accurate context discriminations (Wiltgen & Tanaka, 2013; Winocur & Moscovitch, 2011). This raises the question of whether the context discrimination in Chapter Three was difficult enough to require the HPC. There are several problems with what might be called a more-of-the-same approach. First, assuming that the compliment of environmental cues in a given context determines the detail of the contextual memory representation is not entirely logical. There is no objective way of knowing which and how many cues were included in the contextual memory, nor is there a way to know in advance which cues will be treated as identical (by a rat). Indeed, titrating the ambiguity of a conditioning and novel context may produce differences in discrimination accuracy in the presence and absence of the HPC (Antoniadis & McDonald, 1999; Antoniadis & McDonald, 2000), but perhaps not in a reliable or coherent pattern (Balog, 2016).

There are multiple dimensions which may contribute to memory discriminability (Lewandowsky et al., 2012) – the degree of overlap between stimuli in the chambers is only one. Furthermore, the low-to-medium ambiguity discrimination I employed should have been very easy for intact rats to resolve, yet on the 15 d test rats with hippocampal damage froze less than SHAM rats in the novel context. There is simply no evidence which can be gleaned from my data to support the idea that the memory for the conditioning episode was less detailed in animals with hippocampal damage. On a related caveat, the apparent decrease in discrimination accuracy in the SHAM group compared to the HPC group over

time might be a red herring – insofar that I cannot dissociate between the many mnemonic versus emotional factors that contribute to freezing behaviour values in my data sets. It is certain that hippocampal damage disrupts more than normal learning and memory processes. For example, the vHPC is a key part of the neurocircuitry that contributes to a myriad of affective processes, including normal and abnormal fear responses (Gouveia et al., 2019; Maren et al., 2013). This raises the possibility that the apparent generalization gradient in SHAM rats could be more affective/emotional than mnemonic in nature.

Why Are Object and Picture Discriminations Different?

The networks that support instrumental visual discrimination learning and resultant S-R habits are purported to be anatomically and functionally independent of the HPC (Hirsh, 1974; Mishkin & Petri, 1984). As discussed in Chapter 4, this is not always the case, as illustrated by the paradoxical effects of hippocampal damage on object and picture discriminations. Whereas both discriminations can be learned normally by rats with hippocampal damage, damaging the HPC after learning causes retrograde amnesia for picture, but not object discriminations (Epp et al., 2008; Lehmann et al., 2007; Mumby et al., 1999). According to the heterarchic model, there are a few clues as to why this might be the case. First, it takes a far greater number of trials to learn picture discriminations compared to object discriminations. This is the case for rats (Broadbent et al., 2007; Epp et al., 2008; Lehmann et al., 2007; Chapter 4), monkeys (Zimmermann & Hochberg, 1971), and amazingly New Zealand parrots (O'Hara, Huber, & Gajdon, 2015). Second, rats require extensive overtraining on picture discriminations for the memory to survive hippocampal damage (Driscoll et al., 2005), but object discriminations acquired in a single day over far fewer trials are unaffected (Lehmann et al., 2007). Third, 3-D objects are qualitatively

different memoranda compared to 2-D pictures in multiple domains; including, visual/perceptual, olfactory, and haptic. Object discriminations likely require greater recruitment of visual areas during learning, but also distributed polymodal sensory and associative regions.

It seems to me the complex experiential features coupled with the low-to-medium ambiguity of object discrimination tasks facilitate a faster learning rate and greater resistance to HPC damage compared to picture discriminations. This pattern of spared and impaired performance after hippocampal damage does not fit into any consolidation-based theory of memory, which might predict that more complex discriminations should take longer to become independent of the HPC and that picture discriminations never require the HPC. According to HR and in opposition of traditional views, picture discrimination tasks likely recruit a limited number of sensory and associative regions; therefore, more iterations are required for learning and for the memory to be retrievable by lower levels of the heterarchy.

Future Directions

To summarize, I interpret the heterarchic memory framework as making the following predictions: 1) tasks involving complex, polymodal stimuli require a greater depth of processing (dense coding), which necessarily engages a greater proportion of the heterarchy; 2) simple, unimodal stimuli engage less of the available heterarchy and require less depth of processing; 3) increased engagement of the heterarchy via dense coding decreases the number of iterations required for learning under certain conditions; 4) differences in learning rate should be apparent in tasks that engage more versus less of the heterarchy; 5) following damage to the rat HPC, anterograde memory impairments should

be present only in tasks that require unique hippocampal information processing functions,

or in cases of very brief/limited experience.

References

Alvarado, M. C., & Rudy, J. W. (1995). Rats with damage to the hippocampal-formation are impaired on the transverse-patterning problem but not on elemental discriminations. *Behavioral Neuroscience*, *109*(2), 204–211. https://doi.org/10.1037//0735-7044.109.2.204

Alvarez, P., & Squire, L. R. (1994). Memory consolidation and the medial temporal lobe: a simple network model. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(15), 7041–5. https://doi.org/10.1073/pnas.91.15.7041

Amaral, D. G., Rempel-Clower, N. L., Zola, S. M., & Squire, L. R. (1996). Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation. *J Neurosci*, *16*(16), 5233–5255. https://doi.org/10.1523/JNEUROSCI.16-16-05233.1996

Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(3), 1106–14. https://doi.org/http://hdl.handle.net/2027.42/56234

Antoniadis, E. A., & McDonald, R. J. (1999). Discriminative fear conditioning to context expressed by multiple measures of fear in the rat. *Behavioural Brain Research*, *101*(1), 1–13. https://doi.org/10.1016/S0166-4328(98)00056-4

Antoniadis, E. A., & McDonald, R. J. (2000). Amygdala, hippocampus and discriminative fear conditioning to context. *Behavioural Brain Research*, *108*(1), 1–19. https://doi.org/10.1016/S0166-4328(99)00121-7

Antoniadis, E. A., & McDonald, R. J. (2001). Amygdala, hippocampus, and unconditioned fear. *Experimental Brain Research*, *138*(2), 200–209. https://doi.org/10.1007/s002210000645

Aristotle. (1984). The Complete Works of Aristotle-The Revised Oxford Translation II. *Ed. J. Barnes, Princeton, NJ*. https://doi.org/10.1007/s13398-014-0173-7.2

Axmacher, N., & Rasch, B. (2017). *Cognitive neuroscience of memory consolidation*. Springer.

Balog, R. J. (2016). Effects of cue type and overlap in context conditioning: the role of the hippocampus. Lethbridge, Alta: University of Lethbridge, Dept. of Neuroscience.

Bi, G., & Poo, M. (2001). Synaptic modification by correlated activity: Hebb's postulate revisited. *Annual Review of Neuroscience*, *24*(1), 139–166.

Biedenkapp, J. C., & Rudy, J. W. (2007). Context preexposure prevents forgetting of a contextual fear memory : Implication for regional changes in brain activation patterns

associated with recent and remote memory tests, (2005), 200–203. https://doi.org/10.1101/lm.499407

Bliss, T. V. P., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, *361*, 31. Retrieved from http://dx.doi.org/10.1038/361031a0

Bliss, T. V. P., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, *361*(6407), 31.

Bliss, T. V. P., Gardner-Medwin, A. R., & Lømo, T. (1973). Synaptic plasticity in the hippocampal formation. *Macromolecules and Behaviour*, *193*, 203.

Bliss, T. V. P., & Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology*, *232*(2), 331–356.

Broadbent, N. J., & Clark, R. E. (2013). Remote context fear conditioning remains hippocampus-dependent irrespective of training protocol, training-surgery interval, lesion size, and lesion method. *Neurobiology of Learning and Memory*, *106*, 300–8. https://doi.org/10.1016/j.nlm.2013.08.008

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(40), 14515–20. https://doi.org/10.1073/pnas.0406344101

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2007). Rats depend on habit memory for discrimination learning and retention. *Learning & Memory*, *14*(3), 145–151. https://doi.org/10.1101/lm.455607

Buzsáki, G., & Links, R. (2017). Space and Time in the. *Science*, *358*(October), 482–485. https://doi.org/10.1159/000485630

Bye, C. M. (2017). The role of hippocampal NMDA receptors in encoding and consolidation of spatial information on the spatial version of the water task. Lethbridge, Alta: University of Lethbridge, Dept. of Neuroscience.

Cain, D. P., Saucier, D., Hall, J., Hargreaves, E. L., & Boon, F. (1996). Detailed behavioral analysis of water maze acquisition under APV or CNQX: contribution of sensorimotor disturbances to drug-induced acquisition deficits. *Behavioral Neuroscience*, *110*(1), 86.

Cohen, N., & Squire, L. (1980). Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. *Science*, *210*(4466), 207–210. https://doi.org/10.1126/science.7414331

Corkin, S. (2002). What's new with the amnesic patient H.M.? *Nature Reviews Neuroscience*, *3*(2), 153–160. https://doi.org/10.1038/nrn726

Cowansage, K. K., Shuman, T., Dillingham, B. C., Chang, A., Golshani, P., & Mayford, M. (2014). Direct Reactivation of a Coherent Neocortical Memory of Context. *Neuron*, *84*(2), 432–441. https://doi.org/10.1016/j.neuron.2014.09.022

Crick, F. (1984). Memory and molecular turnover.

De Biran, P. M. (1929). *The influence of habit on the faculty of thinking* (Vol. 3). The Williams & Wilkins company.

de Oliveira Alvares, L., Einarsson, E. Ö., Santana, F., Crestani, A. P., Haubrich, J., Cassini, L. F., … Quillfeldt, J. A. (2012). Periodically reactivated context memory retains its precision and dependence on the hippocampus. *Hippocampus*, *22*(5), 1092–1095. https://doi.org/10.1002/hipo.20983

Devan, B. D., Hong, N. S., & McDonald, R. J. (2011). Parallel associative processing in the dorsal striatum: Segregation of stimulus-response and cognitive control subregions. *Neurobiology of Learning and Memory*, *96*(2), 95–120. https://doi.org/10.1016/j.nlm.2011.06.002

Dong, Z., Han, H., Li, H., Bai, Y., Wang, W., Tu, M., … Wu, X. (2015). Long-term potentiation decay and memory loss are mediated by AMPAR endocytosis. *The Journal of Clinical Investigation*, *125*(1), 234–247.

Driscoll, I., Howard, S. R., Prusky, G. T., Rudy, J. W., & Sutherland, R. J. (2005). Seahorse wins all races: Hippocampus participates in both linear and non-linear visual discrimination learning. *Behavioural Brain Research*, *164*(1), 29–35. https://doi.org/10.1016/j.bbr.2005.05.006

Dudai, Y. (2002). Molecular bases of long-term memories: a question of persistence. *Current Opinion in Neurobiology*, *12*(2), 211–216. https://doi.org/https://doi.org/10.1016/S0959-4388(02)00305-7

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annu. Rev. Psychol.*, *55*, 51–86.

Dudai, Y., Karni, A., & Born, J. (2015). The Consolidation and Transformation of Memory. *Neuron*. https://doi.org/10.1016/j.neuron.2015.09.004

Dusek, J. A., & Eichenbaum, H. (1997). The hippocampus and memory for orderly stimulus relations. *Proceedings of the National Academy of Sciences*, *94*(13), 7109–7114. https://doi.org/10.1073/pnas.94.13.7109

Eacott, M. J. (1998). Acquisition and retention of visual discrimination learning after ablation of perirhinal cortex in the rat, *26*(1), 36–41.

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(8), 1948–53. https://doi.org/10.1523/JNEUROSCI.2975-03.2004

Ebbinghaus, H. (1885). Ueber das Gedächtnis.

Ego-Stengel, V., & Wilson, M. A. (2010). Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. *Hippocampus*, *20*(1), 1–10.

Eichenbaum, H., Fagan, A., Mathews, P., & Cohen, N. J. (1988). Hippocampal system dysfunction and odor discrimination learning in rats: Impairment of facilitation depending on representational demands. *Behavioral Neuroscience*, *102*(3), 331.

Epp, J., Keith, J. R., Spanswick, S. C., Stone, J. C., Prusky, G. T., & Sutherland, R. J. (2008). Retrograde amnesia for visual memories after hippocampal damage in rats. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *15*(4), 214–21. https://doi.org/10.1101/lm.788008

Epp, J., Keith, J. R., Spanswick, S. C., Stone, J. C., Prusky, G. T., & Sutherland, R. J. (2008). Retrograde amnesia for visual memories after hippocampal damage in rats. *Learning & Memory*, *15*(4), 214–221. https://doi.org/10.1101/lm.788008

Fanselow & Gale, 2003. (2003). The Amygala, Fear, and Memory. *Annals of the New York Academy of Sciences*, *16*, 125–134. https://doi.org/10.1111/j.1749-6632.2003.tb07077.x

Fanselow, M. S. (1980). Conditional and unconditional components of post-shock freezing. *The Pavlovian Journal of Biological Science : Official Journal of the Pavlovian*, *15*(4), 177–182. https://doi.org/10.1007/BF03001163

Fanselow, M. S. (1990). Factors governing one-trial contextual conditioning. *Animal Learning & Behavior*, *18*(3), 264–270. https://doi.org/10.3758/BF03205285

Fanselow, M. S. (2009). From contextual fear to a dynamic view of memory systems. *Trends in Cognitive Sciences*, *14*(1), 7–15. https://doi.org/10.1016/j.tics.2009.10.008

Ferbinteanu, J. (2018). Memory systems 2018 – towards a new paradigm. *Neurobiology of Learning and Memory*, (November). https://doi.org/10.1016/j.nlm.2018.11.005

Fortin, N. J., Agster, K. L., & Eichenbaum, H. B. (2002). Critical role of the hippocampus in memory for sequences of events. *Nature Neuroscience*, *5*, 458. Retrieved from http://dx.doi.org/10.1038/nn834

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nature Reviews Neuroscience*, *6*, 119. Retrieved from https://doi.org/10.1038/nrn1607

Frankland, P. W., Filipkowski, R. K., Cestari, V., McDonald, R. J., & Silva, A. J. (1998). The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. *Behavioral Neuroscience*, *112*(4), 863–874. https://doi.org/10.1037//0735-7044.112.4.863

Gale, G. D. (2004). Role of the Basolateral Amygdala in the Storage of Fear Memories across the Adult Lifetime of Rats. *Journal of Neuroscience*, *24*(15), 3810–3815. https://doi.org/10.1523/JNEUROSCI.4100-03.2004

Genzel, L., Rossato, J. I., Jacobse, J., Grieves, R. M., Spooner, P. A., Battaglia, F. P., … Morris, R. G. M. (2017). The Yin and Yang of Memory Consolidation: Hippocampal and Neocortical. *PLoS Biology*, *15*(1), 1–26. https://doi.org/10.1371/journal.pbio.2000531

Genzel, L., & Wixted, J. T. (2017). Cellular and Systems Consolidation of Declarative Memory BT - Cognitive Neuroscience of Memory Consolidation. In N. Axmacher & B. Rasch (Eds.) (pp. 3–16). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-45066-7_1

Gidyk, D. C., McDonald, R. J., & Sutherland, R. J. (2016). *Persistence of long-term contextual fear and visual discrimination memory in the absence of the hippocampus.* Society for Neuroscience Abstracts, San Diego, CA. Online.

Goshen, I., Brodsky, M., Prakash, R., Wallace, J., Gradinaru, V., Ramakrishnan, C., & Deisseroth, K. (2011). Dynamics of retrieval strategies for remote memories. *Cell*, *147*(3), 678–689. https://doi.org/10.1016/j.cell.2011.09.033

Gouveia, F., Gidyk, D. C., Giacobbe, P., Ng, E., Meng, Y., Davidson, B., … Hamani, C. (2019). Neuromodulation Strategies in Post-Traumatic Stress Disorder: From Preclinical Models to Clinical Applications. *Brain Sciences*, *9*(2), 45. https://doi.org/10.3390/brainsci9020045

Gruber, A. J., & McDonald, R. J. (2012). Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behavior. *Frontiers in Behavioral Neuroscience*, *6*(August), 50. https://doi.org/10.3389/fnbeh.2012.00050

Gulbrandsen, T. L. (2015). Hippocampal overshadowing : exploring the underlying mechanisms. Lethbridge, Alta: University of Lethbridge, Dept. of Neuroscience.

Gulbrandsen, T. L., & Sutherland, R. J. (2014). Temporary inactivation of the rodent hippocampus: an evaluation of the current methodology. *Journal of Neuroscience Methods*, *225*, 120–8. https://doi.org/10.1016/j.jneumeth.2014.01.015

Gulbrandsen, T. L., Zelinski, E. L., Gidyk, D. C., McDonald, R. J., & Sutherland, R. J. Memory trace persistence varies by the supporting neural network: an evaluation of the underlying circuitry of an instrumental task. Program No. 159.19. 2012. FENS

Forum of Neuroscience. Barcelona, Spain: Federation of European Neuroscience Societies, 2012

Hardt, O., Nader, K., & Nadel, L. (2013). Decay happens: the role of active forgetting in memory. *Trends in Cognitive Sciences*, *17*(3), 111–20. https://doi.org/10.1016/j.tics.2013.01.001

Hassabis, D., Kumaran, D., Vann, S. D., & Maguire, E. A. (2007). Patients with hippocampal amnesia cannot imagine new experiences. *Proceedings of the National Academy of Sciences*, *104*(5), 1726 LP-1731.

Hassabis, D., & Maguire, E. A. (2009). The construction system of the brain. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1521), 1263–1271. https://doi.org/10.1098/rstb.2008.0296

Hebb, D. O. (1949). The Organization of Behaviour. *Organization*, 62. https://doi.org/citeulike-article-id:1282862

Hirsh, R. (1974). The hippocampus and contextual retrieval of information from memory: A theory. *Behavioral Biology*, *12*(4), 421–444. https://doi.org/10.1016/S0091-6773(74)92231-7

Honey, R. C., & Good, M. (1993). Selective hippocampal lesions abolish the contextual specificity of latent inhibition and conditioning. *Behavioral Neuroscience*. US: American Psychological Association. https://doi.org/10.1037/0735-7044.107.1.23

Jadhav, S. P., & Frank, L. M. (2014). Space, time and memory in the hippocampal formation. *Space, Time and Memory in the Hippocampal Formation*, 1–571. https://doi.org/10.1007/978-3-7091-1292-2

Jadhav, S. P., Kemere, C., German, P. W., & Frank, L. M. (2012). Awake Hippocampal Sharp-Wave Ripples Support Spatial Memory. *Science*, *336*(6087), 1454–1458. https://doi.org/10.1126/science.1217230

James, W. (1890). *The principles of psychology (Vols. 1 & 2). New York Holt*. https://doi.org/10.1037/10538-000

Jasnow, A. M., Lynch, J. F., Gilman, T. L., & Riccio, D. C. (2017). Perspectives on fear generalization and its implications for emotional disorders. *Journal of Neuroscience Research*, *95*(3), 821–835. https://doi.org/10.1002/jnr.23837

Jenkins, J. G., & Dallenbach, K. M. (1924). Obliviscence during sleep and waking. *The American Journal of Psychology*, *35*(4), 605–612.

Ji, D., & Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature Neuroscience*, *10*(1), 100.

Kentros, C., Hargreaves, E., Hawkins, R. D., Kandel, E. R., Shapiro, M., & Muller, R. V.

(1998). Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science*, *280*(5372), 2121–2126.

Kim, J., & Fanselow, M. (1992). Modality-specific retrograde amnesia of fear. *Science*, *256*(5057), 675–677. https://doi.org/10.1126/science.1585183

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. *Neuroscience and Biobehavioral Reviews*, *30*(2), 188–202. https://doi.org/10.1016/j.neubiorev.2005.06.005

Kitamura, T., Ogawa, S. K., Roy, D. S., Okuyama, T., Morrissey, M. D., Smith, L. M., … Tonegawa, S. (2017). Engrams and circuits crucial for systems consolidation of a memory. *Science*, *356*(6333), 73–78. https://doi.org/10.1126/science.aam6808

Korte, M., & Schmitz, D. (2016). Cellular and System Biology of Memory: Timing, Molecules, and Beyond. *Physiological Reviews*, *96*(2), 647–693. https://doi.org/10.1152/physrev.00010.2015

Korte, M., Schmitz, D., Abbott, L., Nelson, S., Abel, T., Martin, K., … Svoboda, K. (2016). Cellular and System Biology of Memory: Timing, Molecules, and Beyond. *Physiological Reviews*, *96*(2), 647–93. https://doi.org/10.1152/physrev.00010.2015

Kuhn, T. S. (1962). *The Structure of Scientific Revolutions*. *International encyclopedia of unified science Vol2 no2* (Vol. 2). https://doi.org/10.1111/j.1468-0149.1963.tb00813.x

Lavenex, P., & Amaral, D. G. (2000). Hippocampal-neocortical interaction: A hierarchy of associativity. *Hippocampus*, *10*(4), 420–430. https://doi.org/10.1002/1098-1063(2000)10:4<420::AID-HIPO8>3.0.CO;2-5

LeBlancq, M. J., McKinney, T. L., & Dickson, C. T. (2016). ZIP It: Neural Silencing Is an Additional Effect of the PKM-Zeta Inhibitor Zeta-Inhibitory Peptide. *Journal of Neuroscience*, *36*(23), 6193–6198. https://doi.org/10.1523/JNEUROSCI.4563-14.2016

Lee, A. K., & Wilson, M. A. (2002). Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron*, *36*(6), 1183–1194.

Lee, J. Q., Sutherland, R. J., & McDonald, R. J. (2017). Hippocampal damage causes retrograde but not anterograde memory loss for context fear discrimination in rats. *Hippocampus*, *27*(9), 951–958. https://doi.org/10.1002/hipo.22759

Lee, J. Q., Zelinski, E. L., McDonald, R. J., & Sutherland, R. J. (2016). Heterarchic reinstatement of long-term memory: A concept on hippocampal amnesia in rodent memory research. *Neuroscience and Biobehavioral Reviews*, *71*, 154–166. https://doi.org/10.1016/j.neubiorev.2016.08.034

Lehmann, H., Glenn, M. J., & Mumby, D. G. (2007). Consolidation of object-discrimination memory is independent of the hippocampus in rats. *Experimental Brain Research*, *180*(4), 755–764. https://doi.org/10.1007/s00221-007-0895-2

Lehmann, H., Lacanilao, S., & Sutherland, R. J. (2007). Complete or partial hippocampal damage produces equivalent retrograde amnesia for remote contextual fear memories. *European Journal of Neuroscience*, *25*(5), 1278–1286. https://doi.org/10.1111/j.1460-9568.2007.05374.x

Lehmann, H., & McNamara, K. C. (2011). Repeatedly reactivated memories become more resistant to hippocampal damage. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *18*(3), 132–5. https://doi.org/10.1101/lm.2000811

Lehmann, H., & McNamara, K. C. (2011). Repeatedly reactivated memories become more resistant to hippocampal damage. *Learning & Memory*, *18*(3), 132–135. https://doi.org/10.1101/lm.2000811

Lehmann, H., Rourke, B. K., Booker, A., & Glenn, M. J. (2013). Single session contextual fear conditioning remains dependent on the hippocampus despite an increase in the number of context-shock pairings during learning. *Neurobiology of Learning and Memory*, *106*, 294–9. https://doi.org/10.1016/j.nlm.2012.10.011

Lehmann, H., Sparks, F. T., Spanswick, S. C., Hadikin, C., McDonald, R. J., & Sutherland, R. J. (2009). Making context memories independent of the hippocampus. *Learning & Memory* , *16*(7), 417–420. https://doi.org/10.1101/lm.1385409

Lewandowsky, S., Ecker, U. K. H., Farrell, S., & Brown, G. D. A. (2012). Models of cognition and constraints from neuroscience: A case study involving consolidation. *Australian Journal of Psychology*, *64*(1), 37–45. https://doi.org/10.1111/j.1742-9536.2011.00042.x

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioural Brain Research*, *88*(2), 261–274. https://doi.org/https://doi.org/10.1016/S0166-4328(97)00088-0

Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: Implications for fear conditioning, extinction and psychopathology. *Nature Reviews Neuroscience*, *14*(6), 417–428. https://doi.org/10.1038/nrn3492

Marr, D. (1971). Simple Memory: A Theory for Archicortex. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *262*(841), 23–81. https://doi.org/10.1098/rstb.1971.0078

Martin, S. J., Grimwood, P. D., & Morris, R. G. M. (2000). S ynaptic plasticity and memory: An Evaluation of the Hypothesis. *Annual Review of Neuroscience*, (Hebb 1949), 649–711. https://doi.org/10.1146/annurev.neuro.23.1.649

Matus-Amat, P., Higgins, E. A., Barrientos, R. M., & Rudy, J. W. (2004). The Role of the Dorsal Hippocampus in the Acquisition and Retrieval of Context Memory Representations. *The Journal of Neuroscience*, *24*(10), 2431 LP-2439.

Mayford, M. (2014). The search for a hippocampal engram, *369*(1633), 1–10.

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: *Psychological Review*, *102*(3), 419–57. https://doi.org/7624455

McDonald, R. J., & Hong, N. S. (2013). How does a specific learning and memory system in the mammalian brain gain control of behavior? *Hippocampus*, *23*(11), 1084–102. https://doi.org/10.1002/hipo.22177

McDonald, R. J., Hong, N. S., Craig, L. A., Holahan, M. R., Louis, M., & Muller, R. U. (2005). NMDA-receptor blockade by CPP impairs post-training consolidation of a rapidly acquired spatial representation in rat hippocampus. *European Journal of Neuroscience*, *22*(5), 1201–1213.

McDonald, R. J., Hong, N. S., & Devan, B. (2017). *Interactions Among Multiple Parallel Learning and Memory Systems in the Mammalian Brain*. *Reference Module in Neuroscience and Biobehavioral Psychology*. https://doi.org/10.1016/B978-0-12-809324-5.21067-5

McDonald, R. J., Jones, J., Richards, B., & Hong, N. S. (2006). A double dissociation of dorsal and ventral hippocampal function on a learning and memory task mediated by the dorso-lateral striatum. *The European Journal of Neuroscience*, *24*(6), 1789–801. https://doi.org/10.1111/j.1460-9568.2006.05064.x

McDonald, R. J., King, A. L., & Hong, N. S. (2001). Context-specific interference on reversal learning of a stimulus-response habit. *Behavioural Brain Research*, *121*(1–2), 149–165. https://doi.org/10.1016/S0166-4328(01)00160-7

McDonald, R. J., King, A. L., Wasiak, T. D., Zelinski, E. L., & Hong, N. S. (2007). A Complex Associative Structure Formed in the Mammalian Brain During Acquisition of a Simple Visual Discrimination Task :, *774*, 759–774. https://doi.org/10.1002/hipo

McDonald, R. J., Ko, C. H., & Hong, N. S. (2002). Attenuation of context-specific inhibition on reversal learning of a stimulus-response task in rats with neurotoxic hippocampal damage. *Behavioural Brain Research*, *136*(1), 113–126. https://doi.org/10.1016/S0166-4328(02)00104-3

Mcdonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. *Behavioral Neuroscience*, *127*(6), 835–853. https://doi.org/10.1037/a0034883

McDonald, R., Murphy, R., Guaracci, F., Gortler, White, N., & Baker, A. (1997). A

systematic comparison of the effects of fornix-fimbria and hippocampal lesions on the acquisition of three configural tasks. *Hippocampus*, *7*, 371–388. Retrieved from http://discovery.ucl.ac.uk/176210/

McGaugh, J. L. (1999). The perseveration-consolidation hypothesis: Mueller and Pilzecker, 1900. *Brain Research Bulletin*. https://doi.org/10.1016/S0361-9230(99)00126-4

McGaugh, J. L. (2000). Memory--a Century of Consolidation. *Science*, *287*(5451), 248–251. https://doi.org/10.1126/science.287.5451.248

McGaugh, J. L. (2018). Emotional arousal regulation of memory consolidation. *Current Opinion in Behavioral Sciences*, *19*, 55–60. https://doi.org/10.1016/j.cobeha.2017.10.003

McGeoch, J. A. (1932). Forgetting and the law of disuse. *Psychological Review*, *39*(4), 352.

McNaughton, B. L. (2010). Cortical hierarchies, sleep, and the extraction of knowledge from memory. *Artificial Intelligence*, *174*(2), 205–214. https://doi.org/10.1016/j.artint.2009.11.013

McNaughton, B. L., Douglas, R. M., & Goddard, G. V. (1978). Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. *Brain Research*, *157*(2), 277–293.

McNaughton, B. L., & Morris, R. G. M. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends in Neurosciences*. https://doi.org/10.1016/0166-2236(87)90011-7

Migues, P. V., Liu, L., Archbold, G. E. B., Einarsson, E. Ö., Wong, J., Bonasia, K., … Hardt, O. (2016). Blocking synaptic removal of GluA2-containing AMPA receptors prevents the natural forgetting of long-term memories. *Journal of Neuroscience*, *36*(12), 3481–3494.

Mills, F., Bartlett, T. E., Dissing-Olesen, L., Wisniewska, M. B., Kuznicki, J., Macvicar, B. A., … Bamji, S. X. (2014). Cognitive flexibility and long-term depression (LTD) are impaired following -catenin stabilization in vivo. *Proceedings of the National Academy of Sciences*, *111*(23), 8631–8636. https://doi.org/10.1073/pnas.1404670111

Milner, B., Corkin, S., & Teuber, H. L. (1968). Further analysis of the hippocampal amnesic syndrome: 14-year follow-up study of H.M. *Neuropsychologia*, *6*(3), 215–234. https://doi.org/10.1016/0028-3932(68)90021-3

Mishkin, M., & Petri, H. (1984). *Memories and Habits: Some implications for the analysis of learning and retention*.

Morris, R. G. M., Anderson, E., Lynch, G. S. a, & Baudry, M. (1986). Selective impairment

of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature*, *319*(6056), 774.

Morris, R. G. M., Garrud, P., Rawlins, J. N. P. al, & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681.

Moscovitch, M., Cabeza, R., Winocur, G., & Nadel, L. (2016). Episodic Memory and Beyond: The Hippocampus and Neocortex in Transformation. *Annual Review of Psychology*, *67*(1), 105–134. https://doi.org/10.1146/annurev-psych-113011-143733

Moser, M. B., Moser, E. I., Forrest, E., Andersen, P., & Morris, R. G. (1995). Spatial learning with a minislab in the dorsal hippocampus. *Proceedings of the National Academy of Sciences*, *92*(21), 9697–9701. https://doi.org/10.1073/pnas.92.21.9697

Müller, G. E., & Pilzecker, A. (1900). *Experimentelle beiträge zur lehre vom gedächtniss* (Vol. 1). JA Barth.

Mumby, D. G., Astur, R. S., Weisend, M. P., & Sutherland, R. J. (1999). Retrograde amnesia and selective damage to the hippocampal formation: Memory for places and object discriminations. *Behavioural Brain Research*, *106*(1–2), 97–107. https://doi.org/10.1016/S0166-4328(99)00097-2

Mumby, D. G., Pinel, J. P. J., Kornecook, T. O. M. J., Shen, M. J., & Redila, V. A. N. A. (1995). Memory deficits following lesions of hippocampus or amygdala in rats Assesment by an object-memory test battery.pdf, *23*, 26–36.

Nadel, L., & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Current Opinion in Neurobiology*, *7*(2), 217–227. https://doi.org/10.1016/S0959-4388(97)80010-4

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation\nafter retrieval. *Nature*, *406*(6797), 722–726. https://doi.org/10.1038/35021052

O'Hara, M., Huber, L., & Gajdon, G. K. (2015). The advantage of objects over images in discrimination and reversal learning by kea, Nestor notabilis. *Animal Behaviour*, *101*, 51–60. https://doi.org/10.1016/j.anbehav.2014.12.022

O'keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map*. Oxford: Clarendon Press.

O'Reilly, R. C., & Rudy, J. W. (2001). Conjunctive representations in learning and memory. Principles of learning in the neocortex and hippocampus. *Psychological Review*, *108*(May), 311–345.

Ólafsdóttir, H. F., Bush, D., & Barry, C. (2018). The Role of Hippocampal Replay in Memory and Planning. *Current Biology*, *28*(1), R37–R50.

https://doi.org/10.1016/j.cub.2017.10.073

Packard, M. G., Hirsh, R., & White, N. M. (1989). Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. *Journal of Neuroscience*, *9*(5), 1465–1472.

Pavlov, I. P. (1927). *Conditioned Reflexes. Oxford University Press* (Vol. 17). https://doi.org/10.2307/1134737

Pickens, C. L., Golden, S. A., Adams-Deutsch, T., Nair, S. G., & Shaham, Y. (2009). Long-Lasting Incubation of Conditioned Fear in Rats. *Biological Psychiatry*, *65*(10), 881–886. https://doi.org/10.1016/j.biopsych.2008.12.010

Poulos, A. M., Mehta, N., Lu, B., Amir, D., Livingston, B., Santarelli, A., … Fanselow, M. S. (2016). Conditioning- and time-dependent increases in context fear and generalization. *Learning & Memory*, *23*(7), 379–385. https://doi.org/10.1101/lm.041400.115

Preston, A. R., & Eichenbaum, H. (2013). Interplay of Hippocampus and Prefrontal Cortex in Memory. *Current Biology*, *23*(17), R764–R773. https://doi.org/10.1016/J.CUB.2013.05.041

Redondo, R. L., & Morris, R. G. M. (2011). Making memories last: the synaptic tagging and capture hypothesis. *Nature Reviews. Neuroscience*, *12*(1), 17–30. https://doi.org/10.1038/nrn2963

Ribot, T. (1881). Les maladies de la memoire [English translation: Diseases of memory]. *New York: Appleton-Century-Crofts*.

Riccio, D. C., & Joynes, R. L. (2007). Forgetting of stimulus attributes: Some implications for hippocampal models of memory. *Learning and Memory*, *14*(6), 430–432. https://doi.org/10.1101/lm.617107

Ritchey, M., Montchal, M. E., Yonelinas, A. P., & Ranganath, C. (2015). Delay-dependent contributions of medial temporal lobe regions to episodic memory retrieval. *Elife*, *4*, e05025.

Roediger, H. L., Zaromb, F. M., & Lin, W. (2017). A Typology of Memory Terms ☆. *Learning and Memory: A Comprehensive Reference*, 7–19. https://doi.org/10.1016/B978-0-12-809324-5.21003-1

Routtenberg, A. (2013). Lifetime memories from persistently supple synapses. *Hippocampus*, *23*(3), 202–206. https://doi.org/10.1002/hipo.22088

Rudy, J. W. (2008). The neurobiology of learning and memory. *Psychology*, *4032*, 5032.

Rudy, J. W. (2009). Context representations, context functions, and the parahippocampal-hippocampal system. *Learning & Memory*, *16*(10), 573–585. https://doi.org/10.1101/lm.1494409

Rudy, J. W., Biedenkapp, J. C., & O'Reilly, R. C. (2005). Prefrontal cortex and the organization of recent and remote memories: An alternative view. *Learning and Memory*, *12*(5), 445–446. https://doi.org/10.1101/lm.97905

Ryan, L., Nadel, L., Keil, K., Putnam, K., Schnyer, D., Trouard, T., & Moscovitch, M. (2001). Hippocampal complex and retrieval of recent and very remote autobiographical memories: Evidence from functional magnetic resonance imaging in neurologically intact people. *Hippocampus*, *11*(6), 707–714. https://doi.org/10.1002/hipo.1086

Sachser, R. M., Santana, F., Crestani, A. P., Lunardi, P., Pedraza, L. K., Quillfeldt, J. A., … de Oliveira Alvares, L. (2016). Forgetting of long-term memory requires activation of NMDA receptors, L-type voltage-dependent Ca 2+ channels, and calcineurin. *Scientific Reports*, *6*, 22771.

Sara, S. J. (1981). Memory deficits in rats with hippocampal or cortical lesions: Retrograde effects, *32*(4), 504–509. https://doi.org/10.1016/S0163-1047(81)90930-4

Scott, G. A., Saucier, D. M., & Lehmann, H. (2016). Contrasting the amnesic effects of lesions and temporary inactivations of the hippocampus on context and spatial memory. *Journal of Behavioral and Brain Science*, (April), 1–27.

Scoville, W. B., & Milner, B. (1957). LOSS OF RECENT MEMORY AFTER BILATERAL HIPPOCAMPAL LESIONS. *Journal of Neurology, Neurosurgery & Psychiatry*, *20*(1), 11–21. https://doi.org/10.1136/jnnp.20.1.11

Sekeres, M. J., Moscovitch, M., & Winocur, G. (2017). Mechanisms of Memory Consolidation and Transformation. In *Cognitive Neuroscience of Memory Consolidation* (pp. 17–44). https://doi.org/10.1007/978-3-319-45066-7_2

Sharma, A. V., Nargang, F. E., & Dickson, C. T. (2012). Neurosilence: Profound Suppression of Neural Activity following Intracerebral Administration of the Protein Synthesis Inhibitor Anisomycin. *Journal of Neuroscience*, *32*(7), 2377–2387. https://doi.org/10.1523/JNEUROSCI.3543-11.2012

Sherry, D. F., & Schacter, D. L. (1987). Sherry-Shacter-the-evolution-of-multiple-memory-systems.pdf, *94*(4), 439–454.

Skelin, I., Kilianski, S., & McNaughton, B. L. (2018). Hippocampal coupling with cortical and subcortical structures in the context of memory consolidation. *Neurobiology of Learning and Memory*, (March). https://doi.org/10.1016/j.nlm.2018.04.004

Sparks, F. T., Lehmann, H., & Sutherland, R. J. (2011). Between-systems memory

interference during retrieval. *The European Journal of Neuroscience*, *34*(5), 780–6. https://doi.org/10.1111/j.1460-9568.2011.07796.x

Sparks, F. T., Spanswick, S. C., Lehmann, H., & Sutherland, R. J. (2013). Neither time nor number of context-shock pairings affect long-term dependence of memory on hippocampus. *Neurobiology of Learning and Memory*, *106*, 316–323. https://doi.org/10.1016/j.nlm.2013.05.008

Squire, L. R. (1992a). Declarative and nondeclarative memory: Multiple brain systems supporting learning and memory. *Journal of Cognitive Neuroscience*, *4*(3), 232–243. https://doi.org/10.1162/jocn.1992.4.3.232

Squire, L. R. (1992b). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological Review*, *99*(2), 195–231. https://doi.org/10.1037/0033-295X.99.3.582

Squire, L. R. (2004). Memory systems of the brain: A brief history and current perspective. *Neurobiology of Learning and Memory*, *82*(3), 171–177. https://doi.org/10.1016/j.nlm.2004.06.005

Squire, L. R. (2009). The Legacy of Patient H.M. for Neuroscience. *Neuron*, *61*(1), 6–9. https://doi.org/10.1016/j.neuron.2008.12.023

Squire, L. R., & Alvarez, P. (1995). Retrograde amnesia and memory consolidation:a neurobiological perspective. *Current Opinion in Neurobiology PG - 178*, *5*, 183.

Squire, L. R., Cohen, N. J., & Nadel, L. (1984). The medial temporal region and memory consolidation: A new hypothesis. *Memory Consolidation: Psychobiology of Cognition*, 185–210.

Squire, L. R., Genzel, L., Wixted, J. T., & Morris, R. G. (2015). Memory consolidation. *Cold Spring Harbor Perspectives in Biology*, *7*(8), a021766. https://doi.org/10.1101/cshperspect.a021766

Squire, L. R., Knowlton, B., & Musen, G. (1993). The Structure and Organization of Memory. *Annual Review of Psychology*, *44*(1), 453–495. https://doi.org/10.1146/annurev.ps.44.020193.002321

Squire, L. R., & Wixted, J. T. (2011). *The Cognitive Neuroscience of Human Memory Since H.M. Annual Review of Neuroscience* (Vol. 34). https://doi.org/10.1146/annurev-neuro-061010-113720

Steinvorth, S., Levine, B., & Corkin, S. (2005). Medial temporal lobe structures are needed to re-experience remote autobiographical memories: evidence from H.M. and W.R. *Neuropsychologia*, *43*(4), 479–96. https://doi.org/10.1016/j.neuropsychologia.2005.01.001

Sternbach, H. (1997). Antibiotics: Neuropsychiatric effects and psychotropic interactions. *Harvard Review of Psychiatry*. https://doi.org/10.3109/10673229709000304

Strange, B. A., Witter, M. P., Lein, E. S., & Moser, E. I. (2014). Functional organization of the hippocampal longitudinal axis. *Nature Reviews Neuroscience*, *15*(10), 655–669. https://doi.org/10.1038/nrn3785

Sutherland, R. J., McDonald, R. J., Hill, C. R., Rudy, J. W. (1989). Damage to the hippocampal formation in rats selectively impairs the ability to learn cue relationships. *Behavioral and Neural Biology*, *52*(3), 331–356. https://doi.org/10.1016/S0163-1047(89)90457-3

Sutherland, R. J., Kolb, B., & Whishaw, I. Q. (1982). Spatial mapping: Definitive disruption by hippocampal or medial-frontal cortical damage in the rat, *4*, 271–276.

Sutherland, R. J., & Lehmann, H. (2011). Alternative conceptions of memory consolidation and the role of the hippocampus at the systems level in rodents. *Current Opinion in Neurobiology*, *21*(3), 446–51. https://doi.org/10.1016/j.conb.2011.04.007

Sutherland, R. J., & McDonald, R. J. (1990). Hippocampus, amygdala, and memory deficits in rats. *Behav Brain Res*, *37*(1), 57–79. https://doi.org/0166-4328(90)90072-M [pii]

Sutherland, R. J., O'Brien, J., & Lehmann, H. (2008). Absence of systems consolidation of fear memories after dorsal, ventral, or complete hippocampal damage. *Hippocampus*, *18*(7), 710–8. https://doi.org/10.1002/hipo.20431

Sutherland, R. J., & Rudy, J. W. (1989). Configural association theory: The role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology*, *17*(2), 129–144. https://doi.org/10.3758/BF03337828

Sutherland, R. J., Sparks, F. T., & Lehmann, H. (2010). Hippocampus and retrograde amnesia in the rat model: a modest proposal for the situation of systems consolidation. *Neuropsychologia*, *48*(8), 2357–69. https://doi.org/10.1016/j.neuropsychologia.2010.04.015

Sutherland, R. J., Weisend, M. P., Mumby, D., Astur, R. S., Hanlon, F. M., Koerner, A., … Cole, C. (2001). Retrograde amnesia after hippocampal damage: recent vs. remote memories in two tasks. *Hippocampus*, *11*(1), 27–42.

Takeuchi, T., Duszkiewicz, A. J., & Morris, R. G. M. (2014). The synaptic plasticity and memory hypothesis: encoding, storage and persistence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1633), 20130288.

Teyler, T. J., & DiScenna, P. (1986). The hippocampal memory index theory. *Behavioral Neuroscience*, *100*(2), 147–154 ST–The hippocampal memory index theory.

Thorndike, E. L. (1913). *The psychology of learning* (Vol. 2). Teachers College, Columbia University.

Thorndike, E. L. (1932). The physiological basis of the strengthening of connections by their after-effects. *The Fundamentals of Learning.* Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=psyc1&NEWS=N &AN=2006-04535-012

Thorndike, E. L., & Jelliffe. (1912). Animal Intelligence. Experimental Studies. *The Journal of Nervous and Mental Disease*, *39*(5), 357. https://doi.org/10.1097/00005053-191205000-00016

Tolman, E. C. (1948). Cognitive maps in rats and men. *Psychological Review*, *55*(4), 189–208. https://doi.org/10.1037/h0061626

Tononi, G., & Cirelli, C. (2014). Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron*, *81*(1), 12–34.

Tse, D., Takeuchi, T., Kakeyama, M., Kajii, Y., Okuno, H., Tohyama, C., … Morris, R. G. M. (2011). Schema-dependent gene activation and memory encoding in neocortex. *Science*, *333*(6044), 891–895. https://doi.org/10.1126/science.1205274

Tulving, E. (1972). Episodic and semantic memory. *Organization of Memory.* https://doi.org/10.1017/S0140525X00047257

Tulving, E. (1985). How many memory systems are there? *American Psychologist*, *40*, 385–398. https://doi.org/10.1037/0003-066X.40.4.385

Tulving, E. (2002). Episodic memory: From mind to brain. *Annual Review of Psychology*, *53*(1), 1–25.

Underwood, B. J. (1957). Interference and forgetting. *Psychological Review*, *64*(1), 49.

Verfaellie, M., Bousquet, K., & Keane, M. M. (2014). Medial temporal and neocortical contributions to remote memory for semantic narratives: evidence from amnesia. *Neuropsychologia*, *61*, 105–112. https://doi.org/10.1016/j.neuropsychologia.2014.06.018

Vnek, N., & Rothblat, L. a. (1996). The hippocampus and long-term object memory in the rat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(8), 2780–2787.

Wang, S. H., Teixeira, C. M., Wheeler, A. L., & Frankland, P. W. (2009). The precision of remote context memories does not require the hippocampus. *Nature Neuroscience*, *12*(3), 253–255. https://doi.org/10.1038/nn.2263

White, N. M. (2009). Some highlights of research on the effects of caudate nucleus lesions over the past 200 years. *Behavioural Brain Research*, *199*(1), 3–23. https://doi.org/10.1016/j.bbr.2008.12.003

White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiology of Learning and Memory*, *77*(2), 125–84. https://doi.org/10.1006/nlme.2001.4008

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

Wible, C. G., Shiber, J. R., & Olton, D. S. (1992). Hippocampus, Fimbria-Fornix, Amygdala, and Memory: Object Discriminations in Rats. *Behavioral Neuroscience*, *106*(5), 751–761. https://doi.org/10.1037/0735-7044.106.5.751

Wiig, K. a, Cooper, L. N., & Bear, M. F. (1996). Temporally graded retrograde amnesia following separate and combined lesions of the perirhinal cortex and fornix in the rat. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *3*(4), 313–325. https://doi.org/10.1101/lm.3.4.313

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science*, *265*(5172), 676–679.

Wiltgen, B. J., Sanders, M. J., Anagnostaras, S. G., Sage, J. R., & Fanselow, M. S. (2006). Context Fear Learning in the Absence of the Hippocampus. *The Journal of Neuroscience*, *26*(20), 5484 LP-5491.

Wiltgen, B. J., & Silva, A. J. (2007). Memory for context becomes less specific with time. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *14*(4), 313–7. https://doi.org/10.1101/lm.430907

Wiltgen, B. J., & Tanaka, K. Z. (2013). Systems consolidation and the content of memory. *Neurobiology of Learning and Memory*, *106*, 365–71. https://doi.org/10.1016/j.nlm.2013.06.001

Wiltgen, B. J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M. G., Parivash, S. N., … Silva, A. J. (2010). The hippocampus plays a selective role in the retrieval of detailed contextual memories. *Current Biology*, *20*(15), 1336–1344. https://doi.org/10.1016/j.cub.2010.06.068

Winocur, G. (1990). Anterograde and retrograde amnesia in rats with dorsal hippocampal or dorsomedial thalamic lesions. *Behavioural Brain Research*, *38*(2), 145–154. https://doi.org/https://doi.org/10.1016/0166-4328(90)90012-4

Winocur, G., Frankland, P. W., Sekeres, M., Fogel, S., & Moscovitch, M. (2009). Changes in context-specificity during memory reconsolidation : Selective effects of hippocampal lesions. *Cold Spring Harbor Laboratory Press*, *16*, 722–729. https://doi.org/10.1101/lm.1447209

Winocur, G., & Moscovitch, M. (2011). Memory transformation and systems

consolidation. *Journal of the International Neuropsychological Society*, *17*(5), 766–780. https://doi.org/10.1017/S1355617711000683

Winocur, G., Moscovitch, M., & Bontempi, B. (2010). Memory formation and long-term retention in humans and animals: Convergence towards a transformation account of hippocampal–neocortical interactions. *Neuropsychologia*, *48*(8), 2339–2356.

Winocur, G., Moscovitch, M., & Sekeres, M. J. (2013). Factors affecting graded and ungraded memory loss following hippocampal lesions. *Neurobiology of Learning and Memory*, *106*, 351–364. https://doi.org/10.1016/j.nlm.2013.10.001

Winocur, G., Sekeres, M. J., Binns, M. A., & Moscovitch, M. (2013). Hippocampal lesions produce both nongraded and temporally graded retrograde amnesia in the same rat. *Hippocampus*, *23*(5), 330–341. https://doi.org/10.1002/hipo.22093

Wixted, J. T. (2004). The Psychology and Neuroscience of Forgetting. *Annual Review of Psychology*, *55*(1), 235–269. https://doi.org/10.1146/annurev.psych.55.090902.141555

Yin, H. H., & Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience*, *7*(6), 464–476. https://doi.org/10.1038/nrn1919

Zelikowsky, M., Bissiere, S., & Fanselow, M. S. (2012). Contextual Fear Memories Formed in the Absence of the Dorsal Hippocampus Decay Across Time. *Journal of Neuroscience*, *32*(10), 3393–3397. https://doi.org/10.1523/JNEUROSCI.4339-11.2012

Zelikowsky, M., Bissiere, S., Hast, T. A., Bennett, R. Z., Abdipranoto, A., Vissel, B., & Fanselow, M. S. (2013). Prefrontal microcircuit underlies contextual learning after hippocampal loss. *Proceedings of the National Academy of Sciences*, *110*(24), 9938–9943. https://doi.org/10.1073/pnas.1301691110

Zelinski, E. L., Hong, N. S., Tyndall, A. V, Halsall, B., & McDonald, R. J. (2010). Prefrontal cortical contributions during discriminative fear conditioning, extinction, and spontaneous recovery in rats. *Experimental Brain Research*, *203*(2), 285–97. https://doi.org/10.1007/s00221-010-2228-0

Zhou, Yi, L., & Riccio, D. C. (1994). Pretest Cuing can alleviate the forgetting of contextual stimulus attributes. *Learning and Motivation*, *25*(3), 233-244.

Zola-Morgan, S., & Squire, L. R. (1993). Neuroanatomy of Memory. *Annual Review of Neuroscience*, *16*(1), 547–563.https://doi.org/10.1146/annurev.ne.16.030193.002555