PATTERNS OF HIPPOCAMPAL AMNESIA AND POPULATION ACTIVITY IN RATS: IMPLICATIONS FOR CATEGORICAL AND SINGLE-PROCESS MODELS OF LONG-TERM MEMORY ORGANIZATION

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Dedication

For the Critchfields.

Abstract

Contemporary views on the organization of long-term memory (LTM) suggest the hippocampus is involved in a unique category of LTM. However, recent experiments illustrate that hippocampal damage before and after a learning episode result in different patterns of amnesia, and many types of memory are affected by damage after the learning episode. These results challenge contemporary views of LTM organization and motivate the present thesis. We describe a concept, termed heterarchic reinstatement (HR) to account for the pattern of amnesia following hippocampal damage. We observed a pattern of results, in both hippocampal activity and amnesia following damage that generally support the HR view, although an experiment using temporary inactivation also reveals limitations to this concept. Thus, we provide a new predictive model of hippocampus and memory, termed the Memory Manifold Theory (MMT), that incorporates the HR concept and our observations along with the broader research literature.

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Abbreviations

ANOVA	Analysis of variance
CA1	Cornu Ammonis Area 1
CA3	Cornu Ammonis Area 3
CWT	Concurrent cue-place water task
DG	Dentate Gyrus
HR	Heterarchic reinstatement
LTM	Long-term memory
MMST	Multiple Memory Systems Theory
MMT	Memory Manifold Theory
NMDA	N-methyl D-aspartate
S+	Positively reinforced conditioned stimulus
S-	Negatively reinforced conditioned stimulus
TPWT	Two-platform water task

Chapter 1

Heterarchic Reinstatement of Long-Term Memory: A concept on hippocampal amnesia in rodent memory research

Abstract

Evidence from human patients and nonhuman animal research highlights the role of the hippocampus in long-term memory (LTM). Decades of experimental work have produced numerous theoretical accounts of the hippocampus in LTM, and nearly all of them suggest that hippocampal disruption produces amnesia for specific categories of memory. These accounts imply that hippocampal disruption before or soon after a learning episode should have equivalent amnestic effects. Recent evidence from lesion and inactivation experiments in rodents illustrates that hippocampal disruption after a learning episode causes memory impairment in a wider range of memory tasks than if the same disruption occurs before learning. Although this finding supports that multiple circuits can acquire and retrieve similar information, it also suggests they do not do so independently. In addition, damage after learning produces amnesia for simple elements of a task as well as complex, conjunctive features. Here we develop an explanation for why anterograde and retrograde hippocampal effects differ. This explanation, the heterarchic reinstatement view, also generates novel predictions.[1]

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1. The hippocampus and LTM

LTM is the ability to recall information long after a learning episode. The period of recall can last hours, days, years, or an entire lifetime. Evidence from clinical research and experimental work with non-human animals emphasizes the role of the hippocampus in LTM. A key finding supporting this conclusion is that damage to the hippocampus causes retrograde amnesia (RA), that is, the inability to recall information from a learning episode that preceded the damage, in addition to an inability to form new long-term memories (anterograde amnesia, AA; Gilboa et al., 2006; Scoville & Milner, 1957; Steinvorth et al., 2005; Sutherland et al., 2010; Squire, 1992). Early on it was also shown that certain types of LTM were not affected by hippocampal damage. Some memories were lost and subjects were unable to acquire certain types of new memories, while other types of memory and abilities remained intact (Scoville & Milner, 1957; Zola-Morgan et al., 1986). Despite the early recognition of these facts, no consensus on their explanation has emerged. In the present discussion, our goal is not to present a comprehensive theory of the hippocampus in LTM, but rather is much more limited. We examine anomalous experimental results on amnesia and their conceptual implications for a modern view of how memory is organized in the brain.

Several theories have been developed to explain memory impairments following hippocampal disruption. Popular models highlight the role of the hippocampus in spatial

(Morris et al., 1982; O'Keefe & Nadel, 1978; Sutherland et al., 1983; Sutherland et al., 1982), temporal (Eichenbaum, 2014; Davachi & DuBrow, 2015), episodic (Nadel & Moscovitch, 1997; Steinvorth et al., 2005; Squire & Zola, 1998), and more generally, relational and configural memory (Cohen & Eichenbaum, 1993; Eichenbaum et al., 1988; Rudy & Sutherland, 1995; Sutherland et al., 1989; Sutherland & Rudy, 1989; Wickelgren, 1979). Although contemporary views differ in their categorization of hippocampal function, they collectively posit two hypotheses: 1) hippocampal disruption will cause memory impairments in a specific range of memory tests; 2) hippocampal disruption before or soon after learning should elicit similar impairments.

The idea that memory should be equally affected if the hippocampus is disrupted before or soon after learning is consistent with the general notion that different brain areas are required for different types of memory (Gold, 2003; Hirsh, 1974; McDonald & Hong, 2013; McDonald & White, 1993; Packard et al., 1989; Packard & White, 1991; Scoville & Milner, 1957; Squire, 1992; White & McDonald, 2002), and that each system stores information more or less independently and in parallel (Gulbrandsen et al., 2013; Packard & White, 1991; Sutherland et al., 2010; White & McDonald, 2002). These types of memory might include that for objects, locations, actions, visual and auditory stimuli, odours, and various outcomes. The segregation of memory functions to different brain areas is a basic tenet of a class of theories that are termed Multiple Memory Systems Theories (MMST; Squire, 1992; White & McDonald, 2002). Indeed, a large body of empirical work details the role of the hippocampus in spatial, temporal, relational, and episodic memory (Schiller et al., 2015). For example, hippocampal damage or

inactivation impairs the ability of animals to acquire spatial (Morris et al., 1982; Sutherland et al., 1982; Sutherland et al., 1983), temporal (Fortin et al., 2002), and relational or configural associations (Eichenbaum et al., 1988; Sutherland & McDonald, 1990; Sutherland et al., 1989). The same damage or inactivation made before or during a learning episode does not impair other types of memory, including non-spatial, non-temporal, and elemental features of an episode (Alvarado & Rudy, 1995; Bangasser et al., 2006; Solomon et al., 1986; Sutherland & McDonald, 1990). *Prime facie*, these findings support contemporary views of hippocampal function. However, damage or inactivation of a brain area prior to a learning episode does not necessarily reveal whether that region is normally involved in learning and memory as a result of the episode. Rather, these approaches demonstrate which functions can be supported by other brain networks. Nonetheless, popular theories on the hippocampus in LTM suggest that its disruption prior to or after learning should result in similar memory deficits. Each popular view of the hippocampus in LTM, including the Standard Model of Systems Consolidation (SMSC; Squire, 1992), Multiple Trace Theory (MTT; Nadel & Moscovitch, 1997), Transformation Theory (Winocur et al., 2013), Indexing Theory (Teyler & DiScenna, 1986), Relational Memory Theory (Cohen & Eichenbaum, 1993), Configural Association Theory (Rudy & Sutherland, 1995; Sutherland & Rudy, 1989), Spatial Mapping Theory (O'Keefe & Nadel, 1978), and the Multiple Memory Systems Theory (Squire, 1992; White & McDonald, 2002) assume that different brain areas are involved in different types of memory. Each popular model suggests that hippocampal damage would specifically impair mnemonic processes to which it uniquely contributes.

Contrary to this basic tenet of popular theories, many investigators have reported that hippocampal disruption before and after learning in rodents do not produce equivalent amnestic effects. Hippocampal damage or inactivation prior to a learning episode causes AA for spatial, temporal, and relational memory, while its disruption after learning results in RA for a much wider range of memory types. This includes RA for spatial and non-spatial, temporal and non-temporal, elemental, and relational types of memory. This outcome is not likely due to non-specific effects of lesion or acute inactivation, since both types of disruption result in similar experimental outcomes (Otchy et al., 2015; Table 1.1). Evidence for the differential effects of hippocampal damage or inactivation on AA and RA are described almost uniquely in rodent literature. As a result, the evidence we discuss is restricted primarily to rodent memory research.

Task	AA	RA	Reference
Context	No	Yes	Sparks et al. (2011b)
Context	No	Yes	Wiltgen et al. (2006)
Context	–	Yes	Broadbent and Clark (2013)
Context	–	Yes	Lehmann et al. (2007a,b,c)
Context	–	Yes	Sparks et al. (2011a)
Context	–	Yes	Sparks et al. (2013)
Context	–	Yes	Wang et al. (2009)
Context	–	Yes	Sutherland et al. (2008)
Home base	No	Yes	Travis et al. (2010)
Home base	No	–	Lehmann et al. (2007a,b,c)
Light	No	Yes	Lehmann et al. (2010)
Object	–	Yes	Sutherland et al. (2001)
Object	No	–	Morris et al. (1986)
Object	No	Yes	Gaskin et al. (2003)
Object	No	Yes	Broadbent et al. (2007)
Picture	No	Yes	Driscoll et al. (2005)
Picture	No	Yes	Epp et al. (2008)
Tone	No	–	Bangasser et al. (2006)
Tone	–	Yes	Sutherland et al. (2008)
Tone	–	Yes	Broadbent and Clark (2013)

Table 1.1 The table illustrates findings within and across studies that demonstrate RA but not AA for several types of memory. Examples have been limited to reports of complete hippocampal damage or inactivation (>70%) resulting in RA but not AA. As we discuss, these findings are anomalous in the context of modern theories on the hippocampus in LTM. Some conflicting results exist with hippocampal lesions on object memory (see Broadbent et al., 2004, 2010). The reason for these differences between reports is unknown, and we suggest merits further investigation (see Section 7).

Table 1.1 illustrates examples wherein complete (>70%) hippocampal damage or inactivation has resulted in RA but not AA for numerous memory types, including

context fear, tone fear, light fear, picture memory, object recognition, and home base memory. Although an exhaustive list of examples may be greater than Table 1.1 demonstrates, including tasks such as paired associate learning (Kim et al., 2015), and earlier reports of context and tone fear conditioning (Frankland et al., 1998; Maren et al., 1997), we have restricted Table 1.1 to cases wherein hippocampal damage or inactivation is extensive (>70%). Several studies have revealed that the extent of RA soon after learning correlates with hippocampal damage (Epp et al., 2008; Lehmann et al., 2007a; Lehmann et al., 2007b; Lehmann et al., 2007c; Sutherland et al., 2008). Therefore, outcomes of studies with incomplete (< 70%) or unreported amounts of hippocampal damage or temporary inactivation should be interpreted carefully (Sutherland et al., 2010).

The prediction that hippocampal disruption introduced before or soon after learning should result in similar, specific deficits in memory is at odds with the experimental outcomes in Table 1.1. Instances wherein hippocampal disruption causes RA but not AA for a given type of memory are anomalies in the context of popular theories of the hippocampus in LTM. As Table 1.1 illustrates, this phenomenon has been observed in a variety of rodent memory tasks, and has been previously explained by a concept termed, "hippocampal overshadowing" (Driscoll et al., 2005; Fanselow, 2009; Maren et al., 1997; Rudy et al., 2004; Sparks et al., 2011b; Sutherland et al., 2010).

2. The hippocampal overshadowing concept

Hippocampal overshadowing is a process that has been invoked to account for instances when amnesia after hippocampal disruption is observed in the retrograde, but not the anterograde direction. It posits that the hippocampus interferes with acquisition and retrieval in non-hippocampal regions (Biedenkapp & Rudy, 2008; Driscoll et al., 2005; Fanselow, 2009; Maren et al., 1997; Rudy et al., 2004; Sutherland et al., 2010). This is analogous to the Pavlovian concept, wherein if two cues equally predict an unconditioned stimulus, animals will show strong conditioning to the more salient of two cues. A similar phenomenon might occur between memory systems, whereby one more dominant system overshadows another system at the time of the learning episode (Maren et al., 1997; Fanselow, 2009; Rudy et al., 2004; Sutherland et al., 2010). There are a small number of proposals on the mechanisms that could mediate hippocampal overshadowing. There could be a competition during association formation between the hippocampal representation and non-hippocampal representations − a competition that the hippocampus normally wins. This could reduce synaptic plasticity between non-hippocampal representations of a cue with outcomes, thereby preventing or retarding non-hippocampal memory acquisition (Biedenkapp & Rudy, 2008; Fanselow, 2009). On a related idea, the output from the hippocampus could simply inhibit non-hippocampal cue representations (see Section 3; McDonald & Hong, 2013). We propose a novel, simpler alternative explanation of RA in cases where AA is absent. Normally the hippocampus creates a representation of cues in the learning episode and the code contained in hippocampal output directly and indirectly to cortical regions interacts with the sensory-driven representations in these areas. Subsequently, reiteration of this code,

through connections to the cortex from the hippocampus, is necessary to reinstate the full target memory, including activation of relevant subcortical areas that participated in forming associations at the time of learning. If the hippocampus is then taken off-line, the sensory driven cortical representations alone do not sufficiently resemble the patterns of cortical activity at the time of learning and thus do not reinstate the target memory. If the hippocampus was off-line for both learning and retrieval, then acquisition and retention will be largely unaffected. This novel interpretation is central to the view of memory organization that we present below (section 7).

On this view, memory retrieval depends on reinstatement of an activity pattern that includes bottom-up sensory input, and top-down feedback from hippocampus and association areas, such as parahippocampal cortex. For example, if a learning episode occurs in an environment composed of sensory features A, B, C, and D, then memory retrieval will require the presentation of A, B, C, and D, in addition to top-down feedback from hippocampus and association cortices, or pattern completion of these features by hippocampus if a subset of features is presented. If the hippocampus is absent during a learning episode, the memory representation includes top-down feedback only from association areas. Reinstatement is achieved in much the same way if the hippocampus is absent during learning and retrieval when all sensory features are presented, wherein an activity pattern is reinstated from both top-down and bottom-up inputs. The same set of fibres from the cortex then project to effectors of behaviour to elicit a response.

Importantly, the new interpretation of how the hippocampus might interact with non-hippocampal systems during learning and memory retrieval makes a novel

behavioural prediction. The prediction has received only very limited experimental evaluation. Sparks et al. (2011b) provided rats with a single context fear conditioning session while the hippocampus was temporarily inactivated. Two memory retrieval tests were performed: one with the hippocampus on-line (RT1), and another where the hippocampus was off-line (RT2). In RT1, rats that had their hippocampi off-line during conditioning did not freeze more than shock-naïve animals. In RT2, the same animals showed similar freezing responses as shocked controls. The hippocampal output during RT1 appeared to interfere with the ability to retrieve a memory encoded in non-hippocampal regions, whereas memory was retrieved when the hippocampus inactivated during RT2. This result was interpreted as direct support for the hippocampal overshadowing concept. However, the inhibitory account of overshadowing does not explain the outcome in RT1. If overshadowing were caused by the hippocampus interfering with plasticity, through either competition or inhibition in non-hippocampal systems, then retrieval would have been intact in RT1 and RT2, since acquisition could not have been retarded while the hippocampus was off-line. Instead, hippocampal output to distributed, non-hippocampal regions during RT1, which did not contain any information about the learning episode, prevented the non-hippocampal representation from being retrieved and thereby led to amnesia. On a cautionary note, the extent of inactivation during local anaesthesia was not assessed in the experiment. It is possible that some portion of hippocampus was not inactivated during encoding or retention testing. It would be ideal in future studies to repeat this type of experiment using methods to

confirm complete inactivation during training and test sessions in additional memory tasks (Gulbrandsen & Sutherland, 2014).

Although our novel interpretation involves a single process model, the evidence summarized above generally supports a dual-role of the hippocampus in LTM that multiple groups have proposed (Biedenkapp & Rudy, 2008; Driscoll et al., 2005; Fanselow, 2009; Maren et al., 1997; McDonald & Hong, 2013; Rudy, 2009; Rudy et al., 2004; Sparks et al., 2011b; Sutherland et al., 2010; Wiltgen et al., 2006). Namely, that: 1) the hippocampus creates a conjunctive representation of information contained in the learning episode, even for tasks that do not require a conjunctive/relational solution; 2) the hippocampus prevents representations in non-hippocampal systems from participating in associations formed during learning (Rudy et al., 2004). Although several groups have suggested possible mechanisms for a dual role of the hippocampus in LTM, these ideas have so far received very little attention.

3. What prevents hippocampus-independent LTM?

A central concern of systems neuroscience is how brain regions interact with one another to gain control of behaviour (McDonald & Hong, 2013). Although several mechanisms have been proposed on how the dual role of the hippocampus in LTM might emerge, and we will briefly review each of these ideas, we suggest that a fresh perspective is necessary to account for the full range of experimental results.

First we consider the dynamic memory systems concept (Fanselow, 2009). This view posits that the most efficient system for supporting memory comes to control

behaviour through a competitive process, where the "winner" prevents learning in other, parallel memory systems (see also Biedenkapp & Rudy, 2008). On this view, alternative circuits are able to provide compensatory mnemonic function in case a primary region is damaged (Zelikowsky et al., 2013). In evolutionary terms, however, it is difficult to imagine the selection pressures that would produce this sort of redundancy. We do not see the compensatory rationale as sufficient reason for the emergence of multiple brain regions with overlapping mnemonic capabilities (see Sherry & Schacter, 1987). Rather, it is more likely that multiple regions are able to acquire similar memories through fundamental associative processes shared among these areas and overlapping input that each area receives.

An additional inhibitory account of overshadowing, which differs from the dynamic memory systems view, is that the hippocampus automatically acquires information during learning that inhibits learning in non-hippocampal regions (McDonald & Hong, 2013). For example, retrograde but not anterograde lesions of the hippocampus in tasks that depend on an efficient S-R behavioural strategy or visual discrimination elicits marked performance deficits (Epp et al., 2008; Ferbinteanu & McDonald, 2001; Sutherland et al., 2001). Automatically acquired context associations encoded in the hippocampal system also interferes with reversal learning in an S-R win-stay task, which is dependent on the dorsolateral striatum (DLS) for task acquisition (McDonald et al., 2006; McDonald et al., 2001). Further experiments revealed that a hippocampus-dependent context representation can interfere with acquisition of a conditioned cue preference task that requires the amygdala (Ferbinteanu & McDonald,

2001; McDonald & White, 1993; McDonald & White, 1995). Importantly, this observation counts against the dynamic memory system idea that the most efficient system dominates. Instead, these data suggest an obligatory role of a hippocampal representation in controlling behaviour. This view shares commonalities with our new perspective in that the hippocampus automatically acquires information during any learning episode that becomes critical for memory retrieval. However, we suggest that it is not necessary to invoke an inhibitory account of these data. On a different interpretation (sections 2 and 7), the automatically acquired hippocampal code may result in less efficient task acquisition due to stimulus conjunctions and relationships between task features projecting to effectors of behaviour, rather than simple elements of the task alone being represented that would enable more efficient responding. Although we suggest that the hippocampus is involved in both simple and complex feature representations, non-hippocampal representations may be more biased toward representing simple elements alone (Rudy & Sutherland, 1995), and thus would be more efficient for certain types of memory-guided behaviour.

Another recent account of the dual role of the hippocampus in LTM is that multiple regions compete for control of an output structure (Gruber & McDonald, 2012; Ito et al., 2006; Ito et al., 2008; McDonald & Hong, 2013). For example, electrophysiological and tracing studies support the ventral striatum (VS), particularly the nucleus accumbens, as both a locus of convergence and possible competitive interaction between hippocampal, amygdalar, and prefrontal inputs (Groenewegen et al., 1999; Groenewegen et al., 1997; O'Donnell & Grace, 1995). Recent work (Gruber et al., 2009a;

Gruber et al., 2009b) suggests that the nucleus accumbens may act as a switch-board to dynamically control which of its inputs, including prefrontal, amygdalar, and hippocampal, determine goal-directed actions. Gruber and McDonald (2012) discussed how this type of interface might determine which regions dominate behavioural control under certain circumstances. On our interpretation, we suggest that a hippocampal code determines activity dynamics in many brain regions to generate overt behaviour, including the VS, following initial experience in any task (section 7).

In addition to the proposals already discussed, there may be other explanations of how the hippocampus gains dominant influence on behaviour and what gives rise to widespread RA following hippocampal damage or temporary inactivation, rather than hippocampus-independent control of LTM. Throughout our discussion it is important to keep in mind that hippocampal and non-hippocampal systems do not perform the same computations nor are each capable of producing the equivalent range of behaviour. Rather, two systems can produce observably similar behaviours in many memory tasks typically used with rodents, albeit based on different computations and motivations. In cases where memory tasks can be solved both in the presence or absence of the hippocampus, such as contextual fear, object recognition, or picture discrimination (Table 1), variants of each task reveal how memories encoded in the absence of the hippocampus may differ in important ways.

4. How do hippocampal and non-hippocampal memories differ?

Despite numerous types of memories being supported in the absence of the hippocampus, there are several distinctions on how hippocampal and non-hippocampal systems encode and retrieve information from a learning episode. Differences may exist in the ability of each system to perform complex feature discriminations, whether the acquisition is automatic or driven by task demands, the manner in which perceptual information is represented, and the rate at which systems acquire information (see Rudy, 2009).

Generally, there are two ways in which a learning context can be represented: as a collection of individual features or elements, or as a conjunction of elements making up the learning context (Fanselow, 2009; Nadel & Willner, 1980; O'Reilly & Rudy, 2001; Rudy et al., 2002; Rudy et al., 2004; Rudy & O'Reilly, 2001; Rudy & Sutherland, 1995; Sutherland & Rudy, 1989). Nadel and Willner (Nadel & Willner, 1980; Nadel et al., 1985), and later Sutherland and Rudy (Sutherland, 1985; Sutherland & Rudy, 1989) proposed that the cortex represents cues as individual features, and that the hippocampus assembles individual features into conjunctive representation of stimulus elements comprising the learning context (see also Cohen & Eichenbaum, 1993; Marr, 1971; Sutherland, 1985). It may be the case that, in the presence of the hippocampus, there is a bias toward conjunctive, rather than feature-based, representation (O'Reilly & Rudy, 2001; Rudy et al., 2002; Rudy & O'Reilly, 2001; Rudy & Sutherland, 1995). Behavioural tasks wherein animals are required to solve nonlinearly separable problems, such as negative or transverse patterning (Fig. 1.1), reveal brain circuits that represent conjunctions or relationships among cues. Over the past three decades experiments have

revealed that there is a subset of nonlinear discriminations that rats cannot solve following hippocampal damage (Fig. 1.1; Alvarado & Rudy, 1995; Driscoll et al., 2005; Sutherland & McDonald, 1990; Sutherland et al., 1989; Sutherland et al., 2010; Whishaw & Tomie, 1991), whereas linear solutions can be formed readily without the hippocampus (Driscoll et al., 2005; Epp et al., 2008; Sutherland & McDonald, 1990). Additional support on the importance of the hippocampus for memory of the relationships between task features comes from results demonstrating that the hippocampus and its output to distributed brain areas is critical for a transitive inference, wherein the indirect relationships between features must be inferred (e.g. A > B; B > C; therefore A > C; Bunsey & Eichenbaum, 1996, Dusek & Eichenbaum, 1997). These results collectively show that animals without a hippocampus cannot learn many complex relationships and conjunctions between task features, supporting that hippocampal and non-hippocampal memories differ.

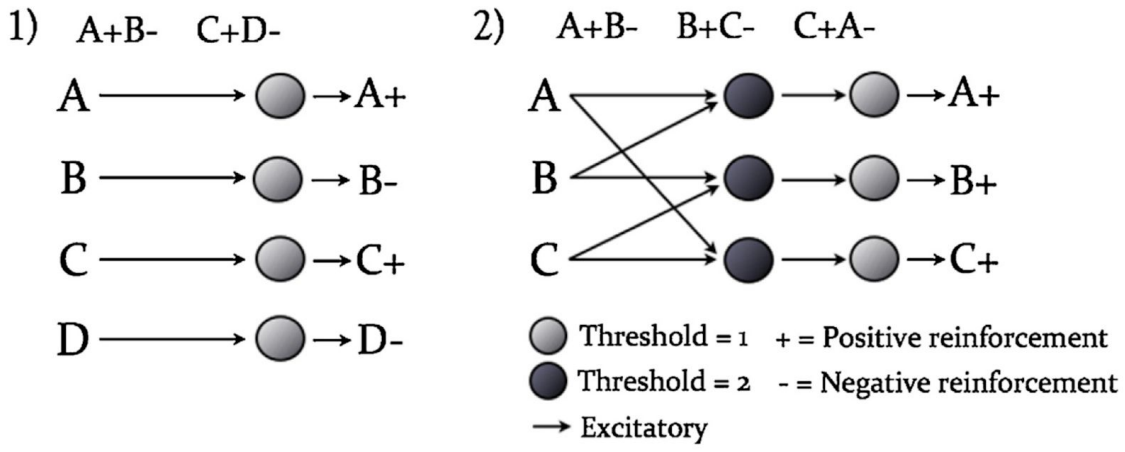


Figure 1.1 Nonlinear separability and the hippocampus. The schematic illustrates how associations between stimulus elements (A, B, C, and D) and a reinforced behavioural response can produce solutions for linearly separable and nonlinearly separable (XOR) problems. Solutions to the first, elemental, problem set (1) can be produced with linear associations between each stimulus element (A, B, C, and D) and output nodes (+ or −). In the latter problem set (2), termed transverse patterning, each element is equally associated with a positively and negatively reinforced output. Therefore, linear associations between stimulus elements and output nodes do not enable correct discriminations between cues when cue combinations (AB, BC, or CA) are presented. A conjunctive layer that consists of nodes that each has a threshold of two excitatory inputs allows for discrimination between reinforced and non-reinforced stimuli. Experiments have shown that animals can solve elemental problems but not transverse patterning problems without the hippocampus (Alvarado and Rudy, 1995, Driscoll et al., 2005). Damage to the hippocampus after learning causes memory loss for both types of problems (Driscoll et al., 2005, Epp et al., 2008).

Details about the setting of an event are often acquired without the intent to commit this information to memory. Thus, much of the information about daily learning episodes and the settings in which they occur are encoded automatically. The hippocampus is likely essential to automatic encoding of information across a wide variety of tasks (Matus-Amat et al., 2004; McDonald & Hong, 2013; Rudy, 2009; Rudy & O'Reilly, 2001), whereas non-hippocampal systems have been argued as not doing so

without explicit task demands or reinforcement (O'Reilly and Rudy, 2001; Rudy, 2009; Rudy and O'Reilly, 2001). Similarly, we suggest that the hippocampus acquires information that is part of a distributed memory trace during any learning experience, and is later necessary for reinstatement of a target memory (section 7). Variants of object recognition and context fear conditioning in rodents clearly illustrate the unique contributions of the hippocampus to automatic memory encoding of several task features.

Animals explore novel objects more than recognized items in a familiar context, and will investigate familiar objects that have changed position since a previous encounter. Although reports are mixed with the anterograde effects of hippocampal damage on the ability of animals to recognize objects (see Broadbent et al., 2004; Broadbent et al., 2010), some reports suggest that hippocampal rats insensitive to changes in the context where familiar objects occur, or changes in object location (Gaskin et al., 2003; Mumby et al., 2002; but see O'Brien et al., 2006; but see O'Brien et al., 2006). In such tasks, novelty is the only reinforcing characteristic of the experience for animals to encode the relationship between objects and the environment. Acquisition of the arrangement of objects and the context in which they occur is therefore considered automatic, and a process to which the hippocampus may uniquely contribute. The impairments observed following damage to the hippocampus in acquiring context information without appetitive or aversive reinforcement supports its role in automatic encoding. This attribute of the hippocampus is further supported by its involvement in context pre-exposure facilitation in fear conditioning.

Fanselow (1990) reported that animals must explore a context for several minutes before receiving a shock in order to develop a strong context-shock association. Animals that were shocked immediately after being placed in a context did not develop a fear memory. Rats also required several seconds when placed back into the conditioning environment prior to the shock delivery in order to condition, even if they underwent context pre-exposure. Fanselow suggested that, during pre-exposure, animals acquire a conjunctive representation or gestalt of the context that becomes associated with the subsequent shock (although he originally used the Pavlovian term, "dynamic stereotype"; see Fanselow, 2009), and immediately retrieve a unitary representation of the context as soon as they enter the context again. Based on configural and relational theories of the hippocampus in LTM (Cohen & Eichenbaum, 1993; Sutherland & Rudy, 1989), it was expected that the hippocampus would have a critical role in forming a conjunctive or gestalt-like representation of cues that comprise the learning context during pre-exposure, and subsequent retrieval upon context re-entry at the time of the immediate shock delivery. Retrieval from a subset of cues is hypothesized to occur through a pattern completion process, whereby an entire representation is retrieved based on partial input (Marr, 1971).

In order to determine the role of the hippocampus in pre-exposure facilitation and pattern completion, Rudy et al. (2002) transported rats to a neutral context (A) several times in a covered transport container and pre-exposed animals for varying intervals of time. Following pre-exposure, rats were transported in the same covered apparatus to a different context (B) and immediately shocked. On the following day, the animals were

taken either to context A or B on a different transportation device, and assessed for freezing behaviour. Animals exhibited increased freezing in the pre-exposed context, rather than the context where they were shocked. From this finding, Rudy et al. (2002) concluded that animals construct a unitary representation of a context during pre-exposure, and this representation can be recalled from a partial set of cues through pattern completion (see also Matus-Amat et al., 2004). This result confirms that fear becomes associated with this context representation at the time of shock delivery, as previously suggested (Fanselow, 1990; Fanselow, 2009; Rudy & O'Reilly, 2001; Sutherland & Rudy, 1989). Damage to the dorsal hippocampus also prevented this effect and context pre-exposure facilitation. These findings support that the hippocampus has a unique role in the conjunctive encoding of a learning context, and that it is this context representation that is rapidly retrieved through a pattern completion process that becomes associated with fear at the time of shock delivery (O'Reilly & Rudy, 2001; Rudy, 2009; Rudy & O'Reilly, 2001). Subsequent investigations on the role of the hippocampus in pattern completion using electrophysiologic population recording and immediate early gene imaging methods have revealed that CA3 makes unique contributions to pattern completion and rapid, automatic encoding (see Leutgeb & Leutgeb, 2007; Yassa & Stark, 2011).

Beyond hippocampal contributions to automatic encoding and pattern completion, it is also important in pattern separation (Gilbert et al., 2001; Lee & Kesner, 2004; Leutgeb et al., 2005; Leutgeb et al., 2007; Leutgeb & Leutgeb, 2007; Yassa & Stark, 2011). While pattern completion is the ability to retrieve an entire representation from a

partial set of cues, pattern separation is the ability to orthogonalize similar inputs based on their non-overlapping elements. Electrophysiologic population recording, immediate early gene imaging, and lesions studies have revealed a unique role of the dentate gyrus and CA3 in pattern separation (Vazdarjanova & Guzowski, 2004; Lee & Kesner, 2004; Leutgeb et al., 2007). While CA3 may perform either pattern completion or separation based on the degree of input similarity between encoded and presented stimuli, the dentate gyrus performs pattern separation on highly overlapping stimulus patterns and representations (Leutgeb et al., 2007; Leutgeb & Leutgeb, 2007). However, as Stark and Yassa (2011) discussed, pattern separation is not unique to the hippocampus. Rather, this computation is performed on various sensory modalities in multiple brain areas, including disambiguation of visual features, odours, objects, and reward value (see also Kent et al., 2016). The hippocampus likely performs unique separation processes in cognitive domains to which it uniquely contributes, such as spatial and temporal aspects of a learning episode (Gilbert et al., 2001; Lee & Kesner, 2004). We suggest, however, that it remains unclear to what extent the hippocampus contributes to pattern separation for memory processes that it is normally involved in but not required, such as contextual fear.

Discriminative fear conditioning to context is a training paradigm that requires animals to discriminate between shock-paired and neutral contexts, wherein the similarity between two training contexts can be manipulated, and thus pattern separation can be examined (Antoniadis & McDonald, 2000; Antoniadis & McDonald, 2001; McDonald et al., 2004a; McDonald et al., 2004b; McDonald et al., 1995). Increasing the similarity between contexts is argued to place demand on pattern separation to discriminate between

a shock-paired and neutral environment. Several experiments supported the role of the hippocampus in the discriminating between shock-paired and neutral contexts (Antoniadis and McDonald, 2000; Antoniadis and McDonald, 2001; Frankland et al., 1998). However, the extent to which hippocampal and non-hippocampal systems differ in their abilities to disambiguate threatening and neutral contexts in this paradigm remains unclear. In contrast to previous results, recent observations in our laboratory support that the hippocampus is required for ambiguous but not distinct contextual discrimination (Lee et al., 2015). We are currently determining the features that animals use to discriminate between contexts when various stimuli, such as colour, odour, geometry, and tactile stimuli, are shared between contexts. McDonald et al. (2004b) suggested that titrating the ambiguity between paired and unpaired contexts would place greater demand on pattern separation processes that require the hippocampus. Indeed, evidence from population recording and immediate early gene studies support that the dentate gyrus makes unique contributions to discriminating highly similar spatial contexts (Leutgeb et al., 2007). Our group is currently resolving these uncertainties further in variants of the contextual discrimination paradigm.

Beyond the contributions of hippocampus to automatic encoding, pattern completion, and pattern separation, another widely held view is that the hippocampus makes unique contributions to these computations because it acquires information faster than non-hippocampal systems (McClelland et al., 1995). However, we suggest that it is unclear whether learning rates between hippocampal and non-hippocampal system actually differ. On the current issue of widespread RA following hippocampal disruption,

this topic is particularly relevant as it relates the competitive learning account of the hippocampal overshadowing concept. If learning rates between hippocampal and non-hippocampal systems do not differ, this outcome would offer no support for the dynamic memory systems view (Fanselow, 2009), as it could not be the case that the hippocampus interferes with learning in non-hippocampal systems if learning rates are equivalent in the presence or absence of the hippocampus.

5. Learning rates in hippocampal and non-hippocampal systems: does the seahorse win all races?

Differences in learning rates across memory systems are computationally advantageous. This type of distribution of labour directly addresses a central issue that Marr (1970) raised: the likelihood of stimuli presenting themselves identically across experiences is miniscule. There is also a need to represent the specific content of experiences. Ideally, memory systems should preserve specific content unique to experiences while also extracting statistical regularities across episodes to give rise to flexible behaviour in novel situations. This goal can be achieved by employing different learning rates across memory systems. McClelland et al. (1995) therefore modelled the hippocampal system as a fast learner and the cortex as a slow learner. Under this framework, the hippocampus stores detail-rich representations of experiences, whereas the cortex extracts invariant characteristics of environments and events across episodes.

O'Reilly and Rudy (2001) employed this principle in a computational model while attempting to replicate multiple published results. They replicated findings that

animals can learn context fear without the hippocampus, but cannot resolve a subset of nonlinearly separable discriminations or acquire conjunctive representations without explicit task demands. Although the use of a complementary learning systems approach can be used to replicate several findings in the experimental literature, the dissociable learning rates of hippocampal and cortical systems were assumed a priori. Current empirical evidence may not adequately support that learning rates actually differ between these systems.

One experiment that is often cited in support of different learning rates between hippocampal and non-hippocampal systems is from Packard and McGaugh (1996), which aimed to determine the contributions of different brain areas to behavioural strategies using a cross maze paradigm (see also Tolman et al., 1946). Rats were released from a single start location in a cross maze and were tasked to approach one of two arms for a food reward. The animal could learn to make a specific turn in order to gain a reward (i.e. a response strategy), or travel to the rewarded location (i.e. a place strategy). Animals were given 7 or 14 days of training in the task, and were tested for their behavioural strategy on days 8 and 16. Upon testing, animals were given either caudate or dorsal hippocampal infusions of saline or lidocaine and released from the opposite start location from training in the cross maze. A response strategy would result in the animal turning toward the opposite location compared to training, whereas a place strategy would result in the animal turning toward the same location as training.

Animals predominantly exhibited a place strategy on day 8, and a response strategy on day 16 (Packard & McGaugh, 1996). Hippocampal contributions were

assessed with lidocaine infusions to dorsal hippocampus, which resulted in animals exhibiting neither place or response strategies on day 8, and no change in behaviour on day 16. By contrast, lidocaine infusion into the caudate did not affect animals' behaviour on day 8, and on day 16 elicited the use of a place, rather than response, strategy. These findings have been interpreted to suggest that animals acquire place and response strategies in parallel, but that each strategy differentially controls behaviour with variable amounts of training (Packard & McGaugh, 1996). Namely, the results show that a place strategy controls behaviour early in training, whereas a response strategy dominates animal behaviour with additional experience (see also Tolman et al., 1946). Packard and McGaugh (1996) also concluded that independent neural systems produce each behaviour: the caudate being responsible for a response strategy, and the hippocampus being necessary for a place strategy.

By contrast to Packard and McGaugh (1996), it might not be the case that each system independently controls behavioural strategies in the cross maze. For example, the hippocampus could be involved in both place and response behaviours, but incomplete hippocampal inactivation spared a response strategy. Subsequent studies have revealed that the extent of hippocampal disruption following training in tasks that do not require the hippocampus for acquisition correlates strongly with RA soon after learning (Lehmann et al., 2007a; Lehmann et al., 2007b; Lehmann et al., 2007c; Sutherland et al., 2008; Epp et al., 2008) and that repeated experience influences the ability of animals to retrieve memory independently of the hippocampus (Sutherland & Lehmann, 2011). Both

of these factors may confound the conclusion that the hippocampus is not involved in a response strategy in Packard and McGaugh (1996).

In order to examine the effects of repeated, distributed experience on the organization of LTM, Lehmann et al. (2009) fear conditioned rats with 10 foot shocks either administered in a single massed session, or in 10 separate sessions distributed over 5 days. Following an equal passage of time after initial conditioning, animals were given complete hippocampal lesions. Lehmann et al. (2009) found that distributed, but not massed training spared context fear memory from hippocampal damage. It could also be that repeated experience in the Packard and McGaugh (1996) study was sufficient to make a response strategy in the cross maze hippocampus-independent. Prime facie, the findings of Packard and McGaugh (1996) and Lehmann et al. (2009) appear to support the view that non-hippocampal systems learn more slowly than the hippocampus (McClelland et al., 1995; O'Reilly & Rudy, 2001).

To appropriately examine learning rates in hippocampal and non-hippocampal systems it is necessary to test learning in the presence or absence hippocampus with lesions or temporary inactivations introduced prior to or during training, respectively. Neither the study of Packard and McGaugh (1996) or Lehmann et al. (2009) met these criteria. Further, it is also necessary to assess the same dependent measure of memory with variable amounts of training, which can be supported in the presence or absence of the hippocampus. It is unreasonable to conclude, for example, that the caudate learns more slowly than hippocampus because response strategies are acquired more slowly than place strategies. As Kim et al. (2015) discuss, it is not necessarily the case that a

behaviour acquired incrementally over many trials is independent of the hippocampus. For example, Kim et al. (2015) found that paired associate memory acquired over many trials depends on the hippocampus in the retrograde, but not anterograde, direction. Response strategies may be acquired slowly due to the characteristics of associations that must be formed to exhibit response behaviour, and not due to a slow learning rate in the brain areas necessary for the target behaviour *per se*. In picture memory tasks where animals must swim toward one of two pictures to escape from a pool, which should depend on caudate for S-R memory, multiple studies have demonstrated that learning rates on problems acquired in the absence of the hippocampus are no different than when hippocampus is present (Driscoll et al., 2005; Epp et al., 2008). The same hippocampal disruption introduced after learning results in RA that correlates with the extent of hippocampal damage (Epp et al., 2008). These findings shed doubt on the view that non-hippocampal systems learn more slowly than hippocampus.

However, two studies from one laboratory suggest that non-hippocampal systems learn more slowly than hippocampus in contextual fear conditioning (Fanselow, 2009; Wiltgen et al., 2006; Zelikowsky et al., 2012; but see Maren et al., 1997). In these studies, animals were given several foot shocks and freezing behaviour was examined between each shock. Rats with hippocampal damage exhibited lower levels of freezing following one, but not three foot shocks (Wiltgen et al., 2006). However, it remains unclear whether these deficits are in fact due to hyper locomotion in hippocampal rats, or an acquisition deficit. Future studies should examine additional measures of contextual fear following variable amounts of conditioning that are not confounded by increased locomotion in

hippocampal animals. Currently, existing data do not provide clear support for different learning rates in hippocampal and non-hippocampal systems.

Although some groups have argued that the non-hippocampal system is less efficient or acquires information more slowly than the hippocampal system (Fanselow, 2009; McClelland et al., 1995), evidence is currently too limited and mixed for this conclusion. Further focused studies are necessary to decide this issue. If future experiments reveal that learning rates do not differ between these systems, this finding would offer no support for the competitive learning account of overshadowing, described above (Rudy et al., 2004; Fanselow, 2009). If memories are acquired at an equal rate (Driscoll et al., 2005; Epp et al., 2008) or more quickly (McDonald et al., 2004b) in the absence of hippocampal function, it could not be the case that the hippocampus normally interferes with learning in other brain regions.

6. Summary

Popular models of the hippocampus in LTM suggest that the hippocampus has a specific role in memory, and that its disruption before or soon after learning should elicit similar types of amnesia. As discussed above, current evidence does not support either of these shared predictions. Instead, studies from various memory tasks and multiple types of hippocampal disruption suggest that hippocampal and non-hippocampal systems can support similar memory-guided behaviours, and that hippocampal disruption after a learning episode causes pervasive RA, while its disruption before learning results in specific memory deficits. Although some concepts have been applied to explain this

phenomenon, such as hippocampal overshadowing, no consensus has emerged. In our discussion of hippocampal and non-hippocampal support of LTM, however, we do not imply that memories encoded in each system are the same.

Although hippocampal and non-hippocampal systems can acquire and retrieve memories that enable similar performance in many tasks, the information each system encodes likely differs in important ways. The characteristics of LTM between these systems might differ in: 1) their ability encode conjunctions and relations between stimuli, 2) whether they encode memoranda automatically, 3) the ability to pattern complete and 4) to pattern separate, and possibly 5) in their learning rates. Thus, non-hippocampal systems cannot be considered a redundant memory system, given that removal of the hippocampus substantially alters the qualities of LTM.

We present a new concept of LTM organization, heterarchic reinstatement, to explain the aforementioned findings and generate new predictions. Notably, the heterarchic model shares several features with popular theories of LTM, such as the configural association (Rudy & Sutherland, 1995; Sutherland & Rudy, 1989), relational (Cohen & Eichenbaum, 1993), and indexing theories (McNaughton, 2010; Rolls, 2013; Teyler & DiScenna, 1986; Teyler and Rudy, 2007), but differs in important ways. We provide several predictions to test our model, below.

7. Heterarchic Reinstatement and the organization of LTM

We propose that a heterarchic view of memory storage and retrieval does well in addressing the experimental results reviewed above. On this view the hippocampus

influences encoding and retrieval of memories through its widespread projections to cortical and subcortical areas. Much attention has been paid in recent years to functions of cortical inputs to the hippocampus; less on outputs from the hippocampus to cortical regions. In the present terminology, a heterarchy is a system of connected structures that assume different hierarchical relationships based on the degree to which each region influences global activity and behaviour. A structure that receives convergent input and projects widely to many brain areas, such as the hippocampus, will be critical for memory retrieval due to its key role in reinstatement of a distributed representation present during learning. If the central structure is disrupted, then the way in which information is encoded and retrieved will be altered, and hierarchical relationships will change.

The hippocampus receives complex, processed polymodal and visuospatial information from association areas through the perirhinal, postrhinal, and entorhinal cortices (Furtak et al., 2007; Lavanex et al., 2002). It also provides distributed feedback to a broad range of cortical and subcortical regions through CA1, the subiculum, fornix/fimbira, and deep layers of the entorhinal cortex (Amaral & Lavenex, 2007; Lavanex et al., 2002; Suzuki & Amaral, 2004; Swanson & Köhler, 1986). It is assumed that performance of complex behavioural tasks depends on patterns of cortical activity that correspond to perceived and anticipated cues in the environment and information related to on-going movements and actions, as in Marr's concept of the "current internal description" (Marr, 1970; Marr, 1971).

Here, as one of its roles, activity in the cortex is proposed to represent the content of LTM, within feature analyzers consisting of neurons that have modular organization,

possibly in sets of minicolumns (Mountcastle, 1997; but see Horton & Adams, 2005; Swindale, 1990). Concurrent activation of feature analyzers gives rise to representations of stimulus conjunctions across multiple sensory modalities that become linked with one another through a Hebbian associative process (Tsunoda et al., 2001). The hippocampus also serves to enhance associative strength between sets of analyzers in the cortex that represent cue conjunctions (Rudy & Sutherland, 1995). For example, if cues A and B consistently co-occur, and are respectively processed in feature analyzers X and Y, hippocampal reinstatement would enhance the synaptic strength of connections between analyzers X and Y such that presentation of A predicts B, and vice versa. In other words, a symmetric association develops between X and Y in which presentation of one stimulus evokes retrieval of the other. It could also be the case that only A predicts B, but not vice versa, in which an asymmetric, hetero-association forms between X and Y, wherein X elicits activation of Y, but Y does not retrieve X. This is particularly important for event storage wherein the timing or sequence of cue presentations predicts outcomes. Indeed, recent findings implicate the hippocampus in memory for temporal order in both rodents and humans (for review see Davachi & DuBrow, 2015; Eichenbaum, 2014). The hippocampus is likely sensitive to cue sequences across a wide range of events due to its extensive reciprocal projections with the cortex. From recent evidence, it seems that discrete feature analyzers might exist in superficial layers of the cortex, in which the collective activity of neurons corresponds to conjunctive representations of a behavioural context (Burke et al., 2005; Xie et al., 2014; but see Takehara-Nishiuchi et al., 2013).

Cortical networks are further assumed to exhibit local attractor dynamics. In the present terminology, an attractor is a pattern to which neighbouring or incomplete patterns tend to converge (Hopfield, 1982; McClelland et al., 1995; Rolls, 2010), and discrete attractors in a network represent distinct memories, perceptions, and thoughts (Hopfield, 1982; Rolls, 2010). In the case of auto-associatively-stored information, memories are represented as simple attractors whose basin of attraction and memory retrieval can be reached in a manner that is not specific to the direction of approach in a high-dimensional state-space, or the order in which a subset of stimuli are presented. Hetero-associatively stored information can also be represented as simple attractors, but the existence of attractor states is not always guaranteed because of asymmetric connections. Therefore, hetero-associative memory is better understood as a set of quasi-attractors whose activity dynamics may exhibit chaotic itinerancy in the state-space (Tsuda, 2015). Retrieval involves the activation of a hippocampal code that, given a partial input, will pattern complete and provide reinstatement in the cortex that activates an appropriate set of feature analyzers (see also Edelman & Gally, 2013). In this framework, features are represented as basic elements in distributed regions of the heterarchy, and conjoined into increasingly complex associations, or conjunctions, as information converges in the system. Representations within hippocampus reflect the conjunction of output from multiple cortical sensory representations together with a spatial position code (Sutherland, 1985). In the present model, CA1 holds both simple elements and complex relational features of a learning episode that outputs to the cortex during memory encoding and reinstatement. As Marr (Marr, 1970; Marr, 1971) described,

the cortex is a system capable of categorizing stimuli, whereas the hippocampal circuit performs an associative function between categories of information received from broad regions of the cortex. As a result, the reinstatement of a memory representation critically depends on hippocampus due to its distributed feedback established during an initial learning experience.

Although we propose that the hippocampus is critically involved in feature representation in many types of memory, the hippocampal representation does not directly acquire affective meaning and elicit overt behavioural responses, but acts indirectly by influencing activity in cortical regions. These cortical regions project to memory effector systems that differ in their control of emotions and/or actions. A memory effector is functionally defined as any region or circuit that is required for triggering a specific type of memory-guided behaviour, resulting in both AA and RA if the region is disrupted. These effectors include the amygdala, frontal cortex, striatum, and cerebellum. For example, in the presence or absence of the hippocampus, the encoding and retrieval of fear memories critically depends on outputs from the amygdala. Animals demonstrate both AA and RA for fear memory if amygdala is inactivated or damaged (Helmstetter & Bellgowan, 1994; Maren, 1999). An exhaustive discussion of each system is well beyond the scope of the present review. Our aim is to describe how the hippocampus might interact with the cortex during any type of memory encoding and retrieval.

We suggest that the hippocampus is essential in various types of LTM due to its afferents from multiple sensory modalities and its distributed efferents across the cortical

mantle and thence to memory effectors. As a result of this heterarchic organization, the activity state that corresponds to the retrieval of memory representation in the cortex, and the elicitation of behavioural responses in memory effectors, requires top-down, hippocampal reinstatement. A central prediction from this framework is that the hippocampus is required for memory retrieval in any task following limited or massed training, such that complete damage or temporary inactivation of the hippocampus causes "global" RA. Earlier evidence supported this prediction, in which memory for non-spatial, positively-reinforced visual stimulus associations were lost following damage to the hippocampus, but not the amygdala (Sutherland & McDonald, 1990), and numerous lesion and temporary inactivation studies have recently corroborated this view (Table 1.1; Epp et al., 2008; Mumby et al., 2002; Sutherland et al., 2008; Sutherland et al., 2001).

An outstanding issue with the global RA hypothesis outlined here are recent findings showing a lack of hippocampal involvement in odour and flavour memories, and some examples of object discrimination (Lehmann et al., 2007b; Mumby et al., 1999; Thapa et al., 2014). However, recent electrophysiologic evidence illustrates that hippocampal units encode object information, conjunctions of object and location, conjunctions of object and context, and their associated outcomes such as food rewards (Komorowski et al., 2009; McKenzie et al., 2014). Additional work may be necessary to elucidate whether global RA also occurs for odour and flavour associations, and the role of repeated experience in hippocampus-independent representation of odour memory, flavour memory, and object identity.

We also suggest that future studies exploit technological advancements, such as optogenetic (Fenno et al., 2011) and chemogenetic methods (Smith et al., 2016; Roth, 2016), to alter hippocampal and cortical activity in order to study the involvement of the hippocampus in LTM and the "global" RA hypothesis. Importantly, however, in using these methods it is also critical that investigators examine the extent of hippocampal inactivation and its relationship to RA (Sutherland et al., 2010). Ideally, these methods should be used in combination with traditional lesion approaches in order to avoid overestimates on the role of hippocampus in LTM (see Otchy et al., 2015).

The model we present (Fig. 1.2) is simple and can account for a broad range of findings in the circumscribed literature that we have discussed, including: 1) RA following the removal of the hippocampus, 2) memory sparing with hippocampal damage in the retrograde direction following repeated experience, 3) that the degree of RA soon after learning is related to the extent of hippocampal lesion or inactivation, 4) the absence of AA in many of the same tasks for which there is RA following hippocampal disruption, 5) the unique contribution of the hippocampus in pattern completion and pattern separation, and 6) between-systems interference when the hippocampus is offline during encoding and online during retrieval.

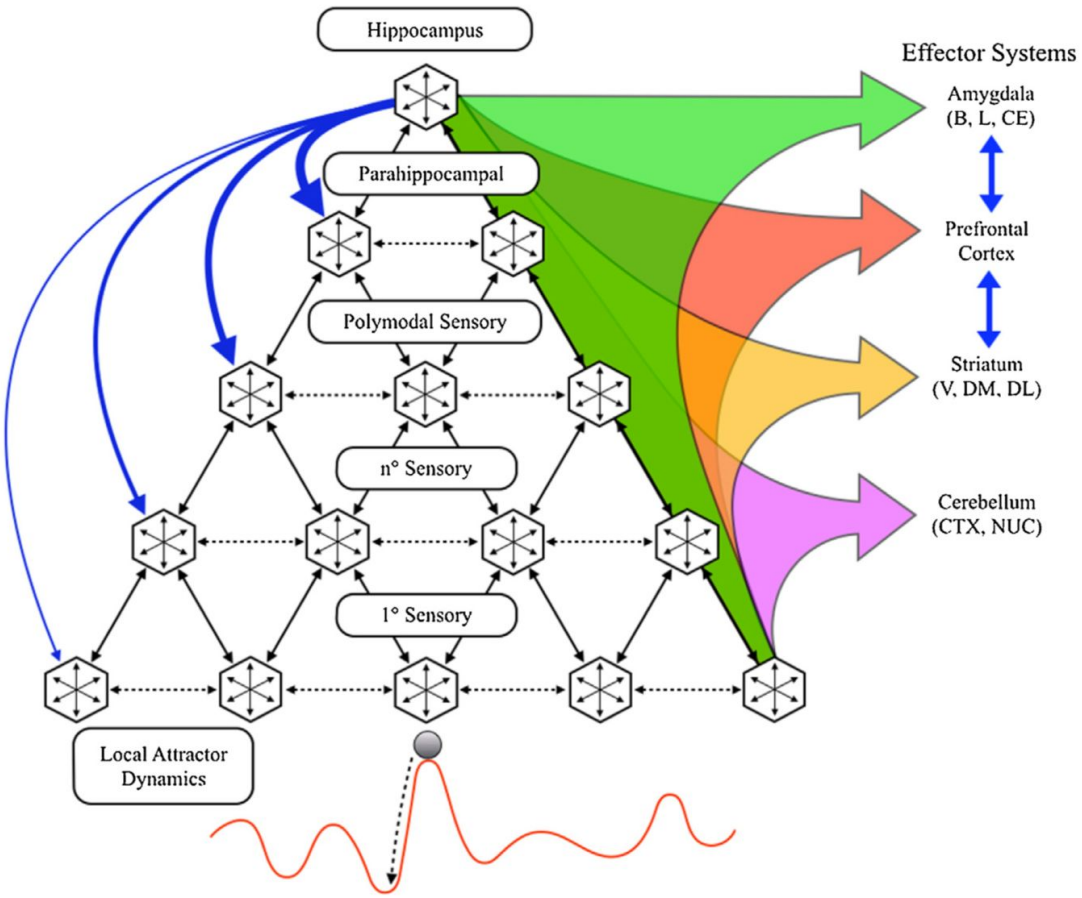


Figure 1.2 The heterarchic reinstatement model. The illustration depicts the central characteristics of the heterarchic model. Within this framework, regions that receive convergent information and send divergent projections to broad areas of the cortex greatly influence global activity dynamics and behavioural output. Information is fed in sequence and in parallel through feature analyzers composed of cortical minicolumns with modular organization, whose activity states exhibit local attractor dynamics in the cortex. The coordinated activity between analyzers gives rise to conjunctive representation of collections of cues in the environment. Due to its distributed, top-down projections, the cortico-hippocampal representation is integrated across memory effector systems that

determine the qualities of a learning episode and generate behavioural responses through their efferents.

Assuming local attractor dynamics, the initial conditions of a network's activity are a primary determinant of whether it will converge to one stable state or another. Our model predicts that hippocampal reinstatement is sufficient to bias each analyzer toward forming an attractor that is hippocampus-dependent for retrieval. With limited training, removal of the hippocampus causes RA due to considerable change in the population firing vector that produces memory recall. This would occur regardless of the type of information represented in cortical analyzers. After a limited amount of exposure to one set of environmental features, top-down reinstatement is necessary to retrieve an entire conjunctive representation due to the limited inter-analyzer connectivity in the cortex (McNaughton, 2010). For many cue conjunctions repeated experience, especially with spaced learning episodes, is sufficient to produce a hetero-association between analyzers in the cortex and memory effectors that does not require the hippocampus for retrieval. As a result, non-hippocampal networks can acquire a conjunctive memory, even if the hippocampus is intact during acquisition, but this requires repeated experience (Lehmann & McNamara, 2011; Lehmann et al., 2009). This non-hippocampal memory appears not to arise with the passage of time alone (Broadbent and Clark, 2013; Lehmann et al., 2007b; Lehmann et al., 2013; Sparks et al., 2013; Sutherland et al., 2008). When the hippocampus is intact, its distributed reinstatement strongly influences the cortical population firing until repeated experience drives retrieval from the "bottom-up." This

perspective offers an alternative to previous suggestions that different learning rates can be explained by plasticity in the non-hippocampal system being compromised when the hippocampus is intact (Biedenkapp & Rudy, 2008; Driscoll et al., 2005; Fanselow, 2009; Maren et al., 1997).

The attractor dynamics of cortical analyzers also suggests that the extent of hippocampal damage should predict memory sparing in the retrograde direction soon after learning. We have reported in several studies that the extent of RA correlates with the extent of hippocampal damage at temporally recent, but not remote, testing periods (Epp et al., 2008; Lehmann et al., 2007b; Sutherland et al., 2008). This may be understood through the notion that memory retrieval involves a hippocampal firing vector projecting to a set of cortical feature analyzers that must settle in an appropriate "attractor basin." If the hippocampus is partially disrupted prior to retrieval, the population firing vector becomes information-poor, and it is increasingly difficult for a set of analyzers achieve necessary activity states that enable memory retrieval. The more similar cortical activity is reinstated compared to encoding, the more likely it is that memory will be retrieved.

In the absence of the hippocampus in the anterograde direction, parahippocampal cortices gain influence over activity dynamics elsewhere in the cortex and in effector systems due to their highly convergent input and distributed feedback. Specifically, the entorhinal, perirhinal, and postrhinal cortices determine the states to which analyzer networks will stabilize in the cortex. As a result, permanent inactivation of the hippocampus has no effect on LTM acquisition in the anterograde direction in cases

where the measure of memory retrieval is insensitive to differences in characteristics of hippocampal and non-hippocampal memories (see Sections 4–5). Notably, the degree of information convergence in the heterarchy is a primary determinant of the extent to which networks can pattern complete and pattern separate (Kent et al., 2016; Fig. 1.3).

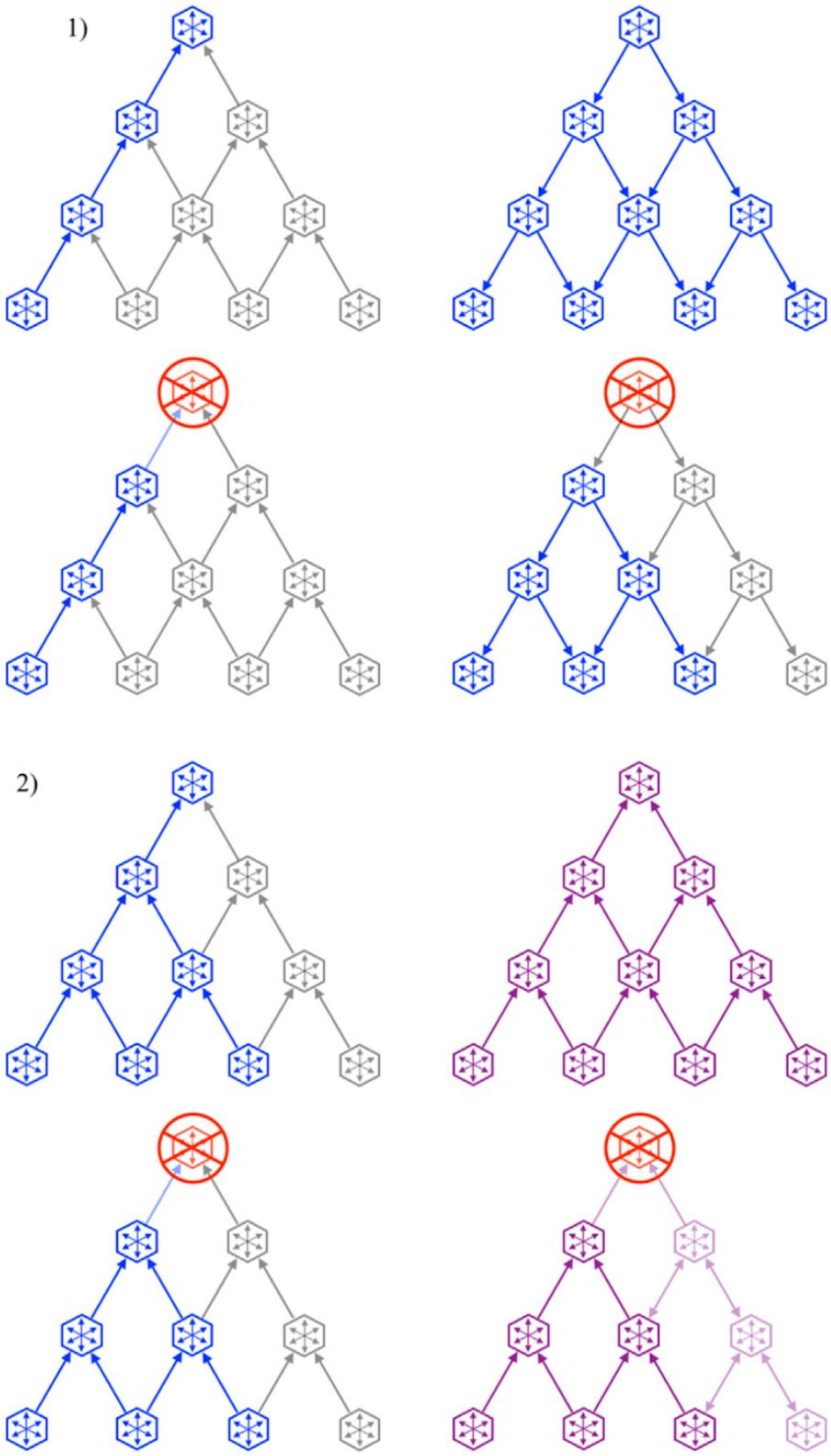

1)
2)

Figure 1.3 Pattern separation and pattern completion. Pattern separation and completion of feature representations arise from convergent input and divergent feedback in the cortico-hippocampal system. The schematic shows that (1) regions higher in the system enable greater degrees of pattern completion through more distributed feedback, and (2) pattern separation with broader and more convergent input that gives rise to feature representation that enables detection of differences between similar inputs.

Limited inter-analyzer connectivity in the cortex necessitates top-down hippocampal reinstatement to bind activity across distributed topographical regions to represent conjunctions of stimuli (Fig. 1.3-1). The unique association fibres of CA3 further provide a mechanism for highly efficient pattern completion from partial input to the hippocampus (Leutgeb et al., 2005; Marr, 1971; McNaughton & Morris, 1987; Rolls, 2013). Feedback from hierarchically lower regions in the system is less distributed than hippocampal efferents. We suggest that, in the absence of the hippocampus, pattern completion is constrained to topographically disparate sets of feature analyzers (Fig. 1.3-1).

Sparsity of coding, defined by the number of active neurons needed to represent a stimulus or conjunction of stimuli, is a determinant of pattern separation capacities. In general, feature representations become increasingly sparse as they approach hippocampus (O'Reilly & Rudy, 2001) – the fewest number of neurons being recruited in the dentate gyrus (Chawla et al., 2005). Regions that are hierarchically lower in the cortico-hippocampal system, such as the parahippocampal cortices, receive less

convergent information from more restricted regions of the cortex. As a consequence, it is more likely that similar patterns of input will be represented equally in parahippocampal cortices. In the absence of the hippocampus, pattern separation is therefore limited (Fig. 1.3-2). Interestingly, these impairments may be ameliorated to some extent with increased conditioning (Lehmann et al., 2009), given that this general computation is supported in various sensory modalities in non-hippocampal areas (Kent et al., 2016; Stark & Yassa, 2011).

In the heterarchic reinstatement model, interference in memory retrieval will occur when non-hippocampal systems, rather than hippocampal output, determine attractor dynamics supporting memory retrieval in analyzer networks, as illustrated in Sparks et al. (2011b). This is shown if the hippocampus is offline during the learning episode and is brought back online during memory testing. If parahippocampal cortices gain influence over a representation in the absence of hippocampal activity, then hippocampal instatement of an unrelated firing vector provides input that is sufficient to destabilize attractors that would otherwise enable successful memory retrieval (Fig. 1.4). As a result, amnesia is elicited due to top-down feedback from the hippocampus that interferes with a memory representation encoded in its absence. Alternatively, if the hippocampus is offline during testing, non-hippocampal reinstatement of a cortical firing vector may be similar enough to that at the time of encoding to enable feature analyzers to stabilize in activity patterns supporting memory retrieval.

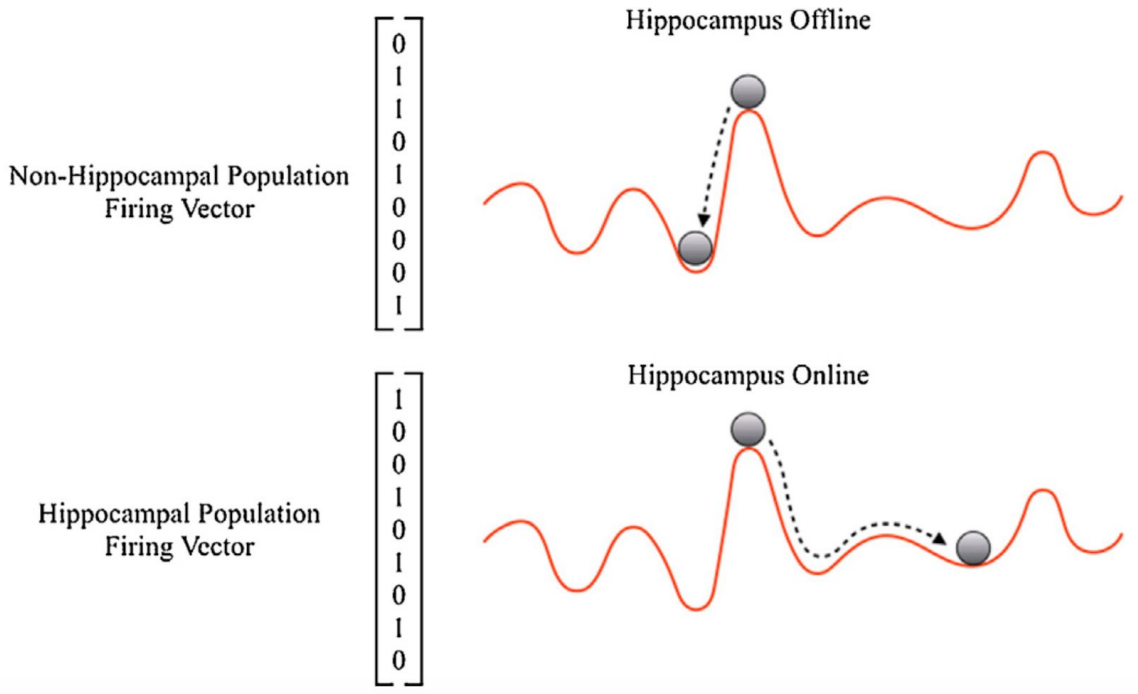


Figure 1.4 Hippocampal activity disrupts retrieval of a non-hippocampal memory. The diagram illustrates how retrieval of a memory encoded while the hippocampus is offline can be disrupted by subsequent hippocampal activity. If a non-hippocampal network establishes a population firing vector corresponding to a learning episode while the hippocampus is offline, subsequent hippocampal activation dramatically changes the cortical population firing vector such that the network does not settle in the appropriate "basin of attraction" that enables memory retrieval.

Due to its simplicity and explanatory capacity, we suggest that heterarchic reinstatement should be considered in future investigations and discussions of the role of the hippocampus in LTM. We provide several predictions to test this model, below.

8. Predictions

1. Following limited experience, hippocampal disruption causes retrograde amnesia in a wide range of memory tasks.
2. Disrupting n neurons of a memory trace in regions that receive highly convergent input (e.g. CA1) will have greater amnestic effects than disrupting n neurons of the memory trace in areas where information is more distributed (e.g. association cortices).
3. Repeated, distributed experience is necessary for non-hippocampal systems to support LTM if the hippocampus is intact and active during learning.
4. The hippocampus enhances inter-analyzer associative strength in cortical networks that gives rise to highly specific, conjunctive representations and rapid learning of new, similar information.
5. Feature analyzers are represented in the connectivity and activity of cell assemblies in superficial layers of the neocortex.

9. Conclusions

In the present review we have discussed evidence that supports a more general role of the hippocampus in LTM. Hippocampal disruption causes RA but not AA in numerous memory tasks, which has remained without clear explanation in popular models of memory organization. The hippocampal overshadowing concept has been discussed in recent years in relation to observations of RA without AA. However, no single previous account of the hippocampus in LTM accounts for these observations. We

have presented the heterarchic reinstatement view of long-term memory to explain several of these findings and generate new predictions.

On this view, the hippocampus receives a broad range of input through convergent cortical afferents, and influences activity dynamics in cortical and subcortical regions through distributed, top-down reinstatement of memory representations. Due to its widespread efferents across the cortical mantle and subcortical regions, the hippocampal representation is essential for memory retrieval. Repeated experience of a learning episode is proposed to drive a hetero-associative process in the cortex that is necessary for non-hippocampal regions to support LTM, independently. We describe how our model can explain a variety of observations on the role of the hippocampus in LTM, including: 1) RA following hippocampal disruption, 2) repeated experience supporting hippocampus-independent memory, 3) that the degree of RA corresponds to the extent of hippocampal lesion or inactivation soon after learning, 4) the absence of AA in many of the same tasks for which there is RA if the hippocampus is disrupted, 5) the unique contribution of the hippocampus in pattern completion and pattern separation, and 6) between-systems interference when the hippocampus is offline during encoding and online during memory testing. Due to its simplicity and explanatory capacity, we hope that future investigators of LTM will examine the principles and predictions of this new framework.

Chapter 2

Relocating cued goals induces population remapping in CA1 related to memory performance in a two-platform water task in rats.

Abstract

The activity of CA1 neurons in the rodent hippocampus represents multiple aspects of learning episodes, including cue and place information. Previous reports on cue and place representation in CA1 have examined activity in single neurons and population recordings during free exploration of an environment or when actions are directed to either cue or place aspects of memory tasks. To better understand cue and place memory representation in CA1, and how these interact during goal-directed navigation, we investigated population activity in CA1 during memory encoding and retrieval in a novel water task with two visibly distinct platforms, using mRNA for immediate early genes Arc and Homer1a as markers of neural activity. After training, relocating cues to new places induces an extensive, perhaps global, remapping of the memory code that is accompanied by altered navigation and rapid learning of new cue-place information. In addition, we have found a significant relationship between the extent of reactivation and overall cue choice accuracy. These findings demonstrate an important relationship between population remapping in CA1 and memory-guided behavior.[2]

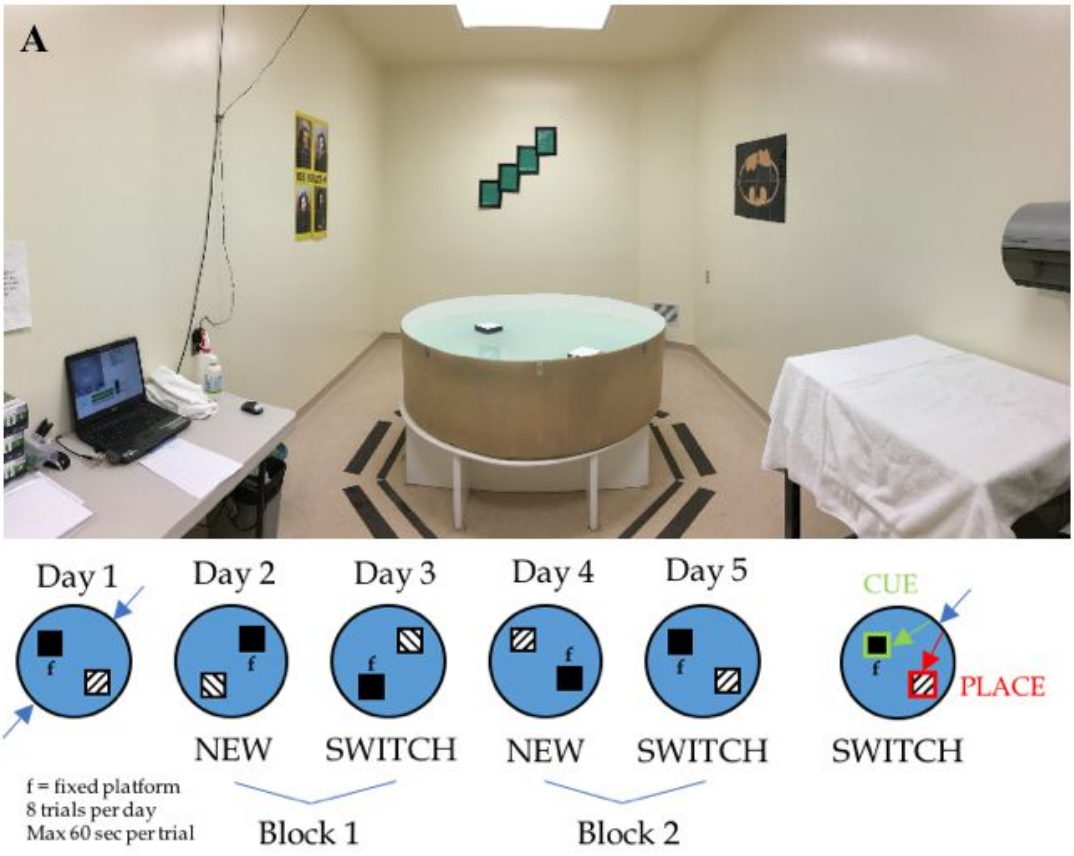
[2] Chapter published as: Lee, LeDuke, Chua, McDonald, and Sutherland (2018). Relocating cued goals induces population remapping in CA1 related to memory performance in a two-platform water task in rats. *Hippocampus, 28*(6): 431-440. Reproduced with permission from John Wiley and Sons.

Introduction

The CA1 region of the rodent hippocampus encodes multiple aspects of a learning episode, including information about cues and places (Komorowski, Manns, & Eichenbaum, 2009; McKenzie et al., 2014; Muller & Kubie, 1987; Sutherland et al., 2001). Although the hippocampus may not be necessary for acquiring cue memory (McDonald & White, 1993; McDonald & White, 1994; Morris, Haggan, & Rawlins, 1986), and in some cases place memory (Day et al., 1999; Hales et al., 2014; Travis et al., 2010), when the hippocampus is present during a learning episode it is necessary for cue and place memory retrieval (Sutherland, O'Brien, & Lehmann, 2008; Sutherland et al., 2001). Several studies have shown that CA1 place cell activity remaps when cues change location in a familiar spatial context (Knierim, Kudrimoti, & McNaughton, 1995; Lee et al., 2004; Muller & Kubie, 1987; Zhang & Manahan-Vaughan, 2015). Specifically, some place cells shift their firing fields in response to cue relocation, while other cells lose their place fields and some begin to exhibit place field activity (Lee et al., 2004; Muller & Kubie, 1987). Previous studies investigating changes in population activity following changes to cue locations have measured unit and population activity while animals freely explore an environment, or while the animal is engaged in distinct cue or place behaviors (Knierim et al., 1995; Leutgeb et al., 2005; McKenzie et al., 2014; Muller & Kubie, 1987; O'Keefe & Nadel, 1978). It remains unclear how changes in CA1 population activity relate to memory performance in goal-directed navigation. Several groups have suggested that CA1 contains a key memory code that is projected to distributed portions of the cortex, and thence utilized for memory-guided behavior (Lee et al., 2016; Marr, 1971;

McNaughton, 2010). Studies on place cell remapping and memory performance have yielded contrasting findings—some groups have reported a relationship between place cell remapping and memory performance (Lenck-Santini, Save, & Poucet, 2001), while others have found no relationship (Jeffery et al., 2003). It remains possible that remapping across the entire population of CA1 neurons is related to memory-guided behavior.

To address this question, we developed a two-platform water task to induce changes in the CA1 population code and determine how changes in the population code are related to cue choice accuracy (Figure 2.1a). The two-platform water task requires animals to discriminate between two, visibly distinct platforms (cues) to escape from a pool filled with opaque water (Morris et al., 1986; Sutherland et al., 2001). One of the cues enables escape from the pool throughout training and is supported on a hidden pedestal, while the other cue does not offer escape and is floating in place. Distal room cues are also visible to the animal on the walls surrounding the pool. The positions of the goal cues remain constant relative to the room for an eight-trial session, and on the following eight trials are shifted 90° clockwise or counter-clockwise relative to distal cues (NEW shift), or are shifted 180° (SWITCH shift). If animals express place memory, they are expected to perform better on NEW than SWITCH shifts, due to cue-place conflict on SWITCH shifts (Figure 2.1a). By contrast, if animals express mostly cue memory, then performance should be equal on NEW and SWITCH cue shifts and choose the correct cue, regardless of its location.


A
Day 1
Day 2
Day 3
Day 4
Day 5
CUE
PLACE
NEW
SWITCH
NEW
SWITCH
SWITCH
f = fixed platform
8 trials per day
Max 60 sec per trial
Block 1
Block 2

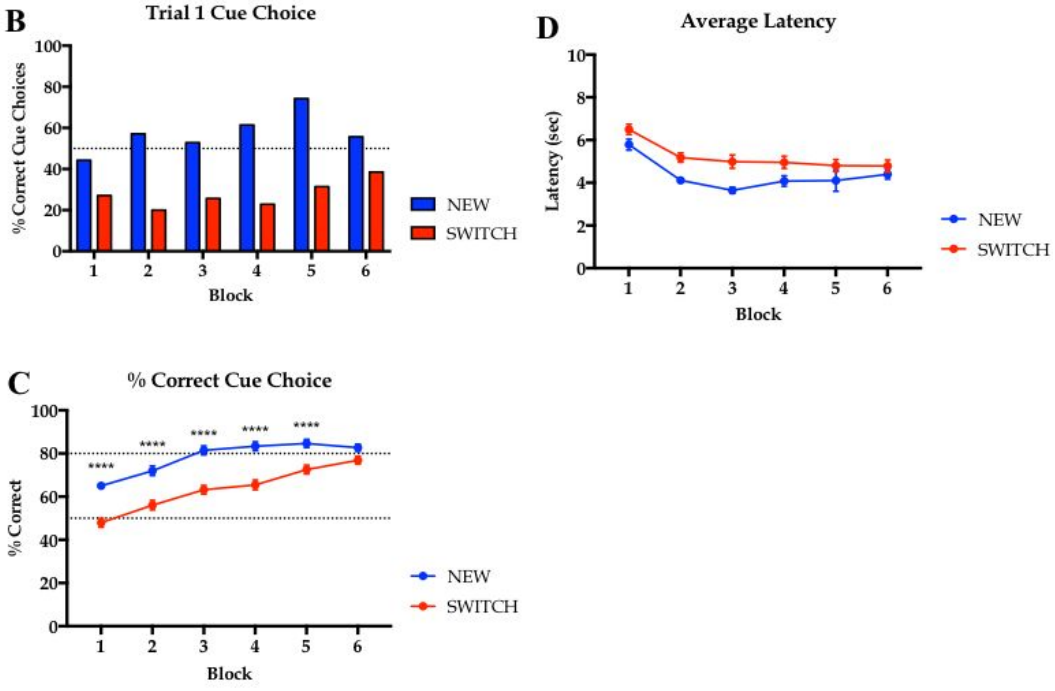

B
Trial 1 Cue Choice
% Correct Cue Choices
Block
NEW
SWITCH
D
Average Latency
Latency (sec)
Block
NEW
SWITCH
C
% Correct Cue Choice
% Correct
Block
NEW
SWITCH

Figure 2.1 Behavioural setup and performance summary of two-platform water task acquisition. (a) Two-platform water task room arrangement and schematic depiction of task design. Training in the two-platform water task alternates between NEW (90°) and SWITCH (180°) cue shifts in a pool filled with opaque room temperature water. One of two visbly distinct platforms (cues) is supported throughout training using a hidden pedistal, while the other is tethered and floating in a stable position. The control of cue and place strategies on navigation are revealed following SWITCH cue shifts when animals are faced with a conflict between a previously reinforced place that is occupied by the incorrect cue (lower panel). (b) Trial 1 percent correct cue choice following NEW and SWITCH cue shifts. The data summary reveals that rats choose the incorrect cue (below chance) that occupies the previously correct place during early phases of two-platform water task training following SWTICH cue shifts, suggesting that place information controls behavior during earlier phases of two-platform water task acquisition. However, a summary of the correct cue choice also suggest that animals do acquire cue memory that assists performance on NEW cue shifts over each eight trial session. (c) Two-platform water task acquisition percent correct cue choice during NEW and SWITCH cue shifts from each eight-trial session. Performance shows a clear division over the eight trial sessions following NEW and SWITCH cue shifts, resulting greater percent correct cue choice in the NEW compared to SWITCH shift condition. This supports that place information controls memory-guided behavior in early task acquisition, and later performance becomes similar in both NEW and SWITCH cue shifts, possibly due to cue memory acquiring greater associative strength (block 6). (d)

Two-platform water task acquisition average latency to the correct cue following NEW and SWITCH cue shifts. A summary of average latency to the correct cue during each eight-trial session reveals a similar pattern as in (c), showing that animals take longer to navigate to the correct cue following SWITCH compared to NEW cue shifts in the two-platform water task.

A summary of performance reveals that NEW shifts, especially during early phases of training, induce initially random platform choice, followed by a rapid learning of the correct cue choice (Figure 2.1b,c). SWITCH shifts result in initial perseveration to navigate toward previously reinforced goal location, which now contains the incorrect cue. As a result, task performance differs in early phases of training when animals are faced with NEW versus SWITCH shifts. Later performance in the two-platform water task is similar on NEW and SWITCH platform shifts, which could suggest a shift from place-controlled to cue-controlled navigation across learning, an observation that is in keeping with previous reports on cue- and place-guided behavior (Morris et al., 1986; Packard & McGaugh, 1996; Tolman et al., 1946). However, the first cue choice in later training does not reveal a strong preference for the correct cue. It is possible that cue memory has gained associative strength and assists with correct choice during each eight-trial acquisition session.

Navigation during NEW shifts in early phases of the two-platform water task suggests rats have relatively poorer recall of which cue is rewarded and they cannot predict which of the novel locations will be rewarded, and thus the NEW shift is treated

as a new learning experience. With SWITCH shifts, rats initially navigate to a previously reinforced location, which contains the incorrect cue, and acquire a new strategy over several trials. We anticipated that a change in the CA1 memory code would be induced by cue shifts in the two-platform water task, and might reflect both new and perseverative navigation strategies in the NEW and SWITCH cue shift conditions, respectively. One method to measure change of the memory code is the amount of similarity in cellular activation that occurs when animals are faced with a NEW or SWITCH cue shifts. To describe population activity that has remained similar, we will use the term "reactivation," and for population activity that has become dissimilar we will use the term "remapping." We generated two, contrasting hypotheses on the role of remapping and reactivation in the two-platform water task. The first hypothesis was that reactivation would benefit correct cue choice in the two-platform water task, while the second hypothesis was that remapping would benefit correct cue choice. The logic behind our second hypothesis is based on our behavioral results, which might suggest that if cue information does not exhibit strong control over navigation, the same memory will be retrieved before the animal shifts its navigation target in the SWITCH shift condition, followed by a small degree of CA1 remapping when eventually changing strategy after initial perseveration to previous goal locations. By contrast, a NEW cue shift could result in greater CA1 remapping and allow the animal to rapidly implement a new navigation strategy and learn new cue-place information. We expected relocating cues would induce remapping in CA1, and our two hypotheses differ on the proposed role of reactivation versus remapping for performance in the two-platform water task.

To investigate this possibility, we used design-based stereology to examine population activity across the entire septal-temporal axis of CA1 and fluorescent in situ hybridization (fISH) to Arc and Homer1a mRNA as markers of neural activity following memory retrieval in the two-platform water task (Figure 2.2; Schmitz & Hof, 2005; Vazdarjanova & Guzowski, 2004). Our results demonstrate an effect of cue relocation on hippocampal remapping in CA1, and that the extent of similarity across all cue shift conditions is positively related to cue choice accuracy in the two-platform water task. In addition, NEW cue shifts in the two-platform water task induce a significant change in the CA1 memory code, while SWITCH shifts induce a non-significant change in population activity compared to SAME cue-place presentations (Figure 2.3). This is the first demonstration using the IEG imaging approach, to our knowledge, of a relationship between remapping across the CA1 septal-temporal axis and performance in a memory task.

2. Materials and Methods

2.1 *Subjects*

Experimentally naïve, male Long Evans rats weighing between 350 and 400 g (Charles River, Raleigh) were used in each of the present experiments following at least one week of acclimation to the University of Lethbridge animal colony room and 5 days of handling by the experimenter.

2.2 *Two-platform water task acquisition*

On the first day of two-platform water task acquisition rats were brought into a room containing a fiber glass swimming pool (2.0 m diameter) filled with room temperature water (~21°C) and several distal cues surrounding the pool (Figure 2.1a). Two visible platforms (cues) with different appearances (one solid black with a rubber lining; the other painted with black and white stripes on PVC imitation wood) located in the center of opposite quadrants in the pool, ~2 inches above the water surface. One of the cues was supported with a hidden pedestal for a given rat throughout training and testing (reinforced cue), while the other cue was floating in place (non-reinforced) and tethered to the bottom of the pool such that it would sink if the animal attempted to escape the pool using the cue. The animal was carefully placed in the water facing the pool wall at one of two locations equidistant from either cue and allowed to swim for a maximum of 60 s per trial with a 10-s timeout following each trial. If the rat did not reach the correct cue by the end of the trial it was placed on the correct platform for 10 s before returning to its holding cage. The cage was also covered with a bath towel to prevent the animal from viewing its surrounding between trials. Each animal swam a total of eight trials per day with between two and four minutes between trials before returning to its home cage for 24 hr. Importantly, given the stable cue contingency and location on a given day, rats could use either a cue or place strategy to navigate to the correct cue. Egocentric strategies (turning response) cannot be used to successfully navigate since starting locations from opposite quadrants of the pool would not be associated with reinforcement of a specific turning response. Thus, manipulations were made of the platform locations to determine which strategy, either cue or place, controlled the animals'

behavior across training. On the following day, the cue contingencies were kept the same for each animal, and both cues were rotated 90° in the pool with respect to the distal cues either clockwise or counter-clockwise (NEW shift). If rats demonstrate a strong cue response they should make correct cue choices on the first trial of the NEW shift. Alternatively, if rats do not have a strong cue memory they might make a random cue choice initially, followed by re-acquisition of the correct cue-place strategy. The difference between cue and place control over the rats' navigation is illustrated on the following day when the animal is returned to the room with the platforms rotated 180° relative to the distal cues from the previous day of training (SWITCH shift). If animals maintain a strong cue strategy, they would choose the correct cue on the first trial and thereafter. However, if they express a strong place strategy they will choose the non-reinforced cue for several trials before correcting their navigation to the correct cue in the opposite location relative to the previous day of training. If animals possess a correct cue representation and place representation they might make an incorrect choice initially and, depending on the associative strength of each aspect, navigate to the correct cue sooner or later in the trials on that day. Each pair of NEW and SWITCH shifts are considered as a single block of training, and each rat experiences the NEW and then a SWITCH shift during a training block. Initial behavioral assessment of task acquisition was carried out for at least six blocks of training (15 days) whereupon performance on latency and percent correct cue choice across the NEW and SWITCH sessions became statistically equal across the eight trials of swimming. For IEG treatment, acquisition

ended following three blocks of training (7 days) when performance tended to rise above an 80% threshold upon a NEW cue shift.

2.3 *IEG Activation*

Following completion of three acquisition blocks in the two-platform water task, rats were given one of three IEG activation treatments to probe neural activity dynamics following different cue shifts. In each condition, rats returned to the room ~24 h after the third block of training with the platforms in the same position as the previous day and were given four swim trials (1-min inter-trial interval; total 5-min session) to assess memory and re-activate the neural ensemble representing the previous cue arrangement. The first four trials of swimming, referred to as "session 1", drive the expression of Homer1a mRNA as a marker of neural activity. Following the completion of session 1, rats were brought back to their home cages for 20 min. Thereafter, rats were given one of three cue manipulations in the following four trials referred to as "session 2". In the SAME condition, rats were returned to the room and swam for four trials (1-min inter-trial interval; total 5-min session) with the cues in the same position as the previous four trials. By contrast, in the NEW condition rats were returned to the room and swam for four trials with the platforms rotated 90° clockwise or counter-clockwise relative to session 1, and in the SWITCH condition the rats swam for four trials with the platforms rotated 180° relative to session 1. The second session was used to drive the expression of Arc mRNA as a marker of neural activity during each cue manipulation. 90 s following the fourth swim during session 2 rats were given a 1.5 ml intraparitoneal injection of

sodium pentobarbital and transported to a separate room for perfusion and tissue collection.

2.4 *Animal Perfusion and Tissue Collection*

Approximately eight minutes following session 2 of the IEG activation rats were perfused intracardially with 100 ml of cold 1× phosphate-buffered saline and diethyl pyrocarbonate (PBS-DEPC) solution followed by 100 ml of 4% paraformaldehyde (PFA) dissolved in 1× PBS-DEPC solution. The brain was immediately removed from the skull and kept at 4°C overnight in 4% PFA in 1× PBS-DEPC solution, and then transferred to 30% sucrose dissolved in 1× PBS-DEPC solution for at least 48 hr prior to sectioning. Before cryosectioning each brain was hemisectioned sagittally down the midline with a sterilized razor blade and then sliced at 40 μm thickness throughout the entire extent of the hippocampus. Every 12th section was collected and mounted on Superfost Plus (Fisher Scientific) ionized slides for fluorescent in situ hybridization (fISH) tissue processing and quantification of IEG expression.

2.5 *fISH Tissue Processing*

Primers flanking portions of Arc intron 1, exon 2 and intron 2 were designed using online software (National Center for Biotechnology Information Primer-Blast). The exact sequences of the primers are as follows and base pair designations match those of GenBank accession number NC_005106: 5′-CTTAGAGTTGGGGGAGGGCAGCAG-3′ (forward primer, base pairs 2022–2045) and 5′-ATTAACCCTCACTAAAG

GG-CCCTGGGGCCTGTCAGATAGCC-3′ (reverse primer tagged with T3 polymerase binding site on 5′ end, base pairs 2445–2466). Polymerase chain reaction (PCR) was performed on genomic rat DNA template using a Taq PCR Kit (New England Biolabs, Ipswich, Massachusetts, USA) and the PCR product was purified using a Qiagen PCR Purification Kit (Life Technologies Inc., Carlsbad, California, USA). A commercial transcription kit (MAXIscript T3; Life Technologies Inc., Carlsbad, California, USA) and Digoxigenin (DIG) RNA Labeling Mix (Roche Diagnostics, Risch-Rotkreuz, Switzerland) were used to generate DIG-labeled Arc intron-specific antisense riboprobes from the PCR template. Fluorescein-labeled Homer1a probes targeting the 3′ untranslated region were generated as previously described (Montes-Rodríguez et al., 2013). Riboprobes were purified with mini QuickSpin columns (Roche Diagnostics, Risch-Rotkreuz, Switzerland).

Fluorescent in situ hybridization was performed as described by Montes-Rodríguez et al. (2013). Briefly, DIG-labeled Arc riboprobe signal was amplified with anti-digoxigenin-POD (1:300; Roche Diagnostics), tyramide signal amplification (TSA) Biotin Tyramide Reagent Pack (1:100; PerkinElmer) and Streptavidin-Texas Red (1:200; Perkin Elmer). Fluorescein-labeled Homer1a probe was detected with anti-Fluorescein-HRP antibody (1:1000; Jackson ImmunoResearch Labs) and amplified with a Fluorescein TSA kit (1:100; PerkinElmer). Nuclei were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI; 1:2000; Sigma-Aldrich).

2.6 *CA1 IEG Quanitfication*

IEG expression was quantified using the optical fractionator method in StereoInvestigator software (version 10.54) from confocal z-stack images collected on an Olympus FV1000 equipped with Fluoview FV10-ASW software (version 4.0). Unilateral traces of CA1 were placed over live images at 20× objective on each section prior to z-stack image acquisition. The counting frames were positioned on a 150 × 150 μm grid over the CA1 trace according to principles of systematic-random sampling. A series of seven z-stack images at 512 × 512 pixels were collected at each sampling site with a 60× oil objective starting at the top of the section every 2 μm for a total 14 μm stack. Image thresholds were set at 720 HV ± 20, 600 HV ± 20, and 575 HV ± 20 respectively in DAPI, FITC, and Texas Red channels and kept constant across imaging a section series such that small Homer1a and Arc transcription foci (2–3 pixels in diameter) could be clearly identified. Z-stack images were imported into StereoInvestigator such that one image from each stack fell above and another below the 10-μm dissector height. DAPI was counted according to optical dissector inclusion–exclusion criteria at each cell's widest point. If included cells contained Homer1a, Arc, or Double Labels, each were counted individually using separate markers.

2.7 *Data Analysis*

Statistical analyses were performed using SPSS (Version 21.0, IBM, Armok, New York, USA), G*Power (Düsseldorf, Germany), and Prism by GraphPad (San Diego, California, USA) software. Behavioral data from percent correct cue choice and latency to the correct cue in SAME, NEW, and SWITCH cue conditions were analyzed using a

mixed-model ANOVA with block and cue shift as factors. Post-hoc LSD pairwise comparisons were performed following significant block X cue shift interaction, comparing performance in cue shift conditions on individual blocks. Initial analyses for effects in imaging data were performed using a mixed-model ANOVA on stereologic estimates of DAPI, Homer1a, Arc, and Double Label marker averages with label and group as factors. Total number of labeled cells was computed and compared across groups to examine a main effect of group on IEG-labeled CA1 cells. The proportion of double labeled cells out of the total labeled population, referred to as similarity index (SI), was calculated for each animal and average SI was compared across groups using a one-way ANOVA. Post-hoc uncorrected LSD comparisons were performed following a significant effect of group on SI. The number of total labeled cells and SI were calculated for each animal using the following equations:

$$QTot = (QH1a + QArc) - QDbl$$

$$SI = QDbl / QTot$$

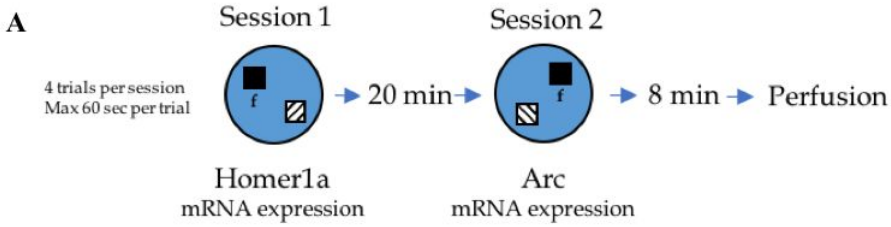
Thus, a SI value of 1 would indicate absolute similarity in Homer1a and Arc IEG expression, whereas a SI value of 0 would indicate absolute orthogonality in the population.

3. Results

3.1 *Two-platform Water Task Acquisition*

A summary of control animal performance (n = 72) in the two-platform water task revealed that rats acquire the correct cue strategy sooner on NEW than SWITCH cue shifts. We found a robust effect of cue shift ($F(1,71) = 134.4$, $p < .0001$), block ($F(5, 355) = 55.41$, $p < .0001$), and a significant shift X block interaction ($F(5,355) = 2.775$, $p = .0179$) on percent correct cue choice (Figure 2.1c). In latency to the reach the correct cue, we also found a significant effect of cue shift ($F(1,71) = 75.71$, $p < .0001$) and block ($F(5,355) = 16.41$, $p < .0001$), but not a significant shift X block interaction ($F(5,355) = 1.145$, $p = .3364$; Figure 2.1d). Trial 1 cue choice also reveals that animals make initial cue choices at a chance level during the first three blocks of acquisition on NEW cue shifts (Figure 2.1b). Later in training, some rats improve in their immediate retrieval of the correct cue during NEW cue shifts on the first trial, although the cue choice does not appear to be greater than chance in block 6. As mentioned previously, cue information may gain some associative strength to assist in better overall performance across the eight trials during NEW shifts. By contrast, SWITCH cue shifts result in rats choosing the incorrect platform in the previously correct place, indicating that rats retrieve the previously reinforced correct cue location in early two-platform water task acquisition. The robust differences between correct cue choice and latency during two-platform water task suggest that, although animals might use visual cues to guide navigation following three blocks of training, place memory maintains strong control on navigation until performance becomes similar in later blocks of two-platform water task acquisition.

In a separate cohort of animals used to probe IEG expression (n = 24) we replicated the effects of two-platform water task acquisition in cue choice over three blocks of training in cue shift ($F(1,23) = 19.46$, $p = .0002$) and block ($F(2,46) = 21.21$, $p < .0001$) prior to IEG treatment, and no significant shift × block interaction ($F(2,46) = 0.7805$, $p = .4642$; Figure 2.2b). Similar effects of cue shift, block, and shift × block interaction occur if only the first three blocks of data are considered from the summary data, above ($F(\text{Shift}(1,71)) = 39.33$, $p < .0001$; $F(\text{Block}(2,142)) = 56.61$, $p < .0001$; $F(\text{Shift} \times \text{Block}(2,142)) = 0.9267$, $p = .3982$). Notably, we found a significant effect of block ($F(2,46) = 4.116$, $p = .0227$) but no significant effect of shift ($F(1,23) = 0.0148$, $p = .9042$) and no significant shift × block interaction ($F(2,46) = 0.6338$, $p = .5351$) in latency to the correct cue in this cohort during acquisition (Figure 2.2c), suggesting that percent correct cue choice is a more sensitive measure to detecting performance changes following cue shifts. After three blocks of two-platform water task acquisition, we sought to examine neural activity dynamics using the IEGs Arc and Homer1a as markers of neural activity following SAME, NEW, or SWITCH cue shifts.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Session 1
Session 2
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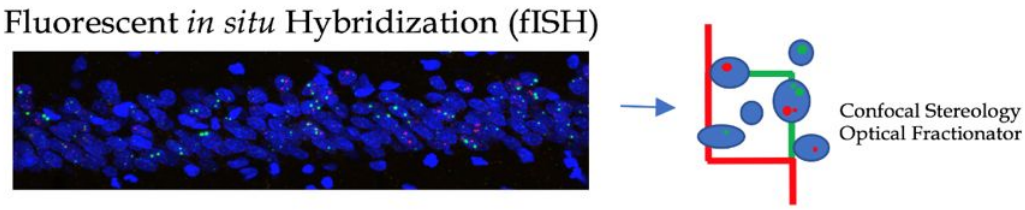
Max 60 sec per trial
20 min
8 min
Perfusion
Homer1a
mRNA expression
Arc
mRNA expression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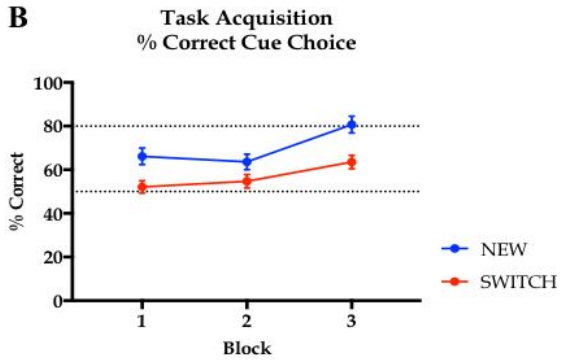
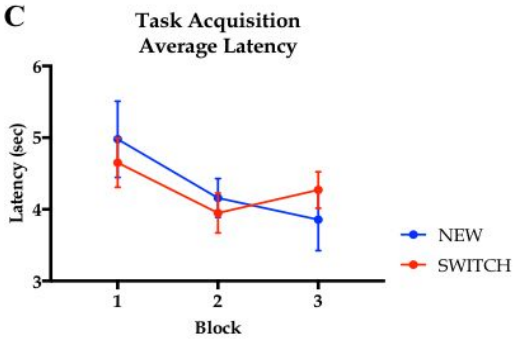
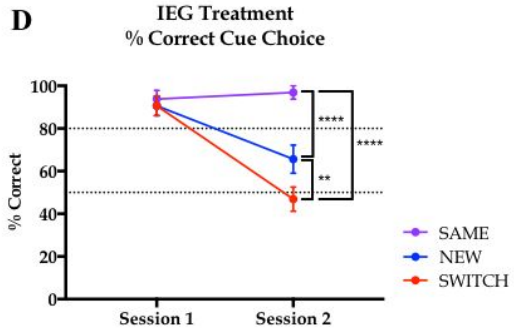
Confocal Stereology
Optical Fractionator


B
Task Acquisition
% Correct Cue Choice
% Correct
Block
NEW
SWITCH


C
Task Acquisition
Average Latency
Latency (sec)
Block
NEW
SWITCH


D
IEG Treatment
% Correct Cue Choice
% Correct
Session 1
Session 2
SAME
NEW
SWITCH

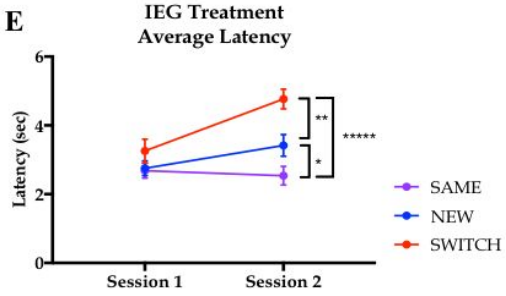

E
IEG Treatment
Average Latency
Latency (sec)
Session 1
Session 2
SAME
NEW
SWITCH

Figure 2.2 IEG Activation task and imaging design and behavioural performance. (a) Schematic diagram of IEG Activation task design and example image of a CA1 confocal z-stack of fISH-processed tissue. In session 1 animals swam four trials with cues in the same position as a previous session to activate Homer1a mRNA expression. This was followed by a 20-min return to the home cage and then a second, four-trial session in which the cues were shifted to NEW and SWITCH arrangements, or not shifted at all in the SAME group. Rats were then perfused and had their brains processed for fISH staining. Folloing fISH tissue processing, DAPI, Homer1a, Arc, and double label markers were estimated using the optical fractionator method adapted for confocal stereology. (b) Two-platform water task acquisition percent correct cue choice. The results from the second cohort of animals used for IEG Activation and quantification displayed similar behavior in percent correct cue choice as animals that performed the extended task in the data summary (Figure 2.1). Correct cue choice was greater following NEW than SWITCH cue shifts across the three acquisition blocks prior to IEG activation. (c) Two-platform water task average latency to the correct cue. The cohort used for IEG Activation and quantification did not display a reliable difference in NEW compared to SWITCH average latency to the correct cue during task acquisition, unlike animals in the data summary. This difference in results across the present experiments suggests that percent correct cue choice is a more sensitive measure to detect differences in navigation strategy in the two-platform water task. (d) IEG Activation percent correct cue choice. Performance in the SAME group in session 1 and 2 suggest that when cues occupy the

same location as the previous session, rats are able to reliably retrieve the correct cue-place memory. However, following a NEW cue shift, there is a drop in session 2 performance due to initially random choice when the cues occupy new places, followed by rapid learning of the correct cue-place strategy. Finally, SWITCH cue shifts during IEG Activation resulted in animals persisting to target the incorrect cue in the previously correct place, causing a greater decline in percent correct cue choice during session 2. (e) IEG Activation average latency to the correct cue. In keeping with percent correct cue choice during IEG Activation, rats were able to quickly navigate to the correct cue in the SAME cue shift condition during session 1 and 2. Differences in average latency performance are evident during session 2, when animals take longer to reach the correct cue during NEW and SWITCH cue shifts due to incorrect cue choices, with the greatest latency to reach the correct cue following a SWITCH cue shift.

3.2 *IEG Activation*

The IEGs were activated in two, four-trial swim sessions separated by twenty minutes (Figure 2.2a). This design allows us to assess Homer1a mRNA expression as a marker of neural activity during the first session, and Arc mRNA expression as a marker of neural activity during the second session. During the first session rats were returned to the room with the cues in the same position as the previous day of training, and were given four swim trials with a one-minute inter-trial interval over a five-minute session. The rats were then returned to their home cage for twenty minutes before coming back to the room with the cues shifted to one of three possible locations: SAME (0° shift), NEW

(90° shift), or SWITCH (180° shift). The rats swam for an additional four trials with 1-min inter-trial intervals over a 5-min session in one of the three shift conditions and were then perfused and had their brains extracted ~8 min after the second session.

Behavioral results from this phase of the task illustrate that each group in the SAME, NEW, and SWITCH cue shift conditions successfully retrieved the correct cue-place strategy during session 1 (Figure 2.2d). Performance in session 2 varied across shift conditions, resulting in a significant effect of session ($F(1,21) = 26.84$; $p < .0001$), shift ($F(2,21) = 17.15$; $p < .0001$), and session × shift interaction ($F(2,21) = 10.41$; $p = .0007$; Figure 2.2d). Although uncorrected post-hoc LSD comparisons revealed no significant differences in percent correct cue choice in session 1, there were significant differences in percent correct cue choice between SAME versus NEW ($p < .0001$), SAME versus SWITCH ($p < .0001$), and NEW versus SWITCH ($p = .0097$) conditions during session 2. We found similar effects in latency to the correct cue, resulting in a significant effect of shift ($F(2,21) = 9.338$, $p = .0003$), session ($F(1,21) = 9.338$, $p = .006$), and shift × session interaction ($F(2,21) = 4.642$, $p = .0214$; Figure 2.2e). In addition, we found significant differences between SAME versus NEW ($p = .0304$), SAME versus SWITCH ($p < .0001$), and NEW versus SWITCH ($p = .0014$) cue shifts in latency to the correct cue in session 2, but no significant differences between shift conditions in session 1. These findings extend the results of the two-platform water task summary in both groups and further show that rats can maintain a reliable memory of the correct cue-place strategy in the SAME cue condition, are able to rapidly encode a new cue-place strategy in the NEW condition, and perform significantly worse following

SWITCH cue shifts due to navigation to the incorrect cue for several trials. We anticipated that the CA1 population would remain stable in the SAME condition, given the accurate performance in both sessions 1 and 2. In general, we expected that cue relocation would cause CA1 remapping following a NEW or SWITCH cue shift. However, SWITCH cue shifts might cause less remapping due to different cues occupying the same locations, while NEW cue shifts might induce greater remapping. Our first hypothesis suggests that reactivation (higher similarity) should benefit performance across all groups, while the second hypothesis suggests that remapping (lower similarity) should benefit performance following shifts.

3.3 *CA1 IEG Expression*

Following Arc and Homer1a mRNA labeling, we estimated the population of DAPI, Homer1a, Arc, and Double Labels across the septal-temporal axis of CA1 using a confocal design-based stereology approach in a randomly chosen, representative subset of animals from the behavioral cohort ($n = 14$; Figure 2.2a). These animals did not differ in their behavior from the greater cohort during session 2 of IEG activation ($F(1, 32) = 2.564$; $p = .1192$). Our results indicate a similar number of DAPI-labeled cells in a single hemisphere of CA1 to previous reports using similar methods (Heggland, Storkaas, Soligard, Kobro-Flatmoen, & Witter, 2015), suggesting that the present confocal design-based stereology approach provides a reliable estimation of cell number (Figure 2.3a). We found a significant effect of label ($F(3,33) = 91.73$, $p < .001$) in our population estimates, but not a significant effect of group ($F(2,11) = 0.6531$, $p = .5395$)

or label × group interaction ($F(6,33) = 0.5856$, $p = .7392$; Figure 2.3a). We normalized the active population of neurons in each animal using the simple calculation: QTot = (QH1a + QArc) – QDbl. A one-way ANOVA showed no significant effect of group on the estimated number of labeled CA1 neurons ($F(2,11) = 0.6383$, $p = .5467$; Figure 2.3b). Following normalization, we sought to determine how similar the population of active neurons was between sessions 1 and 2 in each group using a similarity index (SI) measure. To determine SI we used the following calculation for each animal: SI = QDbl/QTot. Thus, SI measures the proportion of cells labeled in both sessions out of the total population of labelled cells, without assuming any pattern of recruitment to the active population (Witharana et al., 2016). We first examined the relationship between SI and performance during session 2 of IEG treatment to answer if there was a significant relationship between reactivation or remapping and memory retrieval at the behavioral level. A linear regression of SI versus percent correct cue choice in session 2 on all groups revealed a strong correlation between memory reactivation measured with SI and performance of correct cue choice ($R^2 = .5858$, $F = 16.97$, $p = .0014$; Figure 2.3c). When we performed a follow-up regression on animals from the NEW and SWITCH shift groups only we found a trending but non-significant positive correlation between SI and percent correct cue choice ($R^2 = .3556$, $F = 3.863$, $p = .09$). We then sought to further test our prediction that cue shifts in the two-platform water task during session 2 would result in remapping. A one-way ANOVA showed a significant effect of group ($F = 4.694$, $p = .0336$; $\eta^2 = 0.60$; Figure 2.3d), confirming that cue shifts induce a significant change in the

CA1 population code. Uncorrected LSD post-hoc comparisons revealed that NEW ($n = 4$; $p = .0122$, $d = 2.10$, $1 - \beta = 0.76$) cue shifts caused a significantly lower SI score compared to the SAME cue condition ($n = 5$), while SWITCH shifts resulted in a trending but not significantly lower SI ($n = 5$; $p = .0731$, $d = 0.60$, $1 - \beta = 0.59$). We did not find a significant difference between NEW and SWITCH cue shift groups ($p = .2837$, $d = 0.64$, $1 - \beta = 0.13$). Together, these results demonstrate a positive relationship between cue choice accuracy and CA1 remapping, and that remapping might have different functions when animals are faced with SAME, NEW, or SWITCH cue shifts.

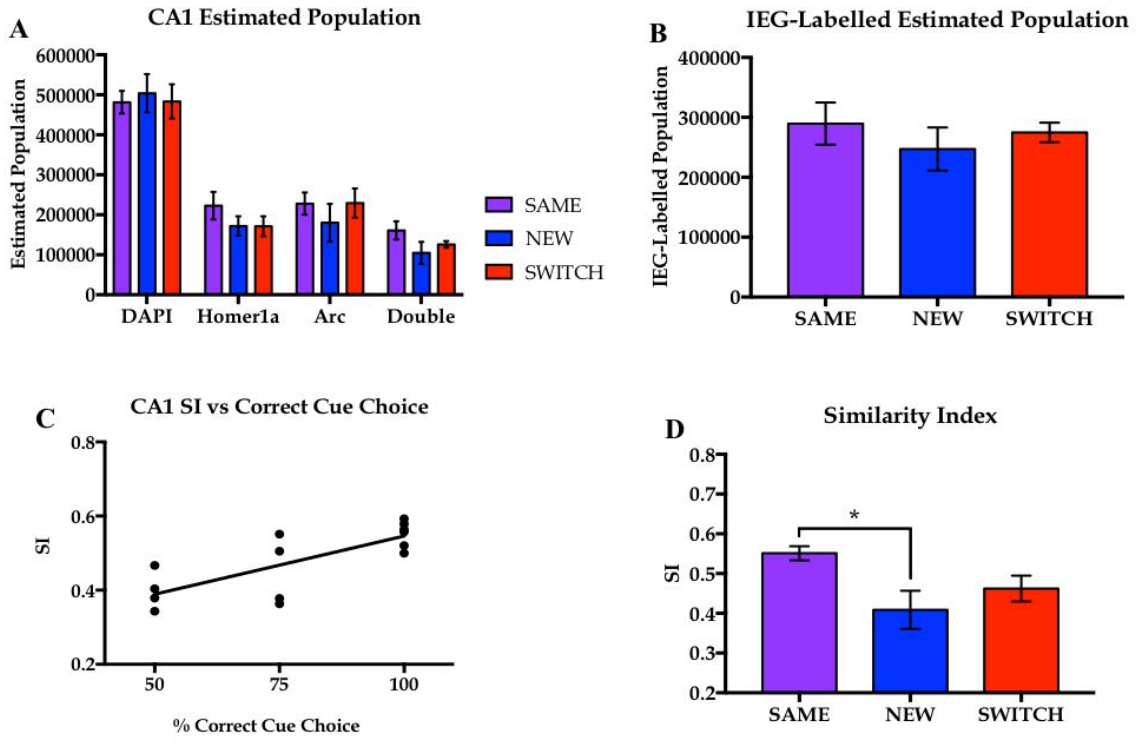


Figure 2.3 IEG quantification results. (a) CA1 estimated population for all markers and IEG Activation groups. Results from stereologic quantification of DAPI and IEG markers showed a significant effect of label but not group or label × group interaction. (b) IEG-labeled estimated population. Following calculation of the total number of cells expressing IEG labels (see Section 2) we compared the estimated population of IEG-labeled cells in CA1 across IEG activation groups. A one-way ANOVA revealed no main effect of group on the total population of labeled cells in CA1. (c) Linear regression of SI and percent correct cue choice in session 2 of IEG activation. We performed a linear regression to examine the relationship between SI as a measure of the extent of CA1 population remapping and percent correct cue choice. Our results demonstrate a significant positive correlation between these measures, suggesting that greater SI results in better performance in the two-platform water task. (d) SI following different cue shifts in IEG Activation. Using SI as a measure of the extent of remapping across the CA1 population, we found a significant effect of cue shift on SI. Post hoc comparisons revealed that SI was significantly lower following a NEW but not SWITCH cue shift compared to the SAME shift condition.

4. Discussion

Our findings demonstrate an important relationship between the extent of CA1 population remapping and memory-guided navigation. We have found a significant correlation between ensemble reactivation and memory retrieval in a two-platform water task, and that relocating cued goals in induces remapping in CA1 related to the learning

of new cue-place information. This finding supports our first hypothesis that reactivation benefits correct cue choice in the two-platform water task. This is the first demonstration, to our knowledge, of a significant relationship between ensemble reactivation across the septal-temporal axis of CA1 and memory retrieval using the IEG method. However, it may also be the case that remapping has a distinct function following cue shifts. NEW cue shifts may result in immediate remapping with initially random cue choice, followed by rapid cue-place learning; SWITCH shifts may result in retrieval of a more similar memory due to cues locating the same positions with worse overall performance due to retrieval of previous place associations. We view this as the most consilient explanation of our behavioral data, although more investigation is clearly needed. We have found a significant difference in SI between groups subjected to SAME and NEW cue shifts, but not between SAME and SWITCH cue shifts. However, we did not find a significant difference between NEW and SWITCH cue shifts. Based on our findings, we cannot rule out another explanation, that remapping could have different functions following SAME, NEW, or SWITCH cue shifts. Importantly, our results support the idea that cue relocation induces population remapping in CA1 and that similarity in the memory code is positively related to cue choice accuracy in the two-platform water task. These findings also add to a growing literature describing the representation of multiple aspects of long-term memory in the rodent hippocampus and its relevance to animal behavior.

Based upon retrograde amnesia effects, a surprisingly broad range of aspects in a learning episode are represented in the rodent hippocampus (Lee et al., 2016; McKenzie et al., 2014; Wood et al., 2000). Hippocampal disruption using either temporary

inactivation or permanent lesions causes robust retrograde amnesia for context fear (Gulbrandsen et al., 2013; Sutherland et al., 2008; Sutherland et al., 2010), context discrimination (Lee et al., 2017), tone fear (Sutherland et al., 2008), fear-potentiated startle (Lehmann et al., 2010), cue memory (Sutherland et al., 2001), picture memory (Epp et al., 2008), home base memory (Travis et al., 2010), spatial memory (Broadbent et al., 2004; Sutherland et al., 2001), and episodic memory (Steinvorth et al., 2005). In a recent review we discussed these findings and their implications for a new view on the role of the hippocampus in long-term memory (Lee et al., 2016). We proposed a new concept, termed heterarchic reinstatement (HR), to account for a broad range of these results. On this view, the output of activity from the hippocampus to the cortex during a learning episode will result in the hippocampal output to the cortex becoming an essential part of most or all target memories. The HR concept predicts that changes in the output of the hippocampus to the cortex will result in changes to the target memory, and task behavior. Thus, HR suggests that population remapping would result in changes at the behavioral level for the many aspects of memory encoded in CA1 cell activity.

Several reports have described that many features of a learning episode are encoded in single-cell and population activity in CA1, including place, visual cues, odors, approach behaviour, and anticipated rewards (Komorowski et al., 2009; McKenzie et al., 2014; Wood et al., 2000). However, some authors have recently questioned whether simple cues represented in hippocampal activity are necessary for guiding animal behavior (Ainge et al., 2012). For example, Ainge et al. (Ainge et al., 2012) described that place unit activity is not controlled by discriminative visual cues, but instead is under

control of the animal's goal location. By contrast, McKenzie et al. (2014) found that place field firing rates can be modified by repeated presentations of a cue in a context-specific location followed by reward. In the current study, we have found that changes in the CA1 memory code are related to changes in visual cue discriminations. Notably, we have examined this relationship following just three blocks of training when spatial memory also has strong control over behavior. It would be interesting in future studies to examine if the relationship between remapping and correct cue choice remains following additional training when animals make responses that may be more strongly controlled by cues.

Previous studies on place cell remapping in the hippocampus have revealed that CA1 has distinct remapping characteristics from the dentate gyrus (DG) and CA3 (Lee et al., 2004; Leutgeb et al., 2007; Leutgeb et al., 2005; Vazdarjanova & Guzowski, 2004). While CA1 tends to show continuous place cell remapping in response to changes in spatial context, CA3 exhibits discontinuous or attractor-like remapping, and the dentate gyrus tends to show remapping following minor changes in spatial context (Lee et al., 2004; Leutgeb et al., 2007). In future studies, it will be important to examine the relationship between remapping in CA3 and the DG to changes in memory-guided behavior. We anticipate that the changes in population activity in the DG-CA3 circuit is the cause of remapping in CA1, and that pattern separation processes may be critical to recognizing shifts in cue orientation relative to previous experience in the two-platform water task and the rapid learning of new cue-place information. Although pattern separation may be a general computation also shared by cortical networks (Leutgeb &

Leutgeb, 2007; Yassa & Stark, 2011), the hippocampal circuit likely provides a unique contribution in its ability to rapidly retrieve a target memory and detect when a spatial context has changed.

The present findings are the first demonstration, to our knowledge, of a significant relationship between cellular reactivation and memory retrieval at the behavioral level applying the IEG imaging approach across the entire CA1 septal-temporal axis. Importantly, we have found that this relationship is robust in a cued navigation task with a simple visual discrimination guiding behavior. In combination with other studies on changes in the memory code and its relation to behavior (Danielson et al., 2016; Dupret et al., 2010; Komorowski et al., 2009; McKenzie et al., 2014), these data suggest that multiple features represented in CA1 activity make an important contribution to memory retrieval. In future studies, it will be important to characterize which representations at the single-unit and population level maintain a significant relationship to memory behavior across training in the two-platform water task or a similar task, and are affected by changes to cue-place presentation in a spatial context. It will also be important to characterize the lasting effects of remapping on behavioral performance, and that remapping measured with IEG activation is not only a transient result of novelty detection (Fyhn et al., 2002). Further, within-subject designs will serve as a powerful tool to examine changes in cue and spatial representation in the hippocampal memory code, and their relation to behavior across the learning experience. In addition, future studies may examine septal-temporal differences in hippocampal neuron population responses across the learning experience. Some models of multiple memory systems would suggest that

the CA1 representation would not maintain a relationship with behavior when cue memory gains control, whereas single-process models such as the HR concept predict there will be a relationship between CA1 population activity for both cue- and place-guided behavior (Lee et al., 2016). Further experiments on this issue will significantly further our understanding of memory organization in the brain.

Chapter 3

Hippocampal damage causes retrograde amnesia and slower acquisition of a cue-place discrimination in a concurrent cue-place water task in rats.

Abstract

Explanations of memory-guided navigation in rodents typically suggest that cue- and place-based navigation are independent aspects of behaviour and neurobiology. The results of many experiments show that hippocampal damage causes both anterograde and retrograde amnesia (AA; RA) for place memory, but only RA for cue memory. In the present experiments, we used a concurrent cue-place water task (CWT) to study the effects of hippocampal damage before or after training on cue- and place-guided navigation, and how cue and place memory interact in damaged and control rats. We found that damaging the hippocampus before training caused a delay in the expression of cue-place navigation strategies relative to intact control animals; surprisingly, place navigation strategies emerged following pre-training hippocampal damage. With additional training, both control and damaged rats used local cues to navigate in the CWT. Damaged animals also show minor impairments in latency to navigate to the correct cue following a cue contingency reversal. By contrast to these anterograde effects, damage made after training causes RA for cue choice accuracy and latency to navigate to the correct cue. In addition, the extent of hippocampal damage predicted impairments in choice accuracy when lesions were made after training. These data extend previous work on the role of the hippocampus in cue and place memory-guided navigation, and show

that the hippocampus plays an important role in both aspects of memory and navigation when present during the learning experience.

Introduction

Multiple environmental features guide navigation, including place information and local visual cues that predict goal locations. Many groups have used behavioural models of navigation in rodents to examine if these features of memory depend on different brain structures (McDonald & White, 1993; Morris et al., 1982; Morris et al., 1986; Sutherland et al., 1982), but few studies have examined the relationship between these aspects of memory and their underlying neurobiology (Devan & White, 1999; McDonald et al., 2004; McDonald & White, 1994). Hippocampal damage reliably impairs the ability of rodents to navigate to places in an environment (Clark, Broadbent, & Squire, 2005; Morris et al., 1982; Morris et al., 1986), while damage to the dorsal striatum impairs cue-guided navigation (Devan & White, 1999; McDonald & White, 1993; McDonald & White, 1994). However, recent work has also shown that neither impairment is absolute; over-training allows lesion animals to express either navigation strategy with less spatial specificity following hippocampal damage (Devan & White, 1999; Hales et al., 2014; McDonald & Hong, 2000; Morris et al., 1990). Some recent concepts on anterograde and retrograde memory also predict that lesions of the hippocampus will cause retrograde amnesia (RA) for a wide range of memory types (Lee et al., 2016). This view contrasts many popular models of the hippocampus and memory, which posit the existence independent memory systems (McClelland et al., 1995; Squire, 1992; White, 2002), and thus damage to the hippocampus would only impair a specific range of memory types, such as place, episodic, or associative memory in the anterograde and retrograde direction.

Experiments on cue-guided navigation and the effects of hippocampal damage on anterograde amnesia (AA) have consistently found that this structure is not necessary for the acquisition and expression of cue-guided navigation strategies (McDonald & White, 1993; Sutherland & Rudy, 1988). For example, Morris et al. (1986) utilized a visible, two-platform water task to test if hippocampal damage would cause AA in cue-based navigation. In this experiment, two visible platforms with distinct visual appearances signalled possible escape locations in opposite quadrants of a pool filled with opaque water. Only one platform was supported with a hidden pedestal and allowed escape, while the other was tethered and floating in place, but would not support the animal. The location of the cues was shifted each swim trial, and therefore the only accurate strategy to escape from the pool was to discriminate between the correct and incorrect visual cues. Morris et al. (1986) found no difference between sham-operated, cortically-lesioned, and hippocampus-lesioned animals in the ability to make correct cue choices. However, using a similar visual cue discrimination task Sutherland et al. (2001) found that hippocampal lesions made after training caused RA (Sutherland et al., 2001). Growing evidence supports that hippocampal damage causes a wide range of RA in different memory tasks, including simple cue discriminations (Epp et al., 2008; Kim et al., 2015; Sutherland et al., 2001), and is not only involved in spatial or associative aspects of memory as the independent memory systems concept and others suggest. This work also shows the effects of hippocampal damage differ in the anterograde and retrograde direction (Fanselow, 2009; Lee et al., 2016; Sutherland et al., 2010). The first goal of our study was to further assess the hypothesis that hippocampal lesions will cause RA but not AA for a

cue discrimination task, and whether a deficit is related to lesion size. We predicted that hippocampal damage would result in RA but not AA for a simple visual discrimination, and that the extent of damage would predict memory performance with post-training hippocampal damage. This expected outcome contrasts with the predictions of popular theories on the hippocampus, which suggest damage would not result in either RA or AA for a simple visual discrimination.

In addition, we aimed to assess how cue and place aspects of memory interact during navigation. In one experiment examining cue and place memory interactions, McDonald and White (1994) trained rats to swim to a single visible platform in a fixed location for three days, and on a fourth day they submerged the platform and trained rats to navigate to the same location without the local visual cue present (McDonald & White, 1994). After repeating this training cycle three times, the visible platform was moved to the opposite pool quadrant. McDonald and White (1994) discovered animals with fornix lesions were impaired at navigating to the submerged platform throughout training, and when the visible platform was moved to a new location, some control rats swam to the previous goal location; others to the visible cue in a new location. In contrast, fornix-damaged animals only swam to the visible cue (see also Devan et al., 1999). Thus, a second goal of the present experiments was to test the hypothesis that hippocampal damage before training in a visually cued navigation task would cause AA for place but not cue-based aspects of navigation, while control animals express place-based strategies in early training, and later show cue-based navigation (Lee et al., 2018; Morris et al., 1986).

We recently developed a novel water task, a concurrent cue-place water task (CWT), adapted from Morris et al. (1986) and McDonald and White (1994), to examine cue- and place-based navigation in parallel, and how these aspects of memory interact (Lee et al., 2018). This task involves distinct patterns of cue shifts that allow us to assess if animals use cue- or place-based features to navigate to a goal location. Recently we found that the hippocampal population activity remaps following changes in cue locations in the CWT, and that changes in population activity are related to the extent of remapping in CA1 (Lee et al., 2018). Based on this finding and previous work from McDonald and White (1994), we predicted that the hippocampus would be critical for tracking cue locations in the CWT, and thus for expressing spatial navigation strategies after pre-training lesions. Based on previous cue discrimination studies, we expected cue-based navigation would remain fully intact.

Previous work using cue-based tasks in the radial arm maze showed that hippocampal damage enhances the ability of animals acquire cue reversal (McDonald et al., 2004; McDonald et al., 2006; McDonald & White, 1995). Following training to discriminate accurately between cues in the CWT, cue contingencies can also be reversed to examine cue reversal learning ability. We anticipated that hippocampal lesions would possibly enhance the ability to reverse a cue strategy with lesions made before training.

Experimental Procedures

Subjects

All procedures were approved by the University of Lethbridge Animal Welfare Committee and meet the Canadian Council of Animal Care guidelines. Experimentally-naïve male Long Evans rats (Charles River; Raleigh, NC) weighing approximately 350 - 450 g were used in the following experiments. Rats were acclimatized to the University of Lethbridge colony room for at least one week following arrival from the breeding facility, and handled by the experimenter for five minutes daily over five days before the start the experiment.

Surgery

Rats sustained hippocampal damage with microinjections of NMDA or sham surgery either before or after training in the CWT, which were procedurally identical to Lee et al. (2017). Thirty minutes prior to surgery rats were given an injection of phenobarbital (30 mg/kg), and metacam (1 mg/kg) upon anesthetic induction with 4% isofluorane dissolved in oxygen. Thereafter, rats were maintained at 1.5-2.5% isofluorane anesthesia and mounted in a stereotaxic frame. Holes were drilled over respective bilateral injection sites and the dura was gently punctured using a 30-gage needle. Lesion rats were given bilateral injections of 7.5 ug/uL NMDA dissolved in 0.9% sterile saline through 30 gage steel cannulae attached to 10-uL Hamilton syringes and microinjection pump at 7 sites bilaterally along the anterior-posterior hippocampal axis at a flow rate of 1.5 uL/min (Table 3.1). Following each injection, cannulae were left in place for a 3.5-minute diffusion period before removing them from the brain. The same procedure

was given to sham-operated rats, except nothing was injected into the brain. Diazepam (5 mg/kg) was also given post-operatively as prophylactic to counter seizure behaviour.

Injection Site	**AP**	**ML (+/-)**	**DV (L)**	**DV (R)**	**Volume (uL)**
1	-3.1	1.5	-3.6	-3.6	0.4
2	-4.1	3	-4	-4	0.25
3	-5	3	-4	-4	0.25
4	-5	5.2	-7.3	-7.3	0.4
5	-5.8	4.4	-4.4	-4.4	0.25
6	-5.8	5.1	-7.5	-7.5	0.5
7	-5.8	5.1	-6.2	-6.2	0.5

Table 3.1 The table depicts the stereotaxic sites relative to bregma and the volume of NMDA injections in the lesion group across experiments in the present study.

CWT Apparatus and Behavioural Procedures

The CWT training methods used here have also been described previously in Lee et al. (2018). The rationale behind the CWT is that distinct changes to cued goal locations can reveal cue- or place-based navigation, and which strategy controls behaviour during learning. The apparatus consists of a 2-meter circular pool filled with room temperature water made opaque with white tempura paint. The pool contains 2 visible platforms

(cues), that extend 5 cm above the water in pool opposite quadrants, and differ in their visual appearance. One cue is solid black and made from plastic with a rubber surface, while the other has bold, black and white stripes made from PVC imitation wood. Throughout training only one cue is positively reinforced (S+) with the use of a hidden pedestal supporting the platform that allows the rat to escape from the water, while the other is floating and tethered in place but does not support the animal to escape from the water (S-). Several distal cues also surround the pool, including posters on the northern, eastern, and western walls, in addition to a table and computer along with miscellaneous items for behavioural monitoring located southwest of the pool, a door to the south, and a computer rack next to a sink and towel dispenser to the southeast.

During each training session, rats are transported to the room in a holding cage on top of a cart covered with a bath towel to occlude their vision of the surrounding area (Figure 3.1). The pool cues are in the centre of randomly chosen, opposite pool quadrants at the start of the experiment. Rats are introduced at one of two, equidistant start locations facing the pool wall on each trial, and they can swim for a maximum of 60 seconds until they reach the correct cue or the maximum time has been reached. If the animal does not reach the correct cue before the end of the trial, the experimenter places the rat onto the correct cue. Following each trial, the rat remains on the cue for 10 seconds and is returned to the holding cage for approximately 1 to 2 minutes before the next trial. During each training session, the cues remain in the same position for 8 trials, and afterwards the rats are returned to their home cages for approximately 24 hours. Importantly, the pool cues remain in constant locations during each swim session so animals can learn the spatial

location of the correct cue on that day. Upon returning to the room, the pool cues are shifted 90° clockwise or counter-clockwise to NEW locations, or 180° to SWITCH locations. These cue shifts probe distinct navigation strategies. NEW shifts reveal if rats learn which cue is correct, and how quickly they can acquire a new cue-place strategy; SWITCH shifts cause previously learned cue and place information to compete, and thus, if place information dominates, it will choose the incorrect cue on several trials. The relationship between cue- and place-based navigation is shown by comparing performance on shift sessions: worse performance on SWITCH compared to NEW shifts implies rats use place-based navigation, while similar performance on both shifts suggest rats use a cue-based strategy. Each NEW cue shift was followed by a SWITCH cue shift, and were together considered a single training block. This pattern was repeated for a total of 7 blocks, which was based on previous work in our lab that showed performance becomes similar (cue-based) at the training block 6 (Lee et al., 2018). We trained for an additional block to ensure retention of cue contingencies after a surgery and recovery period in tests of RA. Following the completion of training, the pool cues were removed for a 60-second spatial probe to examine if animals remembered the recent goal location independent of the local cues. On the following day, cues were shifted to NEW positions relative to the previous training session, and contingencies were held the same or reversed in a 16-trial massed session.

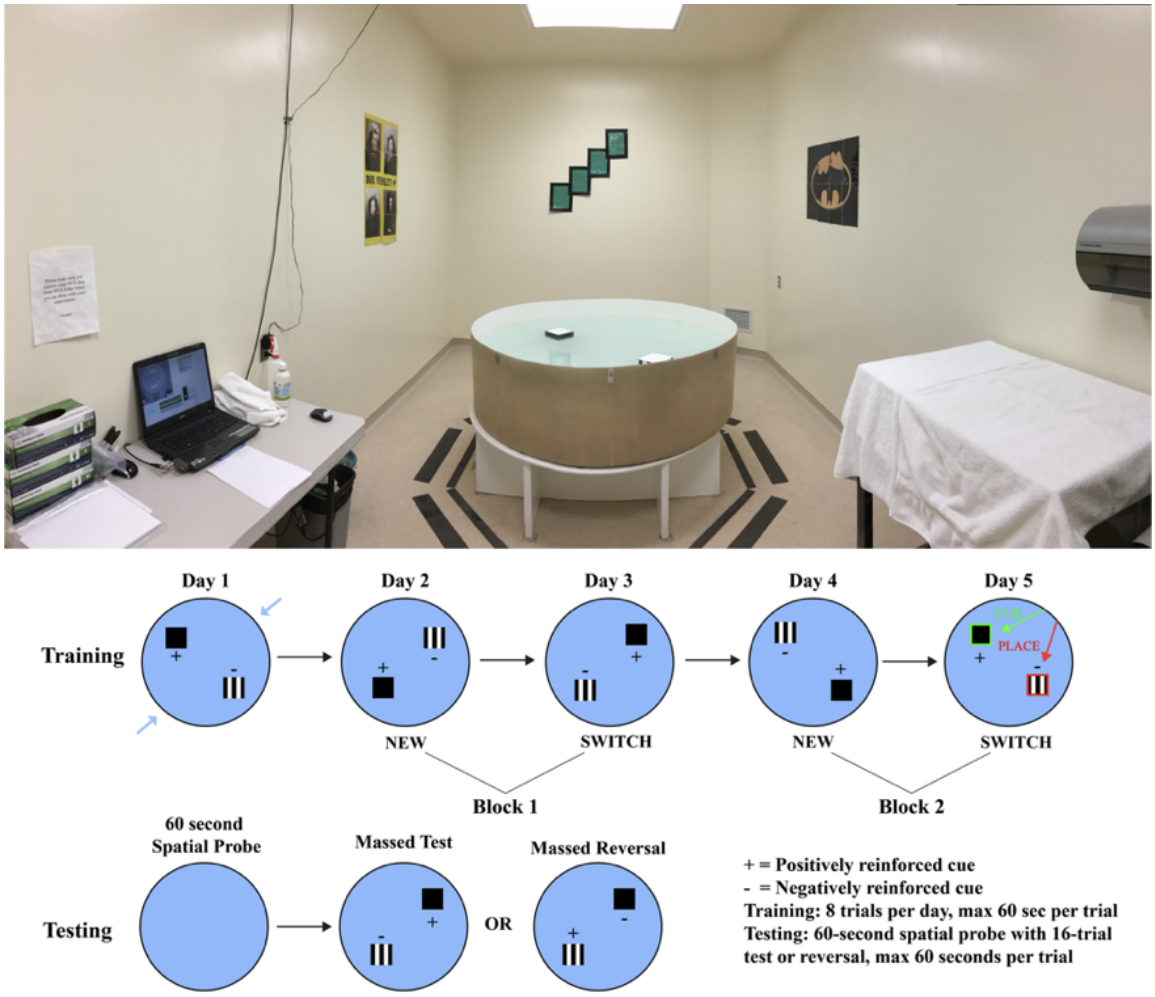


Figure 3.1 The picture shows the training room set-up and apparatus used for the CWT. During each 8-trial session, rats are introduced to at one of two start positions equidistant from the local pool cues that have distinct visual appearances. One of the cues is supported with a hidden pedestal (S+ positively reinforced), while the other is floating in place (S- negatively reinforced), but does not offer escape form the pool. On NEW shift days, the cues are rotated 90° clockwise or counter-clockwise in the pool relative to the previous training session. The following day, cues are shifted 180° relative to the

previous session with the same cue contingencies, but conflicting spatial reinforcement to the previous session, termed a SWITCH shift. Frequent navigation to the incorrect cue on SWITCH shift sessions indicates a place-controlled strategy, whereas navigation to the correct cue regardless of shift indicates a cue-memory controlled navigation strategy.

Animal Perfusion and Tissue Storage

Following the completion of massed training, rats were given an overdose sodium pentobarbital and perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The brain was carefully extracted from each animal and transferred to 4% PFA solution overnight and then held in 30% sucrose solution in PBS with 0.002% sodium azide for at least 48 hours before cryosectioning.

Histology

The Cavalieri estimator method was employed to estimate the volume of remaining hippocampal tissue following NMDA lesions in each experiment. Following cryosectioning at 40 um and cresyl violet staining, every 12th section was sampled for grid point counting at 10 X magnification on a Zeiss AX10 Imager M1 and PCO Sensicam QE High Performance camera connected to Stereo Investigator 10.56 software. Grid points were spaced 120 um apart along the X- and Y-axis of the scaled image in Stereo Investigator. If the upper right corner of a grid point landed on a principle hippocampal subfield, including CA1-3 or the dentate gyrus, the grid point was counted. The estimated hippocampal volume of each lesioned animal was compared to the average

volume of sham control animals to generate a % lesion estimate (% lesion = 100 x (sham volume - lesion volume)/sham volume) (Schmitz & Hof, 2005).

Data Analysis

Data were analyzed using the SPSS and Prism by GraphPad statistical packages. A two-way ANOVA was used to test effects of group, day, block, and interactions for correct cue choice and latency to the correct cue. Uncorrected LSD post-hoc comparisons within groups were evaluated following detection of significant interactions. Relationships between lesion volume and behavioural measures were further examined using a simple linear regression.

Results

Pre-training hippocampal lesions delay CWT acquisition but do not eliminate spatial or cue-based navigation strategies

During CWT acquisition, we found an initial delay in the ability of lesioned rats to navigate to the correct cue in both latency and correct cue choice measures of performance (control n = 24; lesion n = 22). Comparison of correct cue choice training days 1 – 15 revealed a significant effect of group ($F(1,44) = 17.42$; $p = 0.0001$) and day ($F(14,616) = 21.51$; $p < 0.0001$) but not a significant day x group interaction (Figure 3.2A). Latency to find the correct cue showed a significant effect of day ($F(14,616) = 74.63$; $p < 0.0001$), group ($F(1,44) = 18.76$; $p < 0.0001$), and day x group interaction ($F(14,616) = 10.8$; $p <0.0001$; Figure 3.2C). Post-hoc comparisons on latency data

revealed a significant difference between hippocampal and control animals on days 1 – 4, but no differences on days 5 – 15. These results contradict the prediction that lesion animals would show no impairment in the ability to discriminate between cues following pre-training lesions. To examine whether these differences were due to a delay in task performance, we shifted hippocampal lesion data such that days 1 – 15 from controls aligned with days 5 – 19 in the hippocampal group, and termed the shifted data "relative day" (Figure 3.2B and 3.2D). This follow-up analysis revealed an effect of relative day ($F(14,616) = 26.24$; $p < 0.0001$), but no significant group ($F(1,44) = 0.5041$; $p = 0.4815$), or day x group interaction ($F(14,616) = 1.497$; $p = 0.1068$) in correct cue choice, suggesting that both groups similarly improved in task performance following the initial delay in lesion animals. In latency to the correct cue we also found no effect of group ($F(1,44) = 0.4364$; $p < 0.5123$), but we did find a significant effect of relative day ($F(14,616) = 39.85$; $p <0.0001$) and day x group interaction ($F(14,616) = 16.42$; $p < 0.0001$). LSD post-hoc comparisons showed a significant difference between sham and lesion animals only on days 1 and 5, with control animals performing worse than lesion rats on day 1 ($p < 0.0001$) due to higher latency during the first day of acquisition, and lesion rats performing worse than controls on day 5 ($p = 0.0051$).

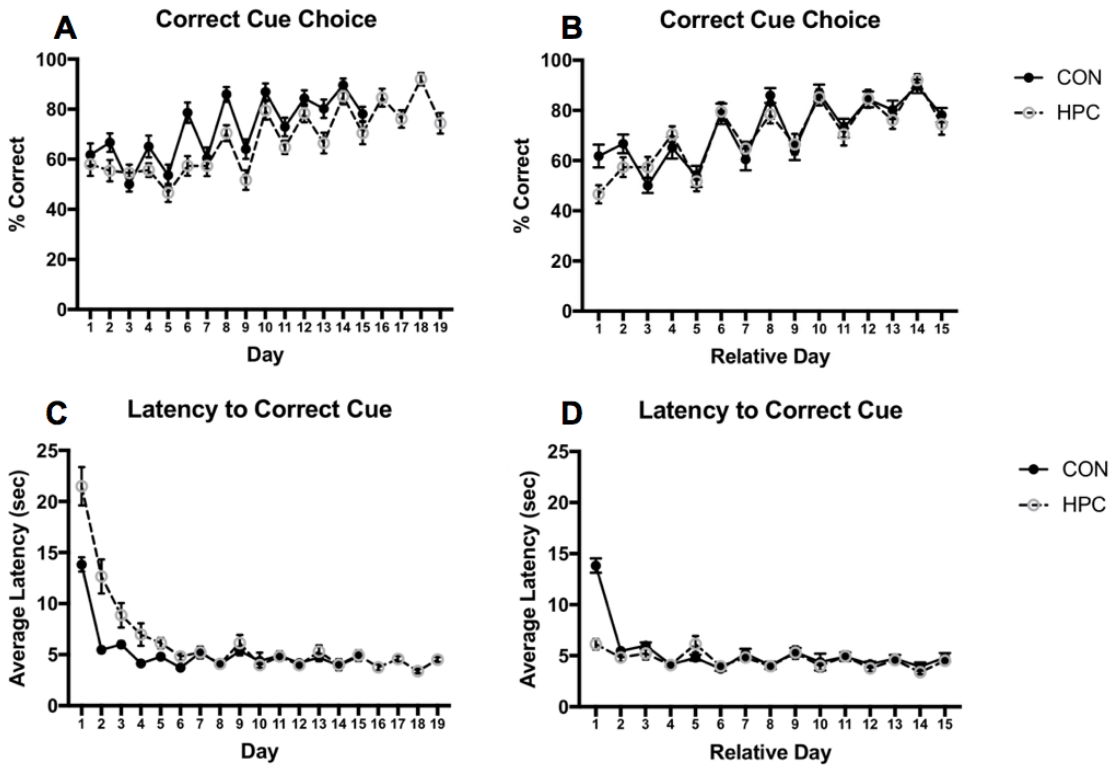


Figure 3.2 Effect of CWT cue shifting on overall latency to the cue choice accuracy and latency to the correct cue in control rats and rats given pre-training hippocampal lesions. (A) Control and lesion animals both show increased cue choice accuracy over the course of training, though lesion animals show an initial delay in task acquisition. (B) When we shifted hippocampal lesion data such that days 1 – 15 from controls aligned with days 5 – 19 in the hippocampal group, control and lesion animals appear similar in cue choice accuracy. (C) Rats given pre-training lesions also show greater average latency to navigate to the correct cue during training, though when lesion animal data are shifted (D) to account for delayed acquisition, latency to the correct cue looks more similar. Differences between lesion and control data when comparing relative day are largely accounted for by greater latency on the first day of learning in control rats. Note the

"saw-toothed" appearance of each graph is due to differences in performance on NEW and SWITCH cue shift sessions across days.

We also compared NEW and SWITCH cue shift days in each group to determine if there was any difference in performance following cue shifts. We expected to find that control but not hippocampal damaged rats would show worse performance in correct cue choice and latency on SWITCH compared to NEW shift days, due to a bias toward choosing a previously reinforced location on SWITCH shifts. In control rats, we found an effect of shift ($F(1,23) = 42.59$; $p < 0.0001$), and block ($F(6,138) = 28.54$; $p < 0.0001$), but no shift x block interaction ($F(6,138) = 1.15$; $p = 0.3366$) in correct cue choice (Figure 3.3A). Similar effects also emerged in latency to the correct cue for controls, revealing a block ($F(6,138) = 10.95$; $p < 0.0001$) and shift effect ($F(1,23) = 37.04$; $p < 0.0001$), but no block x shift interaction ($F(6,138) = 1.811$; $p < 0.1013$; Figure 3.3C). Surprisingly, we found similar effects for hippocampal rats in block ($F(8,168) = 29.06$; $p < 0.0001$) and shift ($F(1,21) = 29.77$; $p < 0.0001$), but no block x shift interaction ($F(8,168) = 1.698$; $p = 0.1022$) for correct cue choice (Figure 3.3B). This result contradicted the prediction that pre-training hippocampal lesions would prevent spatial strategies in the CWT. When we examined latency to the correct cue in hippocampal rats, we also found a significant effect of block ($F(8,168) = 21.16$; $p < 0.0001$) and a block x shift interaction ($F(8,168) = 5.65$; $p < 0.0001$), but not a significant effect of shift ($F(1,21) = 2.924$; $p = 0.1020$; Figure 3.3D). Uncorrected post-hoc LSD comparisons revealed significant differences between NEW and SWITCH performance on block 1 ($p < 0.0001$) and 4 ($p = 0.0054$).

To determine if there was a relationship between lesion size and spatial navigation strategy, we also compared % lesion with the number of days to an 80% correct cue choice criterion (Figure 3.3E), and average % correct difference on NEW and SWTICH cue shifts (average % correct difference = (∑(% correct NEW - % correct SWITCH)) / 100 * number of training days) using a simple regression (mean % lesion = 60.90; SEM = 4.17; min = 31.48; max = 88.81; Figure 3.3F). This analysis revealed no relationship between lesion size and number of days to criterion (R^2 = 0.002217; Figure 3E) or % correct difference measures (R^2 = 0.01577; Figure 3.3F), confirming the prediction that lesions size would not be related to AA in the CWT.

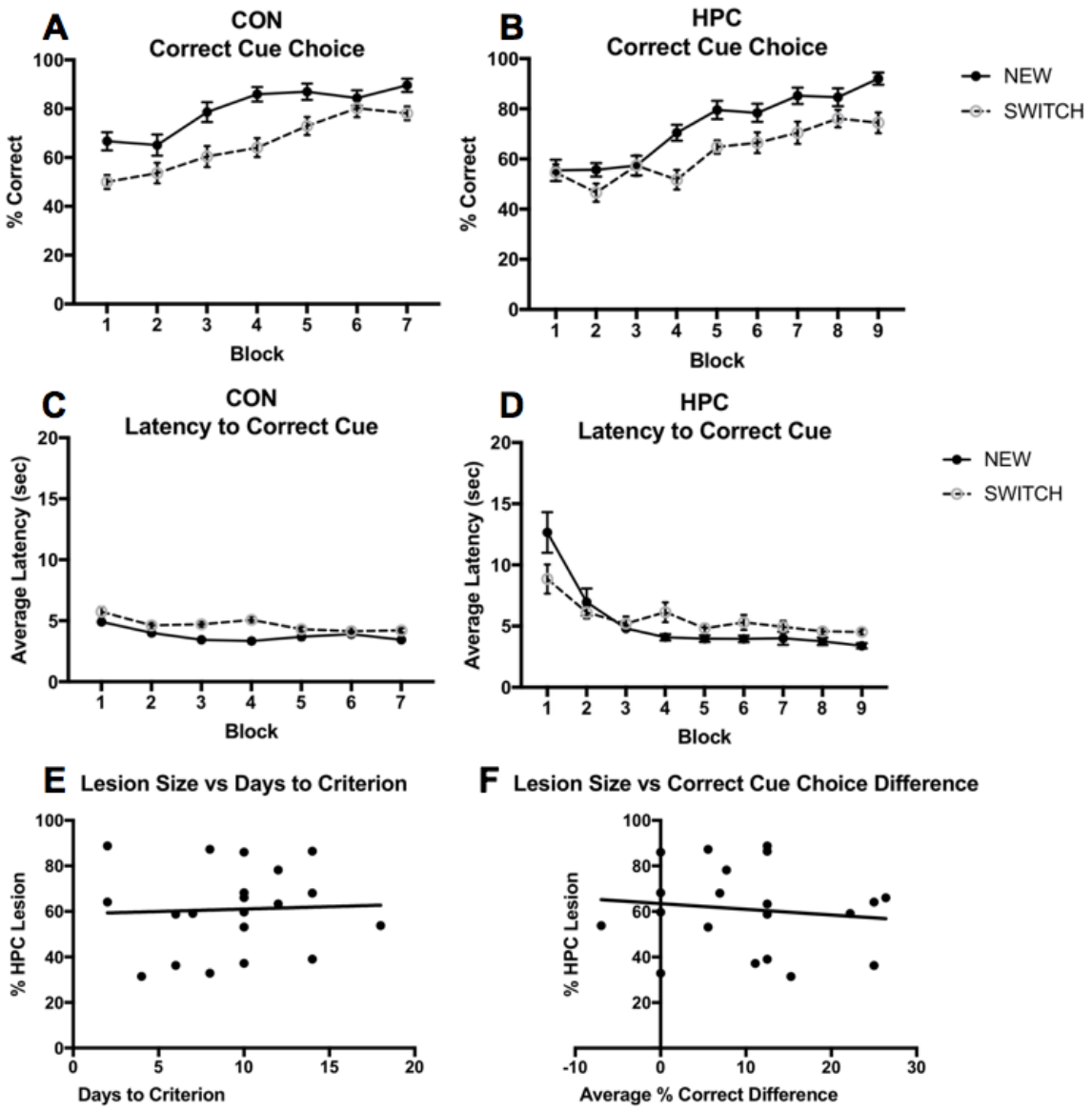


Figure 3.3 Comparison of NEW and SWTICH cue shifts in control and pre-training lesion rats demonstrate an effect of cue shift on choice accuracy in control (A) and lesion rats (B), suggesting that both groups of animals show a spatial bias on SWITCH shift sessions and perform better following a NEW cue shift. Similar effects of cue shift are also present in latency to navigate to the correct cue (C, D). We also examined the effect of lesion size on days to reach an 80% correct cue choice criterion (E) and average

difference between correct cue choice on NEW and SHIFT sessions (F). A simple linear regression revealed that lesion size did not predict either measure of performance when lesions were made prior to training.

Rats do not express independent memory of reinforced locations after training in the CWT

After completion of training, and prior to massed cue memory testing or reversal, we gave rats a 60-second spatial probe with the local pool cues removed to determine if they would express memory for recently reinforced spatial location, independent of local cues to guide navigation (control n = 34; lesion anterograde n = 22; lesion retrograde n = 9). This analysis revealed no effect of quadrant ($F(2,62) = 0.7077$; $p = 0.4034$), group ($F(2,62) = 0.423$; $p = 0.6569$), or quadrant x group interaction ($F(2,62) = 0.4284$; $p = 0.6535$), suggesting that rats rely on local visual cues to navigate at the end of CWT training, and do not use independent spatial memory to perform the task (Figure 3.4).

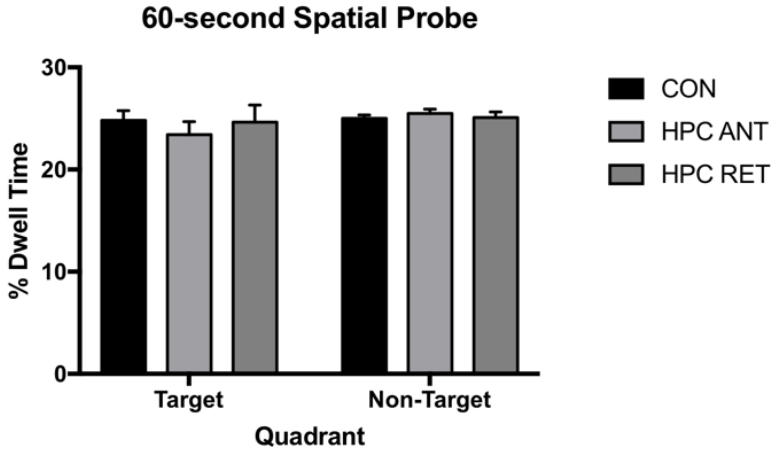


Figure 3.4 Following CWT training, dwell time in target and non-target quadrants during a 60-second spatial probe demonstrates rats do not express spatial memory for recently reinforced locations. This result suggests that control and hippocampus damaged rats rely on local cues to perform the CWT at the end of training, and do not utilize spatial memory alone to navigate to the correct cue.

Pre-training hippocampal lesions do not cause AA for accurate cue choice or latency to a correct cue in a massed test

To ensure that lesion (n = 11) and control rats (n = 12) were trained to a similar level of performance, we assessed cue choice accuracy in a 16-trial massed test with cue contingencies held the same as during training. In correct cue choice measures, we found no effect of trial block ($F(1,21) = 5.222$; $p = 0.0328$), group ($F(1,21) = 0.03223$; $p = 0.8593$), or trial block x group interaction ($F(1,21) = 0.009871$; $p = 0.9218$; Figure 3.5A). Examining latency to the correct cue, we found an effect of trial block ($F(1,21) = 11.52$; $p = 0.0027$), but no effect of group ($F(1,21) = 0.3051$; $p = 0.5865$) or trial block x group interaction ($F(1,21) = 0.6501$; $p = 0.4291$; Figure 3.5B). The lack of significant group and trial block x group interaction terms suggests that 9 blocks of training in the CWT was sufficient to train lesioned rats to similar level of performance compared to control animals, supporting accurate cue strategies in both groups.

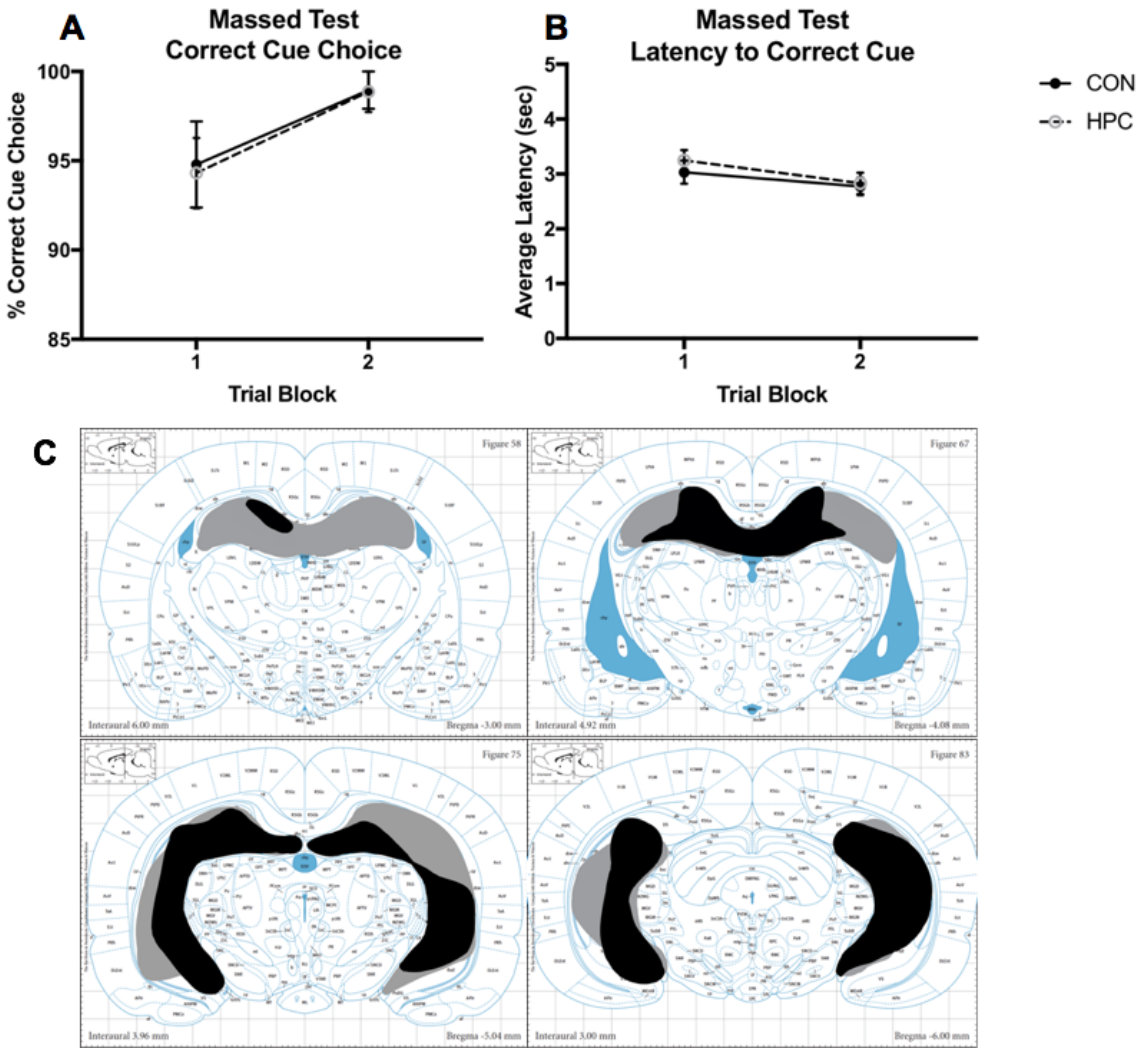


Figure 3.5 Control rats and pre-training lesion rats accurately navigate to the correct cue during a 16-trial massed test when cue contingencies are the same as during CWT training. Similar cue choice accuracy (A) and latency to the correct cue (B) suggests that 9 blocks of training is sufficient for lesion animals perform at a similar level to control rats given 7 blocks of training in the CWT. (C) shows the maximum (grey) and minimum (black) extent of hippocampus damage in the experiment traced over images from Paxinos and Watson (2009).

Pre-training hippocampal lesions impair latency to navigate to a newly correct cue during massed reversal

Based on previous work showing that hippocampal damage affects learning cue reversal, we expected that pre-training hippocampal lesions might improve reversal ability. Therefore, after completion of training we also probed the ability of control (n = 12) and lesion (n = 11) groups to reverse a cue strategy by reinforcing the opposite cue compared to training in a massed reversal session. In correct cue choice measures, our analysis revealed a significant effect of trial block ($F(1,21) = 30.88$; $p < 0.0001$), but not find a significant effect of group ($F(1,21) = 1.857$; $p = 0.1874$), or group x trial block interaction ($F(1,21) = 2.377$; $p = 0.1381$; Figure 3.6A). Latency data also showed a significant effect of trial block ($F(1,21) = 15.81$; $p = 0.0007$), in addition to a significant group effect ($F(1,21) = 5.812$; $p = 0.0252$), but not a significant trial block x group interaction ($F(1,21) = 0.01183$; $p = 0.9144$; Figure 3.6B). The significant effect of trial block, but lack of trial block x group interaction in correct cue choice and latency suggests an effect of cue reversal on performance, and ability of rats to acquire a newly correct cue strategy, though lesion rats were somewhat slower to navigate to the correct cue during massed reversal. This result disconfirms the prediction that lesions rats would be superior to controls at cue reversal.

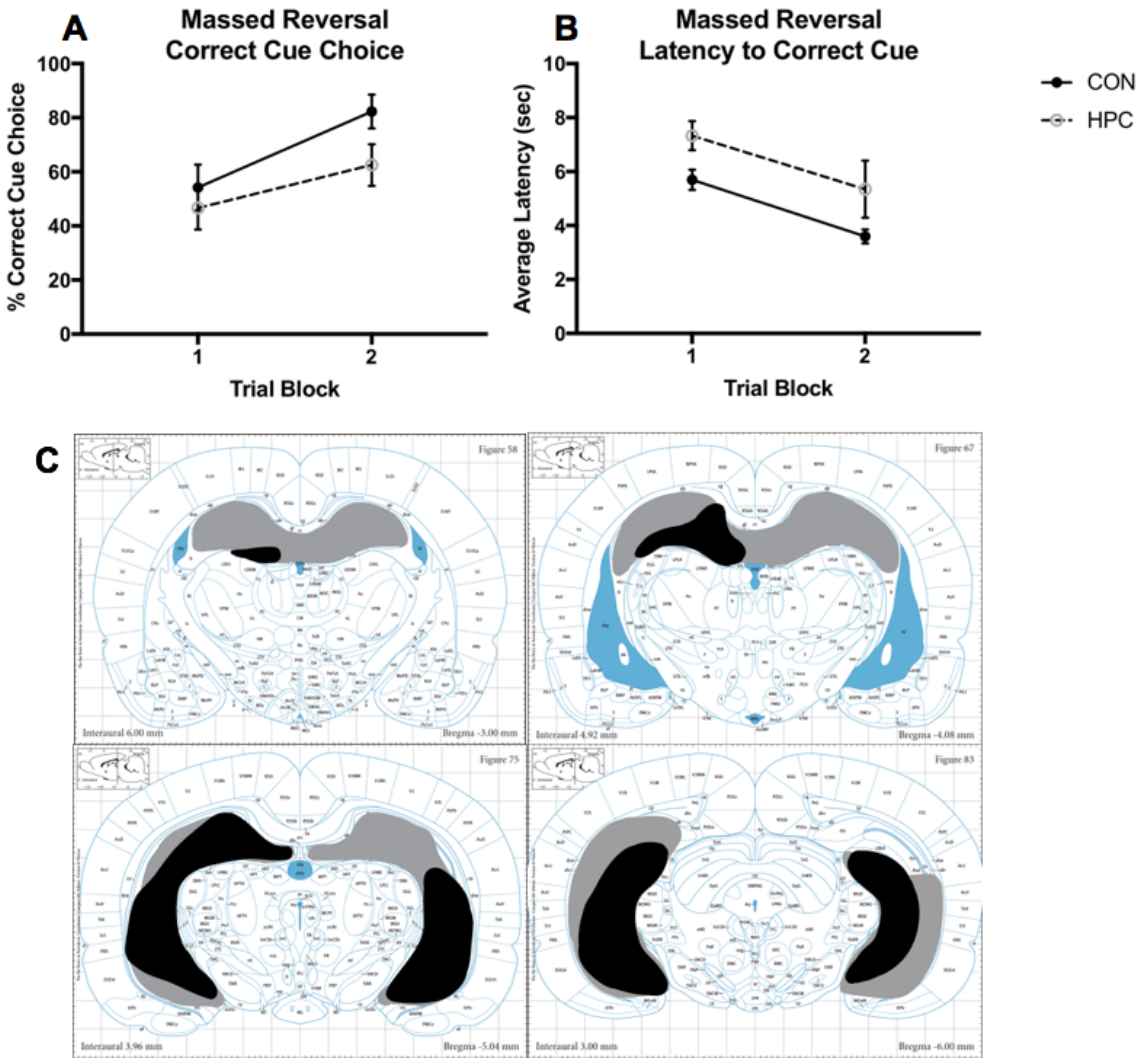


Figure 3.6 Pre-training hippocampal damage impairs latency to navigate to the correct cue but not cue choice accuracy in a 16-trial massed cue reversal. Similar performance in correct cue choice (A) but not greater latency the correct cue in damaged compared to control rats (B) might suggest that hippocampal damaged rats perform a cue reversal with similar accuracy to control animals, though more slowly. However, this result cannot be clearly distinguished from a possible initial delay in learning a new cue contingency, similar to the delay during initial task acquisition when rats are given pre-training hippocampal damage, rather than cue memory reversal ability *per se*. (C) shows the

maximum (grey) and minimum (black) extent of hippocampus damage in the experiment traced over images from Paxinos and Watson (2009).

Post-training hippocampal lesions impair correct cue choice and latency to a correct cue in a massed memory test

Based on previous studies showing differences in AA and RA following hippocampal damage, we anticipated that hippocampal lesions would cause RA for a simple cue discrimination in the CWT, and that lesions size would predict the severity of impaired performance. Thus, in a separate cohort of animals, we studied the effects of post-training hippocampal lesions on cue choice accuracy and latency to the correct cue in a massed test with cue contingencies the same as training (control n = 10; lesion n = 9). To ensure that the groups were comparable, we also examined correct cue choice and latency to the correct cue between groups prior to surgery. This analysis revealed an effect of day ($F(14,238) = 7.991$; $p < 0.0001$), but no effect of group ($F(1,17) = 0.00215$; $p = 0.9636$), or group x day interaction ($F(14,238) = 0.5529$; $p = 0.8991$) in correct cue choice (Figure 3.7A). Similarly, we found a significant effect of day ($F(14,238) = 15.48$; $p < 0.0001$), but no group ($F(1,17) = 0.05855$; $p = 0.8117$) or group x day interaction ($F(14,238) = 1.555$; $p = 0.0929$) in latency to the correct cue prior to surgery (Figure 3.7B). Comparing correct cue choice on NEW and SWTICH shift sessions, we also found a significant effect of block ($F(6,54) = 9.322$; $p < 0.0001$) and shift ($F(1,9) = 38.24$; $p = 0.0002$), but not a significant interaction term ($F(6,54) = 0.8828$; $p = 0.5139$) in control rats prior to surgery (Figure 3.7C). Rats assigned to the lesion group showed similar

effects of block ($F(6,48) = 6.056$; $p < 0.0001$) and shift ($F(1,8) = 13.34$; $p = 0.0065$), but no significant block x shift interaction ($F(6,48) = 1.02$; $p = 0.4237$) in correct cue choice (Figure 3.7D). Comparable results in latency to the correct cue on NEW and SWITCH shift days also revealed an effect of block ($F(6,54) = 4.266$; $p = 0.0014$) and shift ($F(1,9) = 17.02$; $p = 0.0026$) but no block x shift interaction ($F(6,54) = 0.2726$; $p = 0.9474$) in controls (Figure 3.7E). Animals assigned to the lesion group also showed an effect of shift ($F(1,8) = 15.63$; $p = 0.0042$), but no block ($F(6,48) = 0.5449$; $p = 0.7713$) or block x shift interaction ($F(6,48) = 0.5521$; $p = 0.7659$; Figure 3.7F). Despite the lack of a block effect in our lesion group prior to surgery, the general comparison in latency to the correct cue across all days shows that the groups were statistically similar and learned the task before surgery (Figure 3.7B).

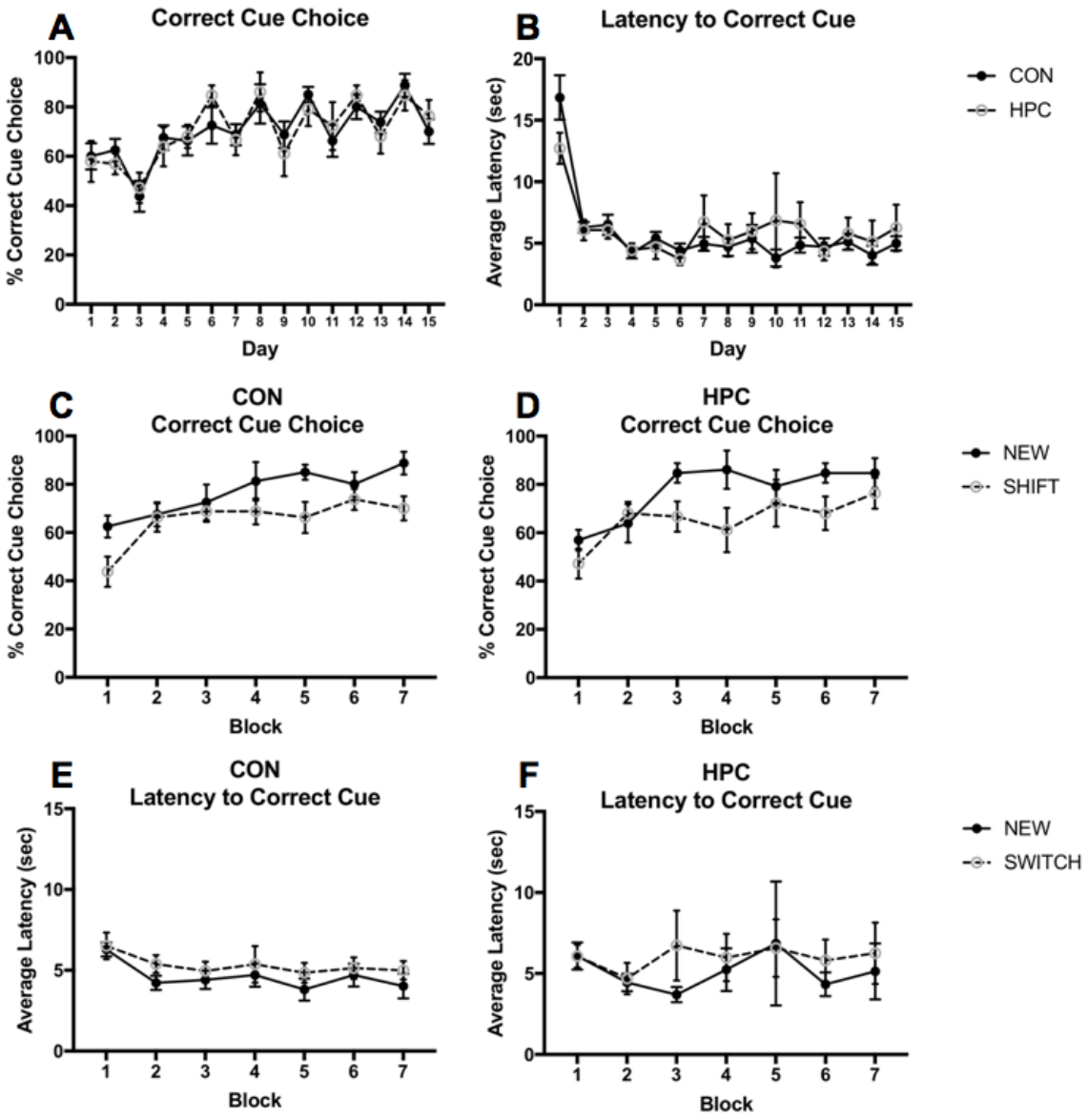


Figure 3.7 Cue choice accuracy and latency to the correct cue in groups of rats prior to sham surgery or hippocampal lesion surgery. We found no effect of group in correct cue choice (A) or latency measures (B) when comparing performance in the two groups prior to surgery. Comparison of the two groups on NEW and SWITCH cue shift sessions also show similar effects of cue shifts on performance in the CWT in animals without hippocampal damage.

Following surgery and a 7- to 10-day recovery period, rats were given a 16-trial massed test with the same cue contingencies as training. This test revealed a significant effect of lesion group ($F(1,17) = 21.22$; $p = 0.0003$) and trail block ($F(1,17) = 10.98$; $p = 0.0041$), but no trial block x lesion group interaction ($F(1,17) = 1.22$; $p = 0.2847$) in correct cue choice (Figure 3.8A). Lesion rats also had a significantly greater latency to the correct cue as shown by a significant group factor ($F(1,17) = 7.397$; $p = 0.0146$; Figure 3.8B). We also found a significant effect of trial block ($F(1,17) = 10.56$; $p = 0.0047$) but no trial block x lesion group interaction ($F(1,17) = 0.004326$, $p = 0.9483$) in latency to the correct cue (Figure 3.8B). To further examine how hippocampal damage is related to cue choice accuracy, we performed a simple regression on % lesion estimates and correct cue choice during the massed test (mean % lesion = 73.96; SEM = 4.77; min = 53.57; max = 87.12), which revealed a significant negative relationship between lesion size and choice accuracy ($R^2 = 0.5745$; $F = 9.452$; $p = 0.0180$; Figure 3.9). These results confirmed the prediction that post-training hippocampal damage would result in RA for correct cue choice in the CWT, and that lesion size would predict the severity of impairment.

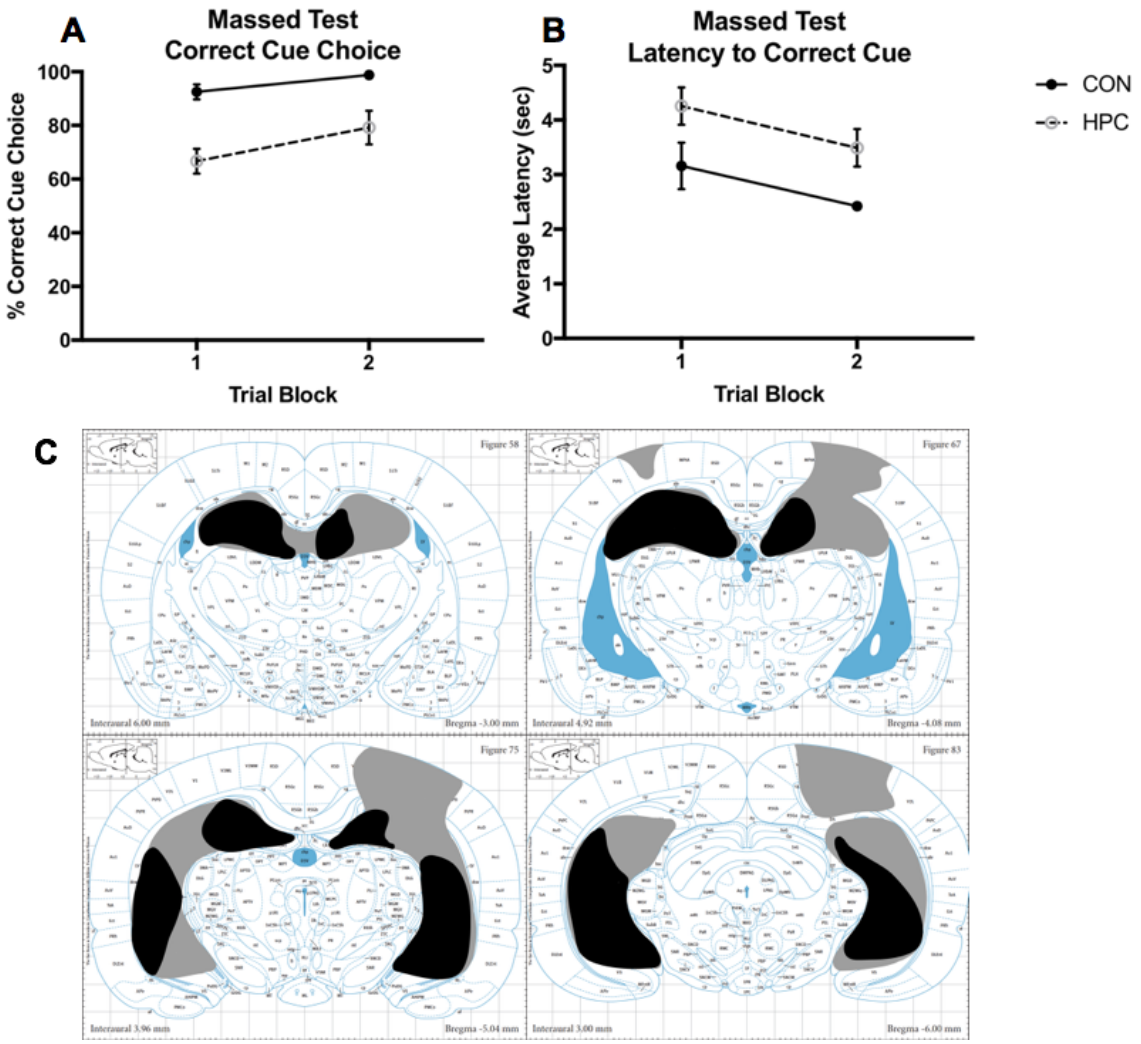


Figure 3.8 Correct cue choice and latency to the correct cue during a massed test with same cue contingencies as during training suggest that hippocampal damage after training causes retrograde amnesia in the CWT. The figure shows that hippocampus damaged animals perform worse than sham-operated animals in both cue choice accuracy (B) and latency to the correct cue (B). (C) shows the maximum (grey) and minimum (black) extent of hippocampus damage in the experiment traced over images from Paxinos and Watson (2009).

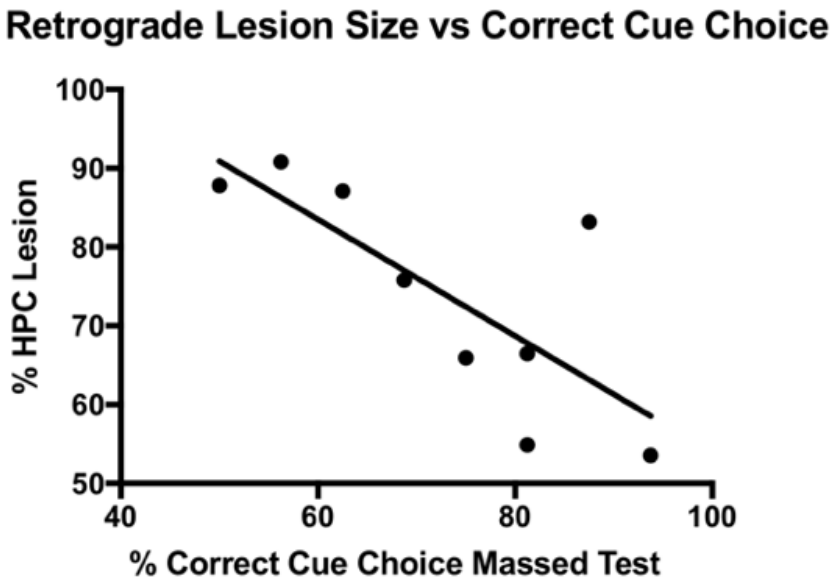


Figure 3.9 A simple linear regression between correct cue choice during the massed test with cue contingencies the same as training and estimated lesion size revealed a negative relationship between the extent of hippocampal damage and choice accuracy.

Discussion

Our findings support the idea that the hippocampus plays an important role in several forms of memory when functionally intact during learning, including a simple visual discrimination in the CWT. Surprisingly, similar features can be learned in its absence, including incidentally acquired spatial information, but perhaps at a slower rate. Previous work from several groups has demonstrated slower learning in rats with hippocampal lesions, including spatial memory tasks and in some cases context fear conditioning (Day et al., 1999; Hales et al., 2014; Morris et al., 1990; Wiltgen et al., 2006; Zelikowsky et al., 2012). Although hippocampal damage does not abolish either spatial or contextual learning, detailed analysis has shown that such information is

represented with less precision in the absence of normal hippocampal function (McDonald & Hong, 2000; Kolarik et al., 2018). The present results are the first to show slower learning in hippocampal rats performing a visual cue discrimination between a single pair of cues. With additional training, we have found lesion rats exhibit similar cue choice accuracy and similar latency to the correct cue as control animals.

We did not find that lesion size correlated with either the number of days to reach an 80% cue choice accuracy criterion, or the magnitude of difference in performance between NEW and SWITCH cue shifts. The latter result was particularly surprising, since the difference between NEW and SWITCH shift sessions is taken as a metric of spatial strategy preference over cue-based strategy in navigation. Based on previous studies in the Morris water task, and effects shown in McDonald and White (1994), we anticipated the opposite result. McDonald and White (1994) found that fornix-lesioned animals faced with a preference test between place- and cue-based navigation exclusively preferred the cue-based strategy (see also Devan & White, 1999). Although hippocampal and fornix lesions have been shown to exert different effects on memory, typically with more aspects of memory spared following fornix lesions, we anticipated hippocampal lesions would eliminate the spatial bias of animals to prefer a recently reinforced spatial location following a SWITCH shift. One possible cause for this discrepancy was that fornix-lesioned animals were not trained to the same level of performance as control or dorsal striatum-lesioned animals in the hidden platform epoch of the task in that study. Based on the present findings, it is possible that fornix-lesioned rats could reach the same level of performance with over-training and show a cue-place split strategy in the

paradigm developed in McDonald and White (1994). Previously, McDonald and Hong (2000) found that overtraining rats with hippocampal damage to navigate to a visual cue and its location containing a hidden platform allows lesion rats to express spatial memory in a no-platform probe trial, although less accurately than control animals. McDonald and Hong (2000) did not assess whether overtraining causes hippocampus damaged rats to express a place or cue preference during a cue-place competition test as used in McDonald and White (1994). How hippocampal damaged animals express cue or place strategies in the competition test with over training would be interesting to assess in future experiments.

In the group of animals faced with a cue contingency reversal during massed training in the CWT, we found impairments in latency to navigate to the newly correct cue in lesion animals, but not a significant effect of trial block or group X trial block interaction. Further, we did not find an effect of group or trial block interaction in correct cue choice during reversal. It remains unclear from the present experiments whether this effect is due to an impairment in cue reversal ability, or a delay in learning a new cue contingency in the CWT, like acquisition following pre-training hippocampal lesions in the same animals. Though previous experiments have shown mixed results on cue memory reversal abilities following complete hippocampal lesions (McDonald et al., 2004; McDonald et al., 2002), the most likely interpretation cannot be decided from the present study.

By contrast to damage sustained before training, which spared the ability of rats to express similar behaviour to control animals in the CWT at a delayed period,

hippocampal lesions made after training result in RA for correct cue choice and latency to the correct cue in a massed training session with the same contingencies. Further, damage extent correlates with the severity of RA in choice accuracy during the massed test. This result corroborates previous studies that have reported hippocampal lesions after learning cause RA for cue (McDonald et al., 2007; Sutherland et al., 2001), object (Gaskin et al., 2003), picture (Driscoll et al., 2005; Epp et al., 2008), tone (Broadbent & Clark, 2013; Sutherland et al., 2008), context (Sparks et al., 2011; Sparks et al., 2011), and context discrimination (Lee et al., 2017). Correlations between lesion size and the severity of RA have also been reported for simple picture discriminations and contextual fear memory (Epp et al., 2008; Sutherland et al., 2008). Notably, popular views on the role of the hippocampus in memory and navigation do not anticipate the present combination of results.

Popular theories on the role of the hippocampus in long-term memory posit that the hippocampus is responsible for a unique set of memory processes, such as episodic memory (Nadel & Moscovitch, 1997), relational memory (Cohen & Eichenbaum, 1993), spatial memory (O'Keefe & Nadel, 1978), or temporal associations to name a few (Eichenbaum, 2017). None of these views on the role of the hippocampus in memory suggest that it would be necessary to remember the reinforcement patterns for a single pair of distinct visual cues. The present experiment, among others (Lee et al., 2016; Sutherland et al., 2010), support that the hippocampus is critical for remembering these simple discriminations. Here we have also found that damage extent correlates with the severity of RA for cue choice accuracy. Further, theories on the hippocampus in spatial

cognition, such as the cognitive mapping theory (O'Keefe & Nadel, 1978), do not predict our finding that lesion rats have a bias toward previously reinforced spatial locations following a SWITCH cue shift in the CWT. The lack of correlation between anterograde lesion size and spatial bias suggests that tissue sparing also does not likely account for this result. Although some popular theories do suggest that hippocampal lesions would retard task acquisition (McClelland et al., 1995), these models also suggest that the hippocampus is involved uniquely in spatial and/or episodic memory. Therefore, we suggest that a different conceptual framework is necessary to account for our observations in the CWT in rats with hippocampal damage.

The wide-ranging RA with hippocampal damage could mean that the hippocampus is involved in a wide range of memory processes, or that the methodology used to study hippocampus and RA exerts non-specific effects on memory after a learning episode (Rudy, 2008). However, the latter account appears less likely, due to several demonstrations of temporary inactivation causing a broad range of RA in memory tasks (Kim et al., 2015; Lee et al., 2016). Nonetheless, alternate methods for hippocampal inactivation should be further assessed in a range of memory tasks to examine this possibility (Smith et al., 2016). In the CWT our group has also recently found that cue shifts induce population remapping in CA1, and that the extent of remapping is related to cue choice accuracy (Lee et al., 2018). This result is in keeping with research on properties of spatial and non-spatial memory coding in the hippocampus (Komorowski et al., 2009; McKenzie et al., 2014; Wood et al., 2000). Current evidence appears to support the conclusion that the rat hippocampus is involved in a wide range of memory types, and

contributes to rapid memory acquisition. Our group recently proposed a concept on the hippocampus and systems-level memory organization that can account for the present findings, termed heterarchic reinstatement (HR; Lee et al., 2016).

The HR view states that the hippocampus has a broad role in memory due to its widespread output to the cortex and subcortical structures, and the hippocampal output to these regions becomes a part of the distributed memory representation during a learning episode. If the hippocampal component is absent upon memory testing - after lesion or temporary inactivation - the target memory cannot be reinstated, and the animal expresses RA. In other words, the similarity in the state of cortical activity during training and testing depends in part on output from the hippocampus to the cortex. This may also be considered analogous to an effect of encoding specificity defined in cortical activity (Godden & Baddeley, 1975). The extent to which the hippocampal code is missing from the cortical representation is expected to scale with the similarity between the original representation and that reinstated upon testing. As a result, we also predict a negative relationship between the extent of hippocampal inactivation or damage and RA in a range of memory tasks. By contrast, when the hippocampus is absent during a learning episode and memory testing, the cortical and subcortical representation remains similar, and no AA results. Similar learning processes may also occur at a slower rate in the absence of the hippocampus. The tri-synaptic circuit is well-suited for pattern completion and separation processes, and distributed connectivity to cortical and subcortical structures allows the hippocampus to complete a distributed representation and aid in fast learning, or separate acute differences between overlapping inputs to aid in memory precision and

interference reduction (Leutgeb et al., 2007; Leutgeb & Leutgeb, 2007; McClelland et al., 1995). We believe the HR view on memory organization is the simplest account of our findings in the CWT, though further work is needed to assess several new predictions from this view.

An important prediction of the HR view is that hippocampal damage will cause RA in a wide range of memory tasks. However, several parameters may affect RA after hippocampal damage, such as the distribution and repetition training, lesion size, and location. For example, Lehman et al. (2009) reported that distributed, repeated context fear conditioning spares memory from complete hippocampal ablation, but training given in a massed, single session results in RA using the same lesion method (Lehmann et al., 2009). It is possible that this aspect of learning may be an important parameter to determine if memory retrieval will depend on intact hippocampus. Some positive-reinforcement paradigms such as conditioned context preference and socially-transmitted food preference require a repeated, distributed pattern of training, and have not shown clear hippocampal amnesia (McDonald et al., 2010; Thapa et al., 2014). However, it is unclear if this effect may be due to the nature of training administration or the type of memory being tested. Methods to observe hippocampal activity during the learning experience may be especially useful to clarify this issue (Gosh et al., 2011). The relationship between lesion size and location related to the RA observed with in a range of memory tasks also deserves further examination. Across several studies, we have found that larger lesions, particularly those affecting the ventral aspect of the hippocampus, cause reliable RA for context fear (Lehmann et al. 2007; Sutherland et al.,

2008), simple picture discriminations (Epp et al., 2008), cue discriminations (Sutherland et al., 2001), and spatial memory. Perhaps output from the ventral hippocampus is significant in broadcasting the hippocampal memory code to the cortex and subcortical structures to reinstate a complete target memory. Finally, multiple aspects of memory, such as cues, spatial information, emotion, and their relationship to context do not affect animal behavior in isolation. Rather, these seemingly distinct aspects of memory interact to guide animal behaviour as a gestalt of mnemonic features (McDonald et al., 2004). We suggest that new tasks to examine multiple aspects of memory in parallel and how they interact will be critical to understand how complex memory representations guide animal behavior.

Chapter 4

Hippocampal damage causes retrograde but not anterograde memory loss for context fear discrimination in rats.

Abstract

There is a substantial body of evidence that the hippocampus (HPC) plays and essential role in context discrimination in rodents. Studies reporting anterograde amnesia (AA) used repeated, alternating, distributed conditioning and extinction sessions to measure context fear discrimination. In addition, there is uncertainty about the extent of damage to the HPC. Here, we induced conditioned fear prior to discrimination tests and rats sustained extensive, quantified pre- or post-training HPC damage. Unlike previous work, we found that extensive HPC damage spares context discrimination, we observed no AA. There must be a non-HPC system that can acquire long-term memories that support context fear discrimination. Post-training HPC damage caused retrograde amnesia (RA) for context discrimination, even when rats are fear conditioned for multiple sessions. We discuss the implications of these findings for understanding the role of HPC in long-term memory.[3]

[3] Chapter published as: Lee, Sutherland, and McDonald (2017). Hippocampal damage causes retrograde but not anterograde memory loss for context fear discrimination in rats. *Hippocampus, 27*(9): 951-958. Reproduced with permission from John Wiley and Sons.

Introduction

Many views hold that the HPC is only involved in specific categories of memory. Popular theories point to a role of the HPC in spatial, temporal, and relational or configural memory processes (Cohen & Eichenbaum, 1993; O'Keefe & Nadel, 1978; Schiller et al., 2015; Squire, 1992; Sutherland & Rudy, 1989; White & McDonald, 2002). Within this framework, several groups have argued that the HPC is also critical for detailed spatial and relational memory supporting context discrimination (Antoniadis & McDonald, 2000; Frankland et al., 1998; Wiltgen et al., 2010; Winocur et al., 2013). By contrast, non-HPC systems, presumably involving other cortical networks are thought to store less detailed features that do not support context discrimination

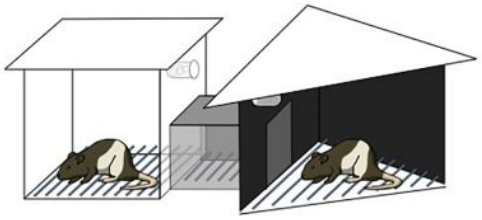
There are several reports that permanent or temporary HPC disruption results in AA and RA for context discrimination and animals that exhibit strong context discrimination have greater immediate-early gene transcription in the dorsal HPC (Antoniadis & McDonald, 2000; Frankland et al., 1998; Wiltgen et al., 2010). However, with multiple, distributed conditioning sessions we found that rats are able to perform contextual fear discrimination when the HPC is damaged after training (Lehmann et al., 2009). This outcome necessarily means that at least one non-HPC system can support context discrimination. Moreover, rats with extensive pre-training HPC lesions can learn object discrimination, elemental picture discrimination, and single-context fear with little or no memory impairment (Alvarado & Rudy, 1995; Driscoll et al., 2005; Epp et al., 2008; Frankland et al., 1998; Gaskin et al., 2003; Maren et al., 1997; Morris et al., 1986; Sparks et al., 2011). Thus, it is not clear why HPC would be necessary for context fear

discrimination as reported in earlier studies, while extensive pre-training HPC damage has little or no effect on other types of discriminative or context fear behavior.

In the present series of experiments we examined the effects of pre-training and post-training HPC damage on context fear discrimination using training conditions similar to previous studies (Antoniadis & McDonald, 2000; Antoniadis & McDonald, 2006; Ferbinteanu & McDonald, 2001), while also addressing potential problems in earlier experiments. Prior studies on the HPC in context fear discrimination used multiple training and extinction sessions to measure context fear (Antoniadis & McDonald, 2000; Ferbinteanu & McDonald, 2001; Frankland et al., 1998). Impairment in HPC animals' performance using this design may be due to memory interference from repeated extinction and training sessions in the same context, rather than an inability to discriminate between shock-paired and unpaired contexts per se. The extent of damage to the HPC in prior experiments on context fear discrimination is also uncertain (Antoniadis & McDonald, 2000; Ferbinteanu & McDonald, 2001; Frankland et al., 1998; Wiltgen et al., 2010). It is possible that less extensive HPC damage or inactivation would allow remaining tissue to control memory acquisition and retrieval, albeit less efficiently. Here, we implemented an extensive (>80% mean lesion volume) HPC lesion approach using a 7-site protocol adapted from (Sparks et al., 2011), and performed all conditioning prior to tests of memory retention to avoid potential effects of interference.

Experimentally, naïve male Long Evans rats (350–450 g; Raleigh, NC) were trained using a conditioning procedure similar to Antoniadis and McDonald (2000), with the exceptions that all conditioning was performed prior to context discrimination tests,

no tactile cues were used to distinguish the contexts, and weaker shocks were used. Animals began experiments after five days of handling and at least one week after arrival at the University of Lethbridge rat colony room. Rats were randomly assigned to groups that received sham or HPC lesion surgery with NMDA (adapted from Sparks et al., 2011), either prior to or after fear conditioning to examine the effects of HPC damage on AA and RA, respectively. On the first day of training, rats were pre-exposed to two contexts located in room 1 for a total of 10 min. The contexts differed in shape, color, and odor (Figure 4.1). Following pre-exposure, rats were assigned to receive foot shocks in one context, and no foot shocks in the other. On shock-paired sessions, rats were transported to room 2, which contained the same context chambers as room 1. The animal was placed in its paired context and allowed 2 min to explore prior to foot shock delivery (0.6 mA, 2 s) at the second, third, and fourth minute. The rat remained in the context for an additional 58 s for a total 5-min session. On unpaired conditioning sessions rats were transported to room 1 and placed into the unpaired context for 5 min, during which no foot shock was delivered. Either before (Experiments 1 and 2) or upon completing training (Experiment 3) each rat was given sham or HPC lesion surgery and was allowed 7–10 days to recover before conditioning or testing. We examined both freezing and context preference as measures of context fear discrimination following either one or three paired and unpaired training sessions (Antoniadis & McDonald, 2000).



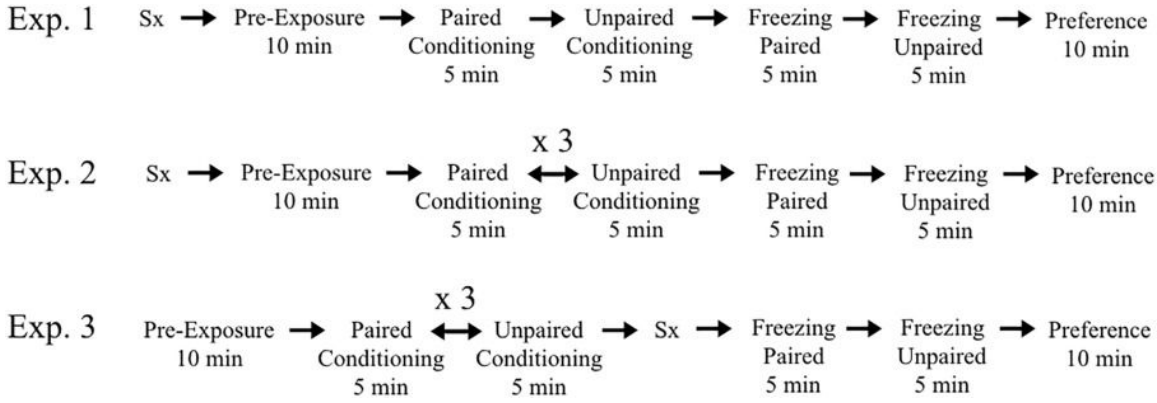


Figure 4.1 The diagram illustrates the design of Experiments 1–3 and provides a depiction of the conditioning and testing apparatus. In experiment 1, rats were given two days of conditioning, including one paired and unpaired day, whereas in experiments 2 and 3 rats were given 6 total days of conditioning (see detailed methods).

After completion of the experiment, animals were perfused with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Brains were then extracted and stored overnight in 4% PFA, and transferred to 0.02% sodium azide in 30% sucrose PBS solution for at least 48 hr prior to crysectioning at −20 °C. Sections were sliced at 40 um thickness and allowed to dry at room temperature before staining with cresyl violet. The volume of spared HPC was quantified using the Cavalieri estimator method (Schmitz & Hof, 2005). Total HPC volume estimates in lesioned rats were then compared

against three control HPC volumes in each experiment to determine the percentage of HPC damage.

In Experiments 1 and 2, we found no evidence that HPC lesions cause AA for contextual fear discrimination in freezing or context preference. The amount of freezing did not differ between contexts ($F(1,19) = 2.384$, $p = .1390$) or groups ($F(1,19) = 0.2103$, $p = .6517$), and there was no context-group interaction ($F(1,19) = 0.2421$, $p = .6284$) when rats were given pre-training surgery and a single paired and unpaired conditioning session in Experiment 1 (Figure 4.2). Although rats did not differ in freezing between paired and unpaired contexts, we found a significant effect of context in preference ($F(1,19) = 22.63$, $p = .0001$), and no significant effect of group ($F(1,19) = 2.506$, $p = .1299$) or context X group interaction ($F(1,19) = 0.2384$, $p = .6309$), suggesting that both groups equally avoided the shock-paired context after a single paired and unpaired conditioning session (Figure 4.2). Following three context-shock pairings in Experiment 2 (Figure 4.3), we found a significant effect of context on freezing ($F(1,20) = 10.06$, $p = .0048$) and no effect of group ($F(1,20) = 1.674$, $p = .2104$) or context X group interaction ($F(1,20) = 0.659$, $p = .4265$). A robust effect of context also emerged in preference ($F(1,20) = 30.85$, $p < .0001$), but we did not find a significant difference between groups ($F(1,20) = 3.675$, $p = .0696$) or a significant context X group interaction ($F(1,20) = 0.1317$, $p = .7205$). Together, the results of Experiment 1 and 2 demonstrate that HPC-lesioned rats are similar to control rats in acquiring and retrieving memories supporting context

discrimination in freezing and preference, and that preference is a more sensitive measure for detecting context discrimination.

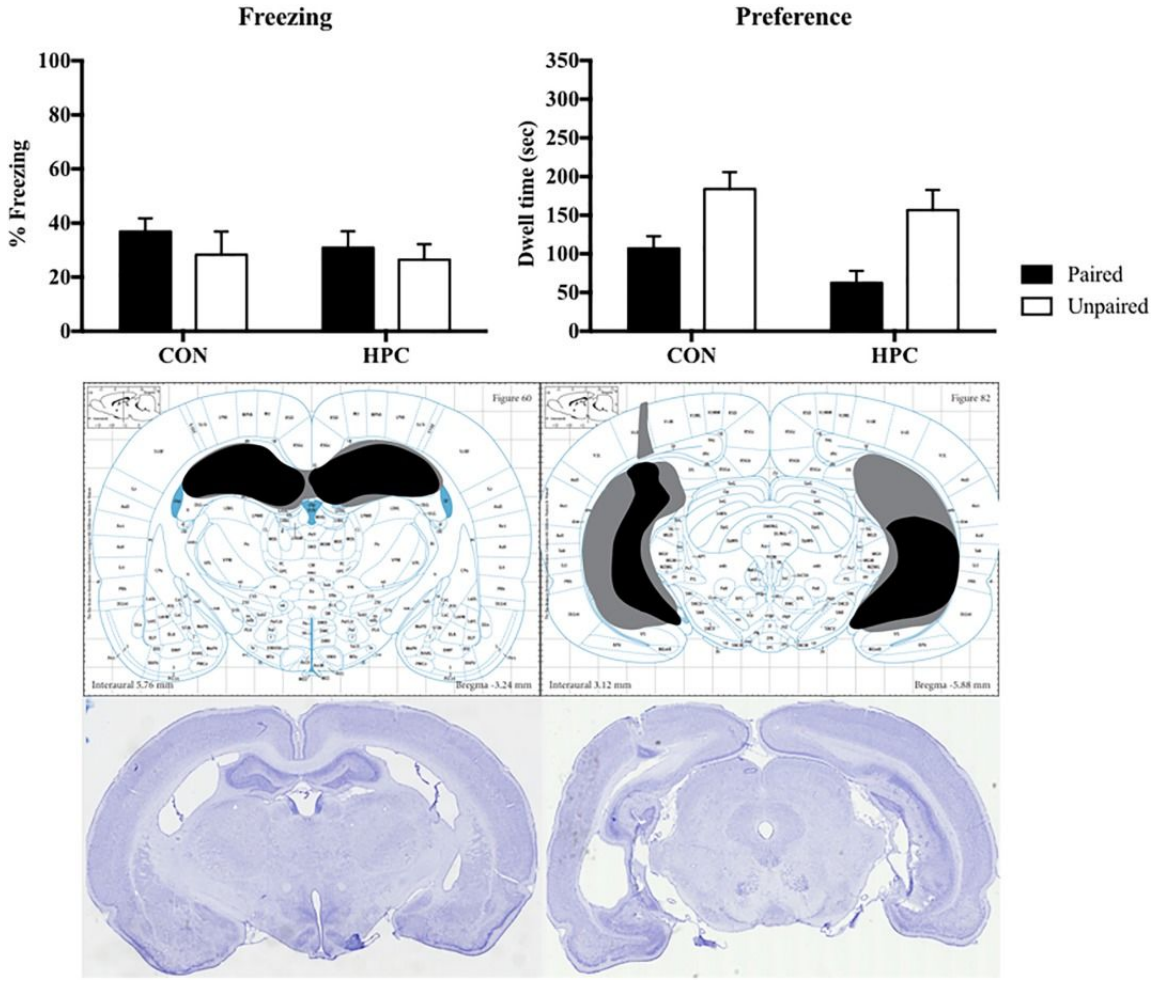


Figure 4.2 Pre-training surgery before a single shock pairing session in experiment 1 resulted in an effect of context in preference but not freezing behavior. We found no difference between HPC lesion and sham rats' ability to acquire context freezing or discriminative preference. The histologic tracings show the largest lesion of the HPC group in gray, and the smallest lesion from the HPC group in black.

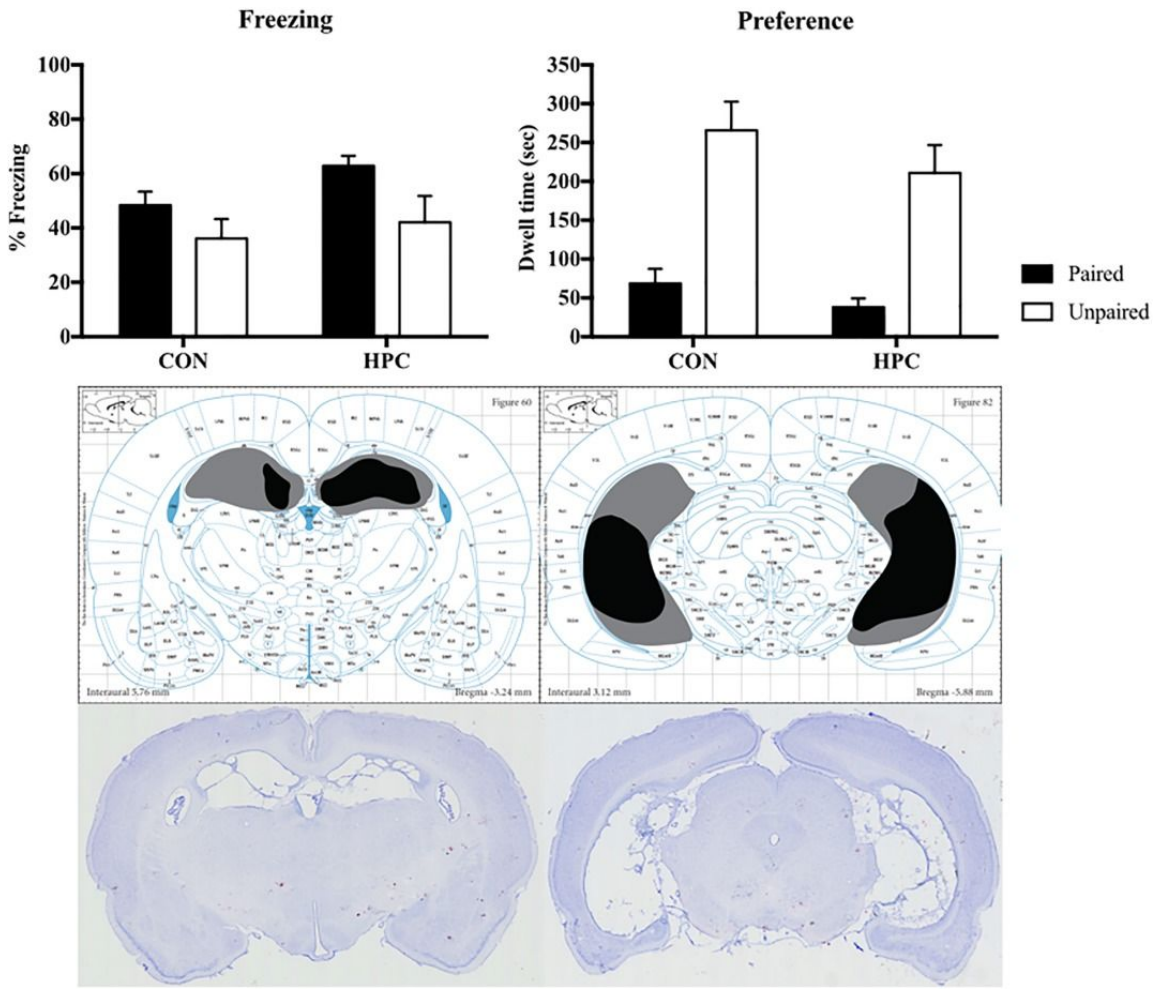


Figure 4.3 In Experiment 2, rats received HPC lesions or sham surgery prior to three context-shock pairing sessions. We found an effect of context present in both freezing and preference measures, but no effect of group or group X context interaction. The histologic tracings show the largest lesion of the HPC group in gray, and the smallest lesion from the HPC group in black.

In contrast to the foregoing, we found that post-training HPC damage produced a different pattern of effects in context fear discrimination. Following three paired and unpaired conditioning sessions, we found a significant effect of context

($F(1,18) = 7.282$, $p = .0147$) and group ($F(1,18) = 11.62$, $p = .0031$) in freezing behavior, and no significant context X group interaction ($F(1,18) = 2.1 = 098$, $p = .647$), suggesting that sham-lesioned rats retrieved context fear memory in freezing tests, while lesioned animals displayed RA for context fear (Figure 4.4). In preference testing, rats exhibited a robust effect of context ($F(1,18) = 28.41$, $p < .001$) and context X group interaction ($F(1,18) = 25.26$, $p < .0001$), but no effect of group ($F(1,18) = 1.021$, $p = .3257$). Follow-up Fischer's LSD post-hoc tests revealed that control ($t(1,18) = 7.323$, $p < .0001$), but not HPC-lesioned rats ($t(1,18) = 0.2148$, $p = .8323$), preferred their unpaired context. The results of Experiment 3 demonstrate that HPC lesions following three paired and unpaired conditioning sessions results in robust RA for context fear discrimination, while sham operated rats retain memory supporting context fear discrimination in both measures. Notably, we have found that fear memory is not retained across a surgical and recovery period after a single paired and unpaired conditioning session in the present design (unpublished observation), further suggesting that the conditioning parameters in Experiment 1 are very near the minimum needed to produce context fear discrimination in rats.

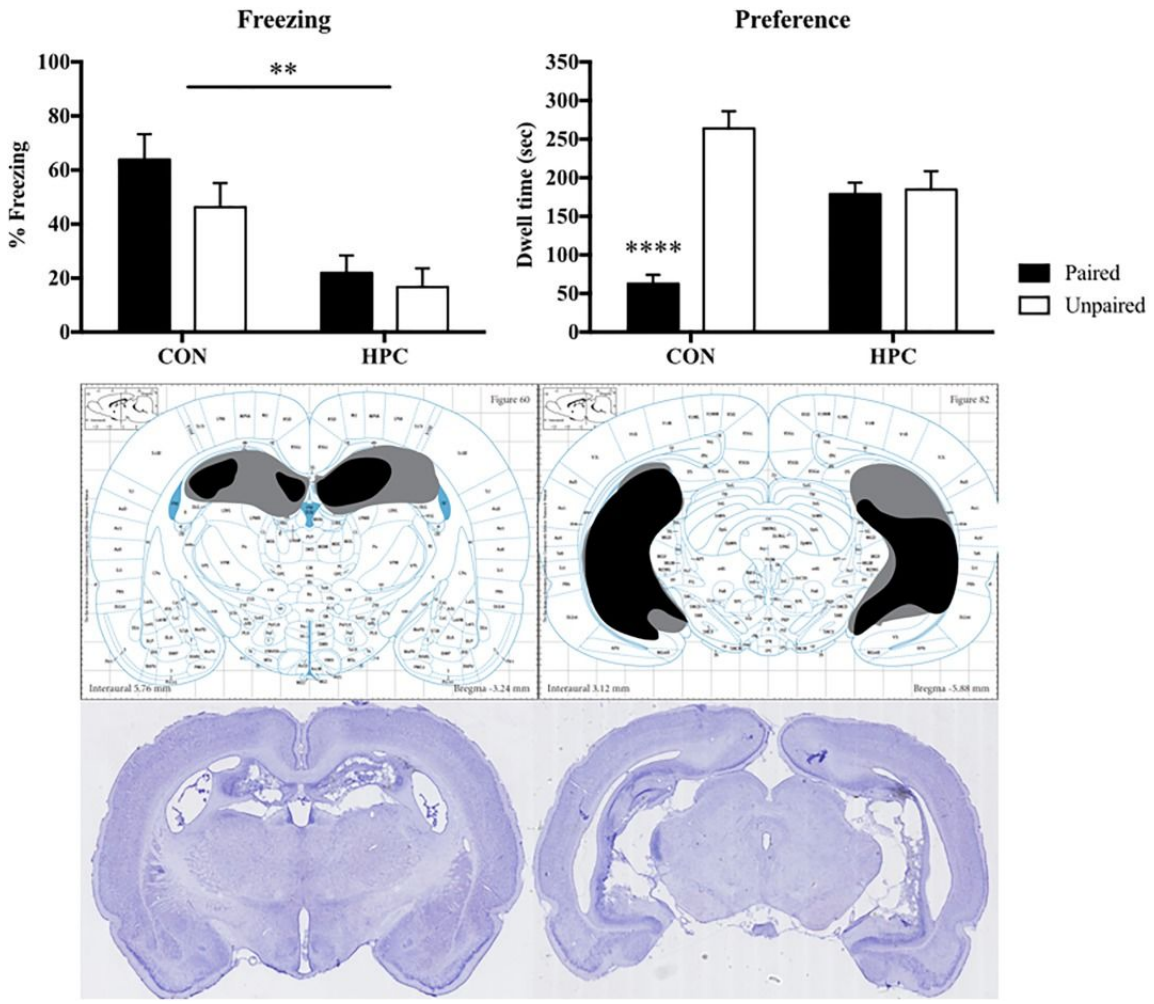


Figure 4.4 Experiment 3 illustrates that HPC damage following three conditioning sessions induces robust RA for both context freezing and preference behavior, while sham-operated rats exhibit context fear discrimination in both measures. The histologic tracings show the largest lesion of the HPC group in gray, and the smallest lesion from the HPC group in black.

Histological confirmation of the extent of HPC lesions in each experiment using the Cavalieri estimator method showed a similar amount of damage across experiments. In Experiment 1, rats in the HPC lesion group sustained an average lesion size of 84%

(sd = 7.695; min = 72.66%; max = 95.86%), while lesioned animals in Experiment 2 received an average HPC lesion of 81.73% (sd = 9.039; min = 62.30%; max = 95.47%), and 86.64% HPC lesion in Experiment 3 (sd = 8.300; min = 68.92%; max = 98.70%). A one-way ANOVA revealed no significant difference in lesion extent across experiments ($F(2,25) = 0.7782$; $p = .4700$). Thus, differences in anterograde and retrograde effects in the present experiments cannot be accounted for by differences in HPC tissue sparing across experiments.

The present experiments demonstrate that extensive HPC damage produces RA, but not AA, for contextual fear discrimination. In contrast to prior reports, the present findings show that at least one non-HPC system can acquire and retrieve long-term memories that are detailed enough to support this type of context discrimination, if the HPC is absent during learning (Antoniadis & McDonald, 2000; Frankland et al., 1998). However, when the HPC is present during learning, it is necessary for retrieval of the target memories. In addition, we have found that animals with pretraining HPC damage perform contextual fear discrimination even with very weak conditioning parameters, suggesting that non-HPC systems supporting context discrimination are roughly equal in efficiency at acquiring and retrieving the target memories. Importantly, we have separately replicated the earlier findings in freezing and preference behavior reported in Antoniadis and McDonald (2000; data not shown), but these results suggest that the deficit in HPC rats is due to an effect of interference with repeated measures, and not a lack of discrimination ability *per se*. The present results do not support the view that the HPC is necessary for efficient acquisition of memory supporting contextual

discrimination (Fanselow, 2009; Winocur et al., 2013). Instead, the present findings add to a growing literature showing that the effects of HPC disruption on AA and RA differ for various types of LTM (Lee et al., 2016; Sutherland et al., 2010).

Multiple studies have reported that HPC damage carried out before learning does not affect memory performance in several tasks, while the same damage after learning produces RA in the same memory tasks. This includes, but is not limited to, memory for context fear (Frankland et al., 1998; Lehmann et al., 2009; Maren et al., 1997; Sparks et al., 2011; Wiltgen et al., 2006), tone fear (Broadbent & Clark, 2013; Sutherland et al., 2008), fear-potentiated startle (Lehmann et al., 2010), object discrimination (Morris et al., 1986; Sutherland et al., 2001), picture discrimination (Alvarado & Rudy, 1995; Driscoll et al., 2005; Epp et al., 2008), and home base memory (Travis et al., 2010). In a recent review, we proposed a potential mechanism for the different effect HPC disruption on AA and RA, termed heterarchic reinstatement (HR; Lee et al., 2016). On this view, the HPC is involved in multiple types of memory retrieval due to its interaction with the cortex during a learning event. We suggest that when the HPC is present during learning it provides output to the cortex, which thence provides information to effectors of behavior, such as the amygdala, to produce a response. If the output from the HPC is lost following a learning event, then the target memory in the cortex is not achieved, and RA results. By contrast, if the HPC is absent during both the learning and retrieval periods, the target memory remains the same, and in many cases AA does not occur. AA will result from HPC damage only if the HPC provides an essential code for guiding a specific set of

behavioral responses, as in certain types of spatial, temporal, and relational memory tests. Indeed, we do not intend to suggest that the HPC makes no unique contributions to LTM.

Many experiments have illustrated the unique contributions of the HPC to LTM processes, particularly in rapid pattern completion and separation (Bakker et al., 2008; Gilbert et al., 2001; Lee & Kesner, 2004; Leutgeb & Leutgeb, 2007; Leutgeb et al., 2007; Yassa & Stark, 2011). Despite the lack of AA with HPC damage in this experiment, context similarity could be titrated to promote separation or completion processes to reveal essential contributions of the HPC to certain forms of discrimination in highly ambiguous circumstances. We predict that HPC damage may result in context discrimination impairments or enhancements if control animals pattern separate or complete a context representation, respectively. Several authors have pointed out that pattern separation and completion are not computations unique to the hippocampus (Kent et al., 2016; Yassa & Stark, 2011). It is possible that differences in pattern completion or separation in HPC-damaged animals could be overcome with additional training in highly ambiguous discrimination tasks.

These findings demonstrate that HPC damage differently affects AA and RA for LTM supporting context fear discrimination. To account for this difference, we suggest that the HPC is involved in memory retrieval when it provides output to the cortex and memory effectors during a learning episode, and thus provides necessary information to retrieve a target memory. Further investigation on the HPC in context discrimination might test AA and RA with HPC disruption in appetitive conditioning parameters and highly ambiguous circumstances.

Detailed Methods

2.1 *Surgery*

Rats were given HPC lesions with microinjections of NMDA or sham surgery either before or after discriminative fear conditioning to context. Briefly, rats were given a preoperative injection of Phenobarbital (30 mg/kg) 30 min prior to surgery and Metacam (1 mg/kg) upon anesthetic induction with 4% Isofluorane dissolved in oxygen. Thereafter, rats were maintained at 1.5–2.5% Isofluorane anesthesia and mounted in a stereotaxic frame. Trephining holes were placed over respective bilateral injection sites and the dura was lightly punctured using a 30-gage needle. HPC lesion rats were given bilateral injections of 7.5 ug/uL NMDA dissolved in 0.9% sterile saline at through 30 gage metal cannulae attached to 10-uL Hamilton syringes and microinjection pump according to the sites and volumes outlined in Table 4.1. The same procedure was given to sham-operated control rats, except nothing was injected into the brain. Diazepam (5 mg/kg) was given postoperatively as an additional prophylactic to counter any seizure behavior.

Injection Site	**AP**	**ML (+/-)**	**DV (L)**	**DV (R)**	**Volume (uL)**
1	-3.1	1.5	-3.6	-3.6	0.4
2	-4.1	3	-4	-4	0.25
3	-5	3	-4	-4	0.25

4	-5	5.2	-7.3	-7.3	0.4
5	-5.8	4.4	-4.4	-4.4	0.25
6	-5.8	5.1	-7.5	-7.5	0.5
7	-5.8	5.1	-6.2	-6.2	0.5

Table 4.1 The table depicts the stereotaxic sites relative to Bregma and the volume of NMDA injections in the HPC lesion group across experiments in this study.

2.2 *Discriminative fear conditioning to context*

On the first day of training in the DFCTC task, rats were pre-exposed to two contexts that differed in color, shape, and odor, which were connected with an alleyway for 10 min. One context was a white square chamber scented with Vic's Vaporub, and the other context was a black triangle chamber scented with isoamyl acetate (Antoniadis & McDonald, 2000). Scents were introduced through a perforated pill bottle fixed to the top right corner of the context chamber with respect to the entrance to the alleyway. After each exposure to a given context, the chambers were cleaned with unscented soap diluted in warm water. During pre-exposure, dwell time in each context was measured between an entrance and exit from each context chamber, wherein the rat placed both forepaws in the chamber and later removed both forepaws, respectively. Prior to conditioning, rats in each group were assigned a shock-paired and unpaired context in a counterbalanced order based on initial context preference, such that each group did not demonstrate a preference for the paired or unpaired context (data not shown). The order of shock-paired and

unpaired conditioning sessions was counterbalanced to ameliorate any effect of shock order on memory acquisition and retrieval. During unpaired conditioning animals returned to room 1, which contained the same apparatus during pre-exposure, except inserts were placed at the entrance of each context to restrict the animal exploration to the unpaired chamber. Rats were allowed to explore for a total of 5 min and then returned to their home cage for 24 hr. During paired conditioning, the entire apparatus was transported to room 2, and the paired chamber was connected to a Kinder Scientific SMSCK Programmable Shocker. The animal's exploration was restricted to the paired context with a door insert placed at the context entrance, and a 0.6 mA, 2-s foot shock was delivered at the second, third, and fourth minute. The animal remained in the context for an additional 58 s prior to being returned to its home cage for 24 hr.

Following either one (Experiment 1) or three (Experiments 2 and 3) paired and unpaired conditioning sessions, rats were returned to either the paired or unpaired context for a 5-min freezing test in room 1. Rats were exposed to either their paired or unpaired context in counterbalanced order to eliminate any effect of testing order on freezing behavior. Freezing was scored by a trained observer from video footage and defined as the absence of movement except for that due to breathing (Antoniadis & McDonald, 2000). The amount of time rats spent freezing in each context was converted into percent freezing [% Freezing = 100 × (seconds freezing/300 s)] for subsequent analysis. After the completion of freezing tests, rats were returned to room 1 for a preference test, wherein animals were introduced to the connecting alleyway used during pre-exposure and allowed a total of 10 min to explore both contexts freely. Dwell time was scored from

video footage by a trained observer as the time between an entrance and exit from each context, wherein the animal placed both forepaws into a context and later removed both forepaws, respectively. If rats successfully acquire and retrieve discriminative context fear memory following conditioning, then animals are expected to freeze more in their paired than unpaired context, and/or spend more time in their unpaired than paired context.

2.3 *Cavalieri hippocammpal volume estimation*

The Cavalieri estimator method (Schmitz & Hof, 2005) was employed to estimate the volume of remaining HPC tissue following HPC NMDA lesions in each experiment. Following cryosectioning at 40 um section thickness and cresyl violet staining, every 12th section was sampled for grid point counting at 10× magnification on a Zeiss AX10 Imager M1 and PCO Sensicam QE High Performance camera connected to Stereo Investigator 10.56 software. Grid points were spaced 120 um apart along the X- and Y-axis of the scaled image in Stereo Investigator. If the upper right corner of a grid point landed on a principle HPC subfield, including CA1–3 and the dentate gyrus, the grid point was counted. The estimated HPC volume of each HPC-lesioned animal was compared to the average HPC volume of three sham control animals in each experiment to generate a %HPC lesion estimate (% HPC Lesion = 100 × (sham volume – lesion volume)/sham volume).

2.4 *Data analysis*

All data in the present experiments were analyzed using the SPSS and Prism by GraphPad statistical packages. Both freezing and preference behaviors were analyzed using a two-way, mixed model ANOVA. Uncorrected LSD post-hoc comparisons within groups were used following significant context X group interactions. Lesion volumes were compared across experiments using a one-way ANOVA.

Chapter 5

Partial hippocampal inactivation causes retrograde amnesia for place navigation memory but not context fear discrimination in rats.

Abstract

Using the lesion approach, we recently discovered that extensive hippocampal damage causes retrograde amnesia (RA) but not anterograde amnesia (AA) for fear discrimination between two distinct contexts in rats (Lee et al., 2017). Here, we implemented pharmacologic temporary inactivation to assess whether temporary blockade of hippocampal activity also produces RA for context fear discrimination and spatial memory in the Morris Water Task (MWT). In addition, we sampled cFos expression 45 minutes following behavioural testing to measure the extent to which our treatment blocked hippocampal activity. Our results show that an estimated 50% reduction of CA1 activity caused RA for place navitgation memory but not contextual fear discrimination, consistent with findings that show complete but not partial hippocampal damage causes RA for context fear memory. These results imply that tests of place navigation memory recall are more sensitive to disruption by interference with hippocampal function than discrimination between distinct contexts.

Introduction

Lesions studies in rodents and non-human primates have demonstrated that post-training hippocampal damage causes retrograde amnesia (RA) for multiple aspects of long-term memory, including visual discriminations (Driscoll et al., 2005; Epp et al., 2008), context memory (Maren et al., 1997; Lehmann et al., 2011; Sparks et al., 2013), tone or light associations (Sutherland et al., 2008; Lehmann et al., 2007), spatial memory (Clark et al., 2005), relational or configural memory (Driscoll et al., 2005), and memory tasks requiring pattern separation and pattern completion (Kim et al., 2015: Matus-Amat et al., 2004). In contrast the effects of hippocampal damage on RA, a more limited range of memory impairments follows pre-training lesions, including anterograde amnesia (AA) for precise spatial locations (Hales et al., 2014; Kolarik et al., 2018; McDonald & Hong, 2000; Ruediger et al., 2012), relational or configural memory (Alvarado et al., 1995; Driscoll et al., 2005; McDonald et al., 1997; Sutherland & McDonald, 1989), and tasks requiring pattern separation and completion (Kent et al., 2016; Fanselow, 1990; Rudy et al. 2002; Sutherland & McDonald, 1989).

Several groups have found a surprisingly wide range of RA following hippocampal damage, but comparatively few studies have replicated these findings using temporary inactivation (Gulbrandsen & Sutherland, 2014). Importantly, lesion methods carry different experimental confounds that might affect the range of RA observed following hippocampal damage, including post-surgical seizure activity and the recovery period prior to memory testing, wherein animals do not have a hippocampus typically for one week or longer, which could affect the maintenance of long-term memory acquired

prior to damage (Sparks et al., 2011; Zelikowsky et al., 2012). It is possible that the wide range of RA following hippocampal damage is not related to the loss of the hippocampal representation *per se*, but rather due to disrupted memory maintenance or post-surgical seizure activity (Sparks et al., 2011).

Experiments using hippocampal inactivation to examine RA have produced mixed results. In fact, some research groups have found different outcomes using reversible inactivation techniques compared with post-training hippocampal lesions. The majority of conflicting findings have been reported in contextual conditioning tasks, such as appetitive or fear conditioning to context (Gulbrandsen et al., 2013; Maren & Holt, 2004; McDonald et al., 2010; Sparks et al., 2011; Stouffer & White, 2006). Surprisingly, however, most studies using temporary inactivation of the hippocampus have not assessed the extent to which their inactivation approach affects hippocampal activity (Gulbrandsen & Sutherland, 2014). Lesion experiments have shown that the extent of hippocampal damage is directly related to the severity of RA in multiple tasks, including tone fear, light fear, context fear, and visual discriminations (reviewed in Lee et al., 2016). This presents an interpretative challenge to understand whether the hippocampus is necessary for memory retrieval in many cases using inactivation.

Our laboratory developed a reversible inactivation method that achieved >80% hippocampal inactivation using the sodium channel blocker ropivacaine hydrochloride (ROP; Gulbrandsen and Sutherland, 2014). ROP infusion 45 minutes prior to memory testing caused RA for contextual fear – a result our laboratory did not find with smaller infusions of muscimol, a GABA agonist (Sparks et al., 2011). It is possible that the mixed

pattern of results in previous tests of memory retrieval may be due to differences in the extent of hippocampal inactivation or the different tasks used.

In a previous lesion study, we found that complete hippocampal damage caused RA but not AA for memory supporting contextual fear discrimination (Lee et al., 2017). To ensure this outcome is due to the loss of the hippocampal memory representation, here we assessed whether temporary hippocampal inactivation causes RA for contextual fear discrimination. In a parallel experiment, we also tested place navigation memory in the Morris Water Task (MWT) following ROP infusion, since published reports using temporary inactivation consistently find disruption in this task with small or large hippocampal inactivation and lesions (Broadbent et al., 2004; Cimadevilla et al., 2005; Clark et al., 2005). Finally, to ensure that our methods inactivated the hippocampus, we quantified cFos expression in CA1 in rats that received ROP or vehicle (VEH) infusions 45 minutes before contextual fear discrimination testing.

Methods

Subjects

21 male Long-Evans rats (Charles River, Raleigh, NC) were used as subjects in the present experiments. Rats were allowed one week of acclimation to the University of Lethbridge colony room and handled by the experimenter for at least 5 days prior to the start of behavioural procedures. Experimental and animal husbandry procedures were approved by the University of Lethbridge Animal Welfare Committee and adhere to Canadian Council of Animal Care guidelines.

Hippocampal cannulation surgery

Permanent stainless-steel guide cannulae targeting the dorsal and ventral hippocampus bilaterally were implanted in all rats (adapted from Gulbrandsen et al., 2013). 30 minutes prior to surgery rats were injected with buprenorphine (Temgesic®, 0.03 mg/kg, sc.; Schering-Plough, Hertfordshire, UK). Animals were then induced to a surgical anaesthetic plane with 4% isoflurane dissolved in 1 L/min oxygen, and subsequently maintained at 1% – 2% isoflurane for the duration of surgery. Animals were given a subcutaneous injection of meloxicam (Metacam®, 5 mg/ml, 0.2 mg/kg, sc; Buehringer Integelheim, Burlington, ON, Canada) to further reduce possible symptoms of pain. The scalp was shaved and cleaned with 4% stanhexidine chloride and 70% EtOH. Following disinfection, the scalp was retracted and 7 trephining holes were placed in the skull with a dental drill above the target cannula placement sites. Two anchoring screws were tapped into place, and 23-gage steel guide cannulae (12 mm targeting dorsal hippocampus; 14 mm targeting ventral hippocampus) were lowered bilaterally into the dorsal and ventral hippocampus according to coordinates in Table 5.1. Cannulae and anchoring screws were secured in place with dental acrylic, and the guide cannulae were occluded with 30-gauge wire until subsequent infusion. Rats were allowed at least 7 days of recovery before the start of infusion and behavioural procedures.

Site	AP	ML	DV

Dorsal HPC	- 3.5	+/- 2.0	- 3.25
Ventral HPC	- 5.6	+/- 5.2	- 6

Table 5.1 Stereotaxic coordinates of cannula tip placements bilaterally in hippocampus according to Paxinos and Watson (2009) with respect to Bregma along anterior-posterior (AP), medial-lateral (ML), and dorsal-ventral (DV) axes.

Hippocampal infusion procedure

To temporarily inactivate the hippocampus, we infused the sodium channel-blocker ROP (Santa Cruz Biotechnology, Dallas, USA; CAS 132112-35-7) dissolved in artificial cerebrospinal fluid (VEH) at one of two concentrations and volumes: the first infusion parameter set used 10 mg/mL of ROP and 0.7 uL infusion volume per site, which has previously been shown to inactivate >80% of the hippocampus; the second parameter set used 15 mg/mL of ROP and 1 uL infusion volume per site (Gulbrandsen & Sutherland, 2014). Animals were removed from their home cage and brought to an infusion room where they had previously been handled by the experimenter and were infused with ROP or VEH vehicle using 30-gauge microinjection needles inserted into all four guide cannulae such that the injector needle tips were flush with the guides and did not protrude into the brain. Injection needles were connected to Hamilton syringes with polyethylene-50 tubing and an infusion pump (Harvard Apparatus, Holliston, MA, USA), and ROP or VEH was infused simultaneously at each

site. Infusion needles were left in place for an additional 4.5 minutes to allow for diffusion, and dummy cannulae were immediately replaced before the animal was returned to its home cage. Based on previous reports of optimal inactivation time periods using ROP (Gulbrandsen & Sutherland, 2014), rats were allowed 45 minutes before the start of behavioral procedures after the diffusion period.

MWT Behavioural Apparatus

Previous studies have found place navigation memory impairments following various types of temporary hippocampal inactivation in the MWT, including the use of sodium channel blockers and muscimol (Broadbent et al., 2006; McDonald et al., 2010). Thus, we sought to replicate these findings to ensure reliability of our approach to block hippocampal activity. Our training apparatus consisted of a 2-metre fibre glass swimming pool filled with room temperature water (approximately 22°C) that was made opaque with the addition of non-toxic, white tempura paint. In the center of the northwest quadrant of the pool we hid a platform approximately 2 cm under the surface of the water that rats could use for escape. Rats could use various distal cues for navigation located outside the pool, including a sink, computer, several posters with different shapes and orientations, a computer rack covered with a black plastic sheet, the experimenter, and the holding cage (Figure 5.1). Throughout the experiment, animal behaviour was monitored with an overhead camera mounted to the ceiling, and connected to the computer equipped with Ethovision XT 11.5 software, which was used for data collection and pre-processing.

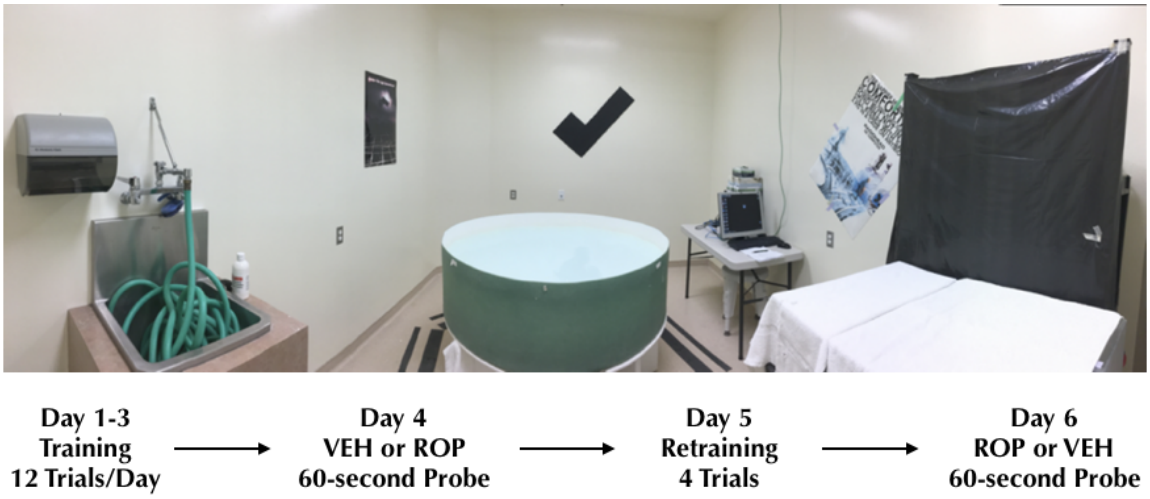


Figure 5.1 Photo and schematic the Morris Water Task behavioural apparatus and procedures.

MWT Behavioural Procedures

Rats were transported from their home cage to the testing room in holding cages on top of a transport cart covered with bath towels to occlude their vision from the surrounding environment. Upon each swim trial, animals were gently placed into the pool facing the wall at randomly chosen principal coordinates such that rats would not start from the same location for more than two trials consecutively, and each start location was used an equal number of times during a training session. Rats swam freely in the pool until they reached the hidden platform or if they did not reach the platform within a 60-second period they were placed onto the platform by the experimenter. After reaching or being placed onto the platform by the experimenter, the animal remained on the platform for 10 seconds and then was returned to its holding cage for an approximate two- to three-minute inter-trial interval. On the first three daily training sessions, rats

were given 12 trials, and returned to their home cage for approximately 24 hours between sessions. Following three training sessions, rats were infused with either 1.0 uL of VEH or 15 mg/mL ROP on the fourth day and returned to the behavioural room for a 60-second, no-platform spatial memory probe. On the fifth day, animals were given four retraining trials with the platform in the same location to ensure that the no-platform probe did not extinguish spatial reinforcement. Finally, a second 60-second, no-platform spatial memory probe was performed on the sixth day with animals given the opposite infusion to the first memory probe, such that an equal number of animals were infused with VEH on the first and second memory test and the same number of animals were infused with ROP in counterbalanced order.

Discriminative Fear Conditioning Behavioural Apparatus

The behavioural training and testing apparatus (Figure 5.2) consisted of two conditioning chambers (contexts) connected with a grey alleyway (16.5 cm long × 11 cm wide × 11 cm high). Each context differed in colour, shape, and size: one context was a black triangle that was 61 cm long × 61 cm wide × 30 cm high, and the other context was a white square context with 41 cm × 41 cm × 20 cm dimensions. Both contexts had steel rod floors that could be connected to a Lafayette Instrument Stimtek SGCG1 scrambled grid current generator to deliver foot shocks (Lafayette Instrument Co., Lafayette, USA). In the scented context fear discrimination, each context also differed in its scent by introducing a small perforated pill bottle located in the upper corner of the wall containing a doorway that linked each context to the alleyway. The white square was

scented with Vicks® VapoRub™ (eucalyptus), and the black triangle with isoamyl acetate (banana). We also performed a context fear discrimination experiment without added scents to assess possible differences in scented versus unscented task sensitivity to hippocampal disruption, as some studies suggest that odour and flavour memories may be retrieved independent of the hippocampus (Lee et al., 2016; Thapa et al., 2014). In the unscented task, identical boxes were used, except no scented pill bottles were inserted into the contexts. Finally, doorways into each context could be opened or closed according to the epoch of conditioning and testing procedures.

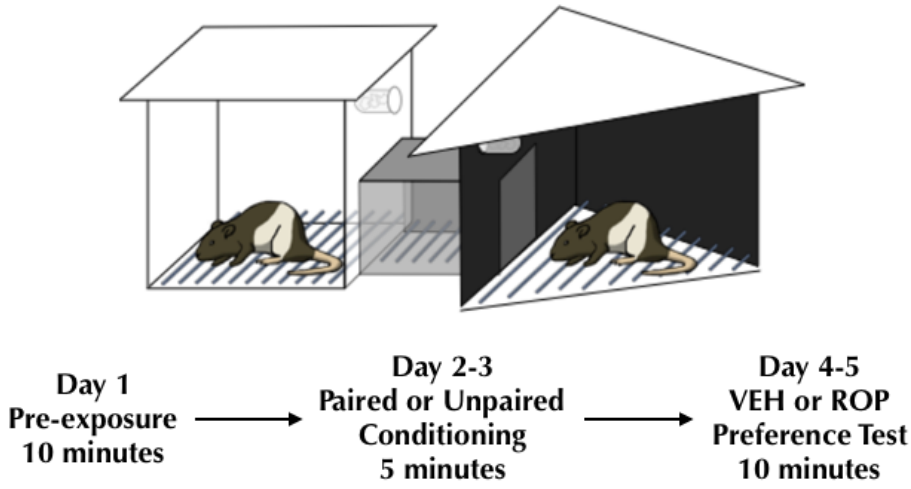


Figure 5.2 Schematic of the context fear conditioning apparatus and behavioural procedures.

Discriminative Fear Conditioning to Context Behavioural Procedures

On the first day of behavioural training, rats were transported to room A, containing the apparatus on a Plexiglas table with a mirror tilted 45 degrees beneath the

table, and a camera directed at the mirror such that rats could be monitored from underneath. During pre-exposure, rats were individually placed into the connecting alleyway and allowed 10 minutes to freely explore both chambers and the alleyway. Dwell time in both contexts was scored as a measure of initial context preference, such that placement or removal of both forepaws from a context was marked as the start and end of entry, respectively. Following pre-exposure, rats were assigned to shock pairing in one context and no shock in the other context, such that groups did not express any initial preference for the to-be-paired or unpaired context (Figure 5.3A-B). On consecutive days, rats experienced shock-paired or unpaired conditioning in the two chambers. During unpaired conditioning, animals were returned to room A containing the same apparatus, but with doors closed to the connecting alleyway. Rats were placed into the unpaired context for 5 minutes, and then removed. The chambers were cleaned with 70% EtOH and then warm water and dried with a clean towel. Animals were then returned to their home cage for 24 hours before the next conditioning session. During shock-paired conditioning, rats were brought to a new room containing the same apparatus and monitoring system, but with different extra-apparatus cues, such as a computer rack and miscellaneous behavioural equipment. Rats were confined to the shock-paired chamber and allowed to explore for 5 minutes, and a series of 0.6 mA, 2-second scrambled foot shocks were manually delivered at the second, third, and fourth minute of conditioning using a Lafayette Instrument Stimtek SGCG1 scrambled grid current generator (Lafayette Instrument Co., Lafayette, USA). After the shock-paired conditioning was complete, animals were returned to their home cage for approximately 24 hours. On the final two

days of the experiment, we performed a context preference test, wherein animals were returned to room A containing the same behavioural apparatus and monitoring equipment, and the doors to each context were opened to allow animals to freely explore both contexts and the connecting alleyway. Rats were placed into the alleyway and dwell time was measured using identical scoring procedures as during pre-exposure. If animals learned and remembered which context was shock-paired or unpaired, we expected rats to spend more time in the unpaired than shock-paired context. In our scented context discrimination experiment, we infused rats with 0.7 or 1.0 uL VEH at each site 45 minutes before pre-exposure and conditioning. Rats that underwent unscented context fear conditioning were trained in separate cohort, and not infused with VEH during the pre-exposure or conditioning task epochs. Both groups were infused with 0.7 uL – 1 uL of VEH or 10 mg/kg – 15 mg/kg of ROP 45 minutes before preference testing. In the scented task, six rats were given the smaller infusion parameter, and four the larger infusion, while six rats were given the small infusion and six rats the larger infusion in the unscented discrimination. Additionally, six animals that underwent unscented context fear conditioning and testing were previously trained and tested in the MWT.

Animal Perfusion and Tissue Collection

At the end of experiments, rats were briefly anesthetized with 4% isofluorane dissolved oxygen at a flow-rate of 4 L/min and given an overdose intraperitoneal injection of sodium pentobarbital. Six animals were perfused 45 minutes following scented context preference testing to determine changes in CA1 cFos expression

following VEH or ROP infusion. Once the rat became non-responsive they were then perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA) dissolved in PBS. Brains were carefully extracted from the skull and stored in 4% PFA dissolved in PBS for 24 hours at 4°C, and then transferred to 30% sucrose solution containing 0.02% sodium azide in PBS for at least 48 hours at 4°C prior to cryosectioning with a freezing sliding microtome at 40 um section cut thickness. Sections were collected and stored in a 12-section series in PBS solution containing 0.02% sodium azide at 4°C until subsequent immunohistochemical staining.

Immunohistochemistry

cFos protein is a molecular marker of cellular activity related to learning and memory events. We performed fluorescent immunohistochemical staining to reveal hippocampal activity blockade during contextual fear discrimination preference testing following the infusion of VEH or ROP in six randomly selected animals (four rats with smaller infusion parameter; two rats with larger infusion parameter). A 12-section series was rinsed in 1X PBS for seven minutes, and then incubated overnight under light agitation on a Fisher Scientific 2314FS agitator (Thermo Fisher Scientific, Hampton, USA) in 1X PBS containing 0.3% Triton X and 1:250 dilution of rabbit anti-cFos IgG primary antibody (ab190289, Abcam, Cambridge, UK). Approximately 24 hours later, sections were washed in PBS three times for seven minutes and transferred to PBS containing Alexafluor 488 goat anti-rabbit IgG secondary antibody for approximately 24 hours (ab150073, Abcam, Cambridge, UK). During the last 15 minutes of secondary

antibody incubation, sections were stained with 4′,6-diamidino-2-phenylindole (DAPI) and washed three times in PBS for seven minutes. Sections were then mounted onto 1% gelatin-coated slides, allowed to dry and cover-slipped with fluorescent mounting medium.

CA1 cFos Quantification

cFos protein expression was quantified using the optical fractionator method in StereoInvestigator software (version 10.54, MBF Bioscience, Williston USA) from confocal z-stack images collected on an Olympus FV1000 equipped with Fluoview FV10-ASW software (version 4.0, Olympus Corporation, Shinjuku, Japan). Bilateral traces of CA1 were placed over live images at 20× objective on each section prior to z-stack image acquisition. The counting frames were positioned on a 250 × 250 μm grid over the CA1 trace according to principles of systematic-random sampling. A series of seven z-stack images at 512 × 512 pixels were collected at each sampling site with a 60× oil objective starting at the top of the section every 2 μm for a total 18 μm stack. Image thresholds were set at 720 HV ± 20 and 600 HV ± 20 respectively in DAPI and FITC channels and kept constant across imaging a section series such that cFos expression could be clearly identified. Z-stack images were imported into StereoInvestigator such that one image from each stack fell above and another below the 14-μm dissector height. cFos was counted according to optical dissector inclusion–exclusion criteria at each cell's widest point.

Statistical Analysis

Statistical analyses were performed using Prism by GraphPad software (San Diego, California, USA). To assess MWT behavioural performance, we used one-way or two-way repeated measures ANOVA and Bonferroni post-hoc comparisons following a significant interaction term. Upon a significant interaction, we also used a two-tailed, one-sample t-test to compare % dwell time in target and non-target quadrants to a 25% chance level of performance. We also used a repeated-measures two-way ANOVA to analyze context fear discrimination, with the addition of a paired t-test to analyze initial context preference during pre-exposure. cFos expression in CA1 was also compared following VEH and ROP infusion using an unpaired, two-tailed t-test. Alpha of 0.05 was used for the threshold of statistical significance in each analysis.

Results

Hippocampal ROP Infusion Does Not Impair Contextual Fear Discrimination

Recently we found that hippocampal damage using the NMDA lesion approach caused RA, but not AA, for context fear discrimination in rats. The present experiment was designed to assess if RA also occurs for context fear discrimination in rats using a temporary inactivation with ROP. In addition, we examined whether the presence of RA is related to the infusion size or odour cues to discriminate between shock-paired and unpaired contexts.

To ensure that rats did not have an innate preference for their paired or unpaired context that could affect subsequent preference testing, we assigned animals to be

shocked in either the white square or black triangle context such that dwell time during pre-exposure was matched. Paired t-tests of pre-exposure dwell times showed no difference between initial paired and unpaired context preference during pre-exposure in either the scented (Figure 5.3A; $t = 0.3032$; $p = 0.7686$) or unscented task (Figure 5.3B; $t = 0.08379$; $p = 0.9347$). A two-way repeated measures ANOVA in the scented contextual fear discrimination showed a significant effect of context (Figure 5.3C; $F(1, 18) = 5.321$; $p = 0.0332$), but not an effect of treatment ($F(1, 18) = 0.1664$; $p = 0.6881$) nor context x treatment interaction ($F(1, 18) = 0.094$; $p = 0.7627$), suggesting that hippocampal inactivation did not affect contextual fear discrimination. Surprisingly, in the unscented task we found a similar effect of context (Figure 5.3D; $F(1, 20) = 4.824$; $p = 0.0400$) but not an effect of treatment ($F(1, 20) = 0.005483$; $p = 0.9417$) or context x treatment interaction ($F(1, 20) = 2.878$; $p = 0.1053$). To determine whether context discrimination was related to the infusion size and the presence of context odours, we transformed each rat's dwell time in paired and unpaired contexts into a single preference score (Preference Score = dwell time unpaired – dwell time paired). A two-way ANOVA with treatment and scent as factors showed no effect of treatment (Figure 5.3E; $F(2, 36) = 0.4821$; $p = 0.7797$), odour ($F(1, 36) = 0.3347$; $p = 0.5665$), or treatment x odour interaction ($F(2, 36) = 0.2505$; $p = 0.7797$), suggesting that neither the size of infusion or context odours affected the ability of rats to discriminate between the shock-paired and unpaired contexts.

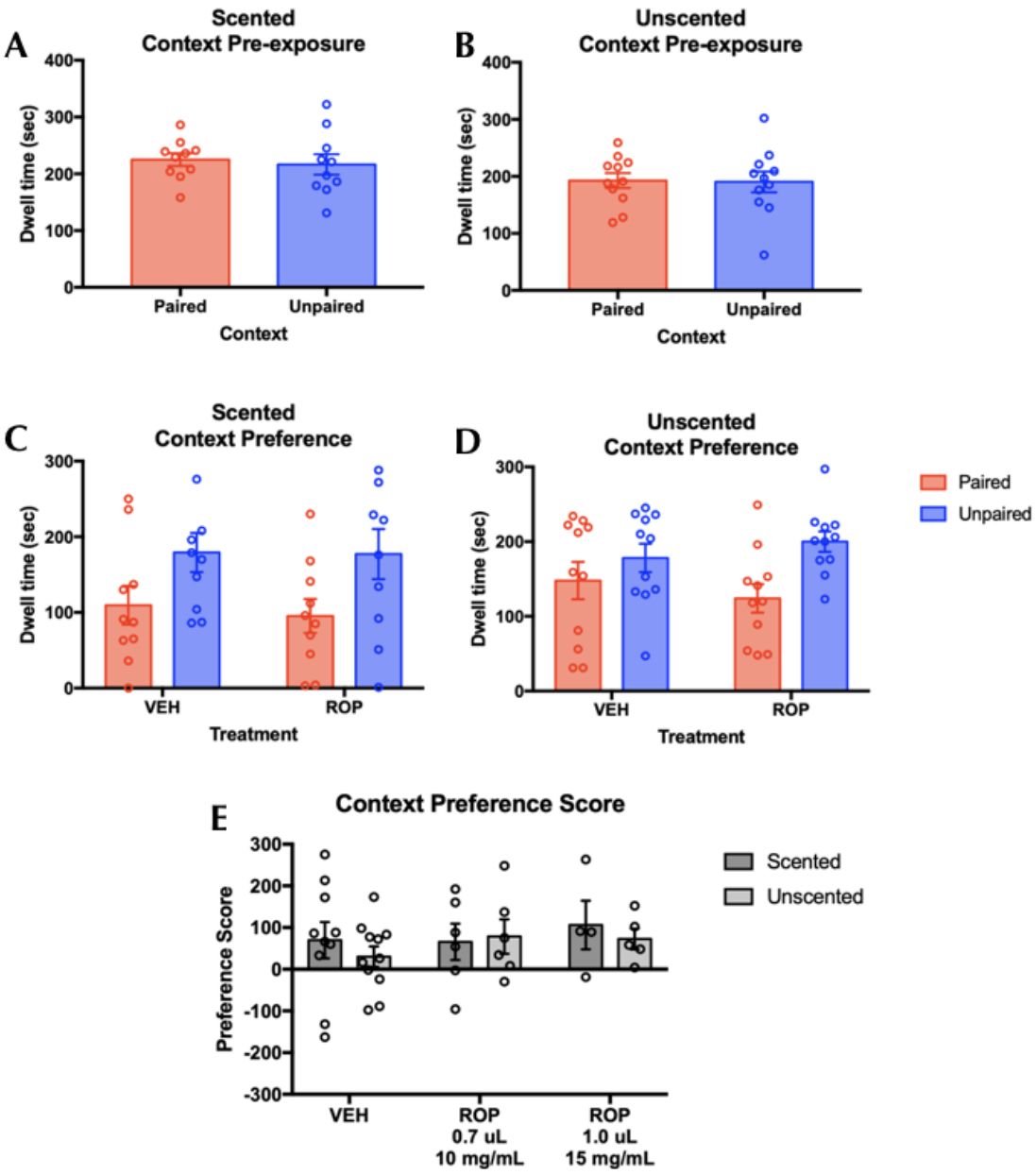


Figure 5.3 Hippocampal ROP infusion does not affect discrimination between distinct contexts. Figures A and B show initial context preference for paired and unpaired contexts during pre-exposure, which were not significantly difference in either the scented (A) or unscented (B) discrimination task. Following conditioning, figures C and D show that neither VEH or ROP infusion affected context discrimination when contexts

were scented (C) or unscented (D). Transforming context preference into a single preference score for each animal showed no effect of treatment or odour on performance.

Hippocampal ROP Infusion Causes Retrograde Place Navigation Memory Loss in the MWT

To ensure that rats learned to navigate to the hidden platform we examined latency to the hidden platform, path length, and average % dwell time in the target vs non-target quadrants during training. A one-way repeated measures ANOVA revealed a significant effect of training day on both latency to the hidden platform (Figure 5.4A; $F(1.867, 9.334) = 29.76$; $p = 0.0001$) and path length (Figure 5.4B; $F(1.596, 7.98) = 24.81$; $p = 0.0005$). *Post hoc* analyses also showed a significant ($p < 0.05$) decrease in latency and path length to the hidden platform except between days 3 and 5, suggesting that rats achieved asymptotic performance on day 3 of training. Our analysis of quadrant preference during training showed a significant effect of quadrant (Figure 5.4C; $F(1,10) = 177.3$; $p < 0.0001$), and quadrant x day interaction ($F(3, 30) = 5.305$; $p = 0.0047$), but not training day ($F(3, 30) = 0.6339$; $p = 0.5990$). Our *post hoc* comparisons of target and non-target quadrant % dwell time showed that target quadrant dwell time was significantly greater than non-target quadrant dwell time on day 1 ($t = 4.366$), day 2 ($t = 8.037$), day 3 ($t = 8.283$) and day 5 ($t = 9.427$), confirming that rats learned the target quadrant where the hidden platform was located.

On day 4 and 6, we performed a 60-second, no-platform probe to assess target and non-target quadrant % dwell time as a dependent measure of spatial memory following

VEH or ROP infusion into the hippocampus. Previous work demonstrated that hippocampal damage or temporary inactivation reliably produces retrograde amnesia in this phase of MWT performance (Broadbent et al., 2006; Clark et al., 2005; McDonald et al., 2010; Morris et al., 1982; Sutherland et al., 1982; Sutherland et al., 1983). Similar to these reports, our two-way repeated measures ANOVA revealed a significant effect of quadrant (Figure 5.4D; $F(1, 10) = 27.2$; $p = 0.0004$), and quadrant x treatment interaction ($F(1, 10) = 6.867$; $p = 0.0256$), but not a significant effect of treatment ($F(1, 10) = 1.261$; $p = 0.2878$). Our post-hoc analyses showed significantly greater target compared to non-target % dwell time following VEH ($t = 5.626$) but not ROP infusion ($t = 2.111$), and that target % dwell time was significantly greater than 25% chance level following VEH ($t = 3.753$; $p = 0.0133$) but not ROP treatment ($t = 1.87$; $p = 0.1204$). These results confirm that temporary hippocampal inactivation during a no-platform probe causes retrograde memory loss for the hidden platform location, corroborating previous studies that have used related approaches to temporarily inactivate the hippocampus in rats (Broadbent et al., 2006; McDonald et al., 2010).

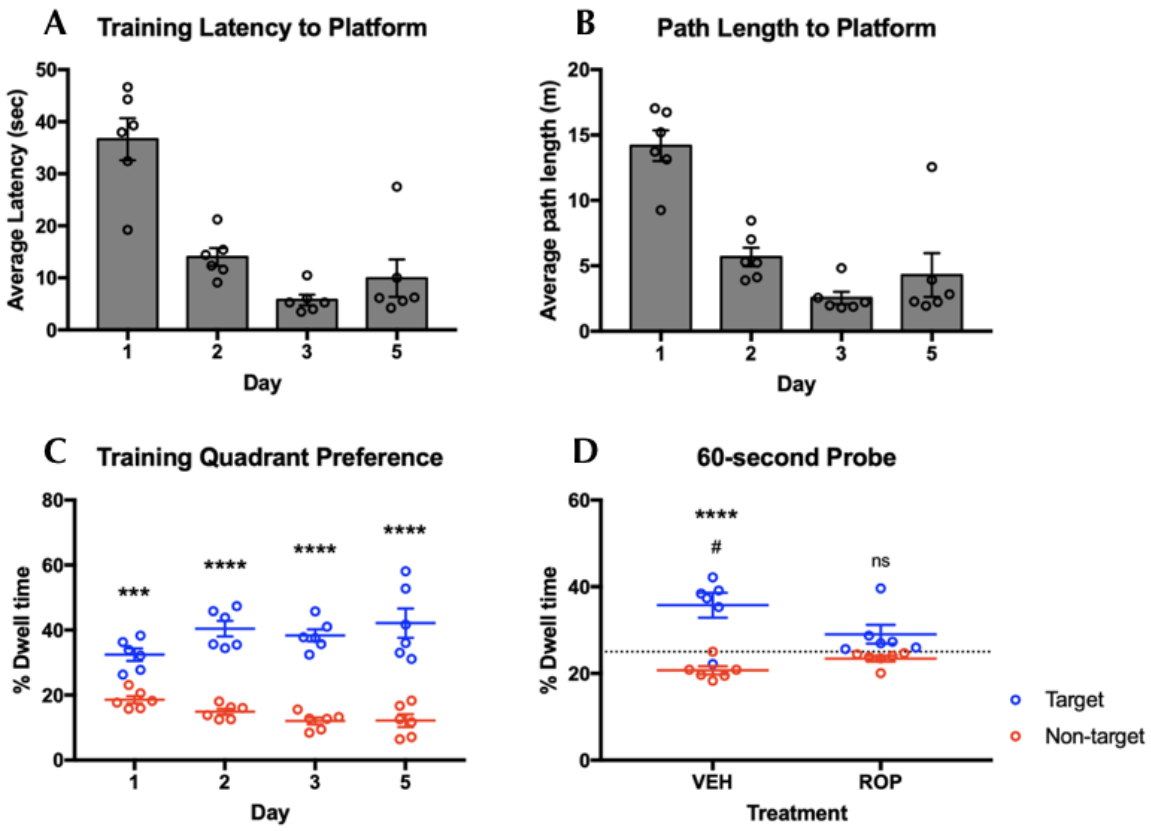


Figure 5.4 Hippocampal ROP infusion causes spatial memory loss in the MWT. Figure A-C illustrate that rats successfully learned to navigate to the target quadrant and escape from the pool based on latency (A), path length (B), and quadrant preference measures (C) during training. Figure D shows that we also found that animals significantly preferred the target quadrant following VEH but not ROP infusion (*), and that VEH target quadrant preference was significantly greater than 25% chance level (#).

ROP Infusion Reduces CA1 cFos Expression

Quantification of cFos expression in CA1 across the hippocampal axis revealed a significant reduction in activity following ROP infusion (Figure 5.5; $t = 7.424$; $p = 0.0018$). Specifically, ROP infusion reduced CA1 cFos expression by 50.60% compared

to VEH controls. Although we did not achieve previous levels of inactivation measured throughout hippocampal sub-regions using this method (Gulbrandsen et al., 2013), the reduction in activity suggests that ROP treatment in the MWT was due to partial hippocampal inactivation. This result is also critical for our interpretation of the contrasting results in contextual fear discrimination.

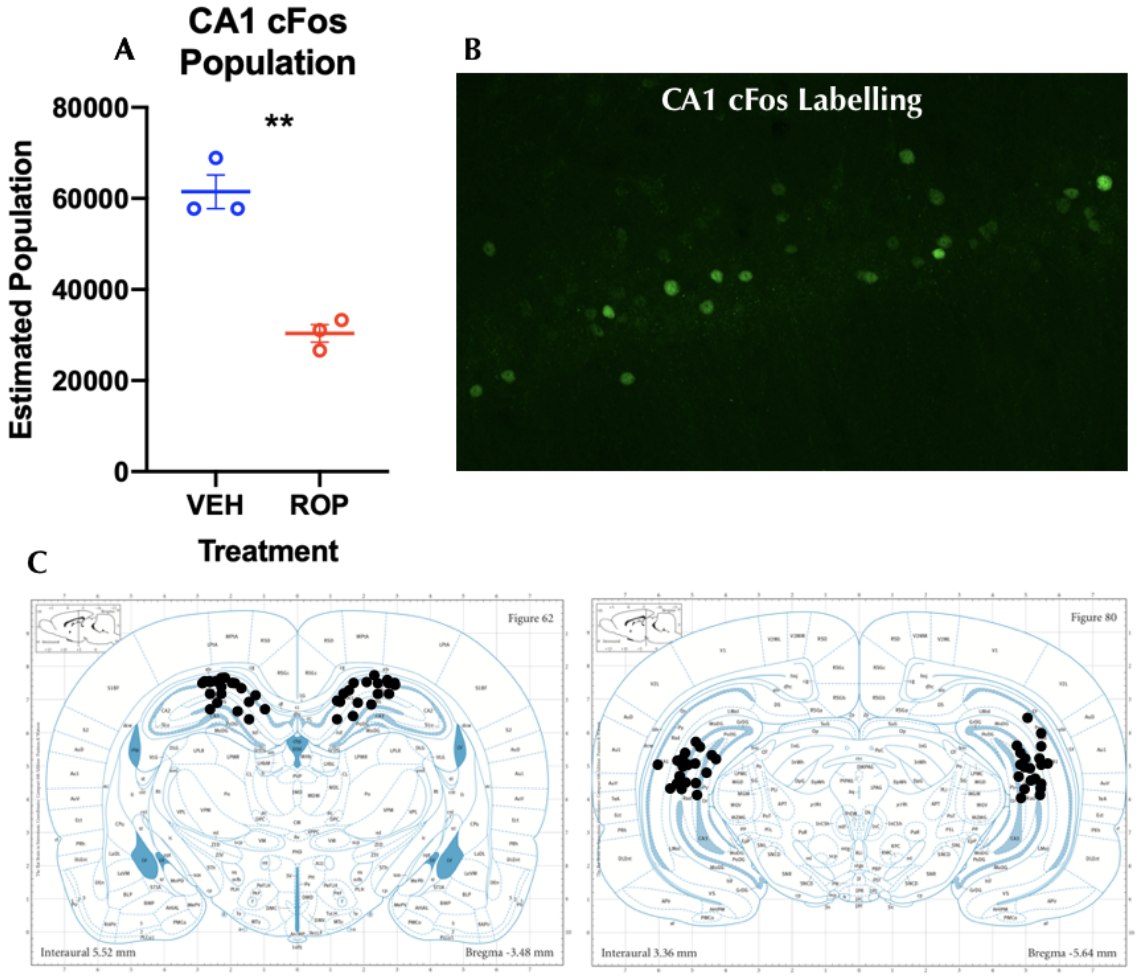


Figure 5.5 ROP infusion significantly reduces hippocampal cFos expression in CA1. Using a design-based stereology approach, our quantification of cFos protein expression in CA1 revealed a significant reduction in the estimated cFos population following ROP

compared to VEH infusion based on animals included from both small (4) and large (2) infusion parameter groups (A). Figure B shows an example of cFos labelling quality. Cannulae placements were also approximated (C) and confirmed to be in the hippocampus throughout all experiments.

Discussion

In the present experiments, we found that hippocampal ROP infusion caused partial CA1 inactivation and RA for place navigation memory in the MWT. We interpret these results to suggest that spatial navigation is a more sensitive measure to detect RA following inactivation than context fear discrimination. Previously our research group found that complete hippocampal inactivation (>80%) resulted in RA for context fear memory (Gulbrandsen et al., 2013). Importantly, several groups have shown that lesion size correlates with the severity of RA for contextual fear memory. Recently, Scott et al. (2016) found similar discrepant results to our own using the sodium channel-blocker tetrodotoxin (TTX). Their group infused the hippocampus with TTX 30 minutes before context fear memory testing or a place navigation memory test in the MWT. Similar to the outcomes of our experiment, Scott et al. (2016) discovered that hippocampal TTX infusion caused impairment in place navigation but not context fear memory. They also included lesion groups with partial (~50%) or complete (~80%) hippocampal lesions using NMDA after context fear conditioning. Similar to previous studies that varied lesion size after conditioning, they found complete but not partial lesions caused RA for context fear. In our previous study, we damaged ~80% of the hippocampus before or after

discriminative context fear conditioning and observed RA but not AA. The current pattern of results supports that complete but not partial hippocampal damage or inactivation causes RA for contextual fear memory (Sutherland et al., 2010). Given the similarity in results using inactivation and lesion methods across studies, we suggest this outcome is likely not due to confounding variables of the method used.

We used the MWT to assess RA following intracerebral ROP infusion based on previous observations that partial hippocampal inactivation using sodium channel-blockers or musicmol impairs performance in this task (Broadbent et al., 2006; McDonald et al., 2010; Scott et al., 2016). Similar to studies that have infused muscimol or lidocaine into the dorsal hippocampus, we found an effect on place navigation memory in the MWT with ~50% hippocampal inactivation with the sodium channel-blocker ROP. Experiments using temporary inactivation methods have reported impairments in several spatial memory tasks, including win-shift behaviour and reward location recognition (Black et al., 2004; Chang & Gold, 2003; Gaskin et al., 2005; Klement et al., 2005). Importantly, these results do not imply that the hippocampus is necessary for all spatial memories.

Rats with hippocampal damage can learn to express spatial behaviour, but not precisely or as quickly as control animals. We recently performed a lesion study in a concurrent cue-place water task and found that rats with the hippocampus intact learned a cue-place discrimination more rapidly than damaged rats (Chapter 3). However, animals with damage expressed spatial strategies similar to controls with continued training, supporting that spatial memory can be acquired in the absence of the hippocampus.

Several studies using the lesion method have shown slower acquisition of spatial behaviour in the absence of the hippocampus (Hales et al., 2014; Day et al., 1999) and that performance is less precise (McDonald & Hong, 2000). A study conducted in humans revealed an effect of hippocampal damage on the precision of spatial memory, but subjects with lesions were still able to express knowledge of general target locations (Kolarik et al., 2018). These outcomes support that the hippocampus is not necessary for spatial memory, but aids in rapid and precise acquisition.

A possible cause for the difference outcomes following partial hippocampal inactivation in context fear discrimination and the MWT could be the amount of precision required to express memory-based behaviour that appears equal to controls. Specifically, pattern separation and pattern completion processes that hippocampus performs on distributed cortical information (Rolls, 2010) might be essential for expressing accurate memory in the MWT, but not the context fear discrimination paradigm used here. During navigation to a hidden platform, animals must predict and complete the spatial sequence to be traversed from a start location to a hidden goal and disambiguate the target from other similar non-target trajectories. By contrast, the contexts used in the current fear discrimination paradigm differed in several sensory dimensions, including colour, shape, and odor. It is likely that a coarse discrimination ability following partial hippocampal inactivation supports memory performance that is similar to control animals. If this account is correct, partial hippocampal inactivation would cause amnesia for fear discrimination between similar contexts due to increased demand for pattern separation,

which we recently observed following ventral hippocampal damage (McDonald et al., 2018).

Differences in task sensitivity observed here, combined with the effects of hippocampal damage on RA but not AA, are lacking a clear explanation from contemporary models of LTM organization (Lee et al., 2016; Sutherland et al., 2010). We recently provided a concept termed heterarchic reinstatement (HR) to account for differences in AA and RA that have appeared in several memory tasks (Lee et al., 2016). However, this view does not provide a coherent explanation of differences in task sensitivity to RA, such as the MWT and context fear discrimination. The present combination of results in this study and beyond suggest a revised model of memory organization is needed to make sense of these observations.

General discussion

The experiments presented here are part of an effort to clarify the role of the hippocampus in multiple aspects of long-term memory (LTM) using the heterarchic reinstatement (HR) concept as a working model (Lee et al., 2016). Our findings primarily support two predictions of this concept: 1) extensive hippocampal damage causes RA but not AA for multiple aspects of LTM; 2) hippocampal population activity in CA1 represents multiple features of LTM. As discussed in previous chapters, the HR concept is uniquely equipped to account for most observations we have made in these experiments. However, there are points of failure in the HR concept along with other popular models of LTM organization.

A prominent feature of HR is its conceptual departure from the categorization of different types of LTM and their dependence or independence of the hippocampus. The most frequently cited models of hippocampal contributions in LTM suggest that the hippocampus is involved in one type of memory, but not another, including the Multiple Trace Theory (Nadel & Moscovitch, 1997), the Standard Model of Systems Consolidation (Squire, 1992), Configural Association Theory (Sutherland & Rudy, 1989), and the Cognitive Mapping Theory (O'Keefe & Nadel, 1978). The distinction that certain types of memory involve the hippocampus or other brain structures, such as the amygdala, striatum, or cerebellum, stems from basic view that there are multiple, independent memory systems in the brain that have unique functions. This general view, which is implied in each categorical model, is the standard Multiple Memory Systems Theory (MMST; Squire, 1992; White & McDonald, 2002). There are two observations

that the MMST is largely based on: 1) damage to distinct brain structures causes dissociable memory impairments; 2) neurons in distinct brain areas have dissociable aspects of memory coding in their firing characteristics. For example, landmark experiments on the cognitive effects of hippocampal damage revealed impairments in spatial memory (Morris et al., 1982; Sutherland et al., 1982), consistent with observations of place cell firing in the hippocampus (O'Keefe & Dostrovsky, 1971), which together support the Cognitive Mapping Theory (O'Keefe & Nadel, 1978). However, hippocampal damage does not only cause spatial memory impairments, nor do hippocampal neurons encode only spatial information (McDonald et al., 1997; McKenzie et al., 2014; Sutherland et al., 1989, 2001; Wood et al., 2000). These types of counter observations have repeatedly resulted in the application of new categories of which types of memory depend on the hippocampus, and which do not. In reviewing the range of memory impairments caused from complete hippocampal damage after learning, it appears that *no single category* accounts for the breadth of RA that has been reported (Lee et al., 2016; Sutherland et al., 2010). Proposing that a brain structure is uniquely involved in a type of memory also assumes that damage before or after a learning episode will have similar amnestic effects. As demonstrated and discussed throughout the present work, this assumption is incorrect.

By contrast to the standard MMST and its various forms, the HR concept suggests that the hippocampus is involved in memory more generally, regardless of the type of memory and its remoteness from a learning episode. Although our previous discussion of this view admits that the hippocampus may have some unique contributions to LTM, it

does not make a *coherent* account of why hippocampal damage causes AA for some tasks and not others, or why partial damage or inactivation would cause RA in some tasks but not others. For example, the HR concept does not address if the same relationship exists between hippocampal lesion size and severity of RA for place navigation memory performance and contextual fear memory retrieval. In conceiving the HR view, this possible difference in task sensitivity to hippocampal damage was largely unexamined, due to our focus on issues with categorization of memory types as being hippocampus-dependent or independent.

In Chapter 5, we temporarily inactivated the hippocampus to determine its effects on RA in context fear discrimination and spatial navigation in the MWT. Surprisingly, we observed that post-training hippocampal inactivation caused RA for spatial memory but not context fear discrimination; *prime face* these results contradict our previous findings in Chapter 4 using the lesion method, and the first prediction of HR view (Lee et al., 2016; Lee et al, 2017). However, our quantification of cFos expression in CA1 following hippocampal infusion of ROP revealed a partial (~50%) decrease in activity. Based on these outcomes, we concluded that tests of place navigation memory are more sensitive to hippocampal interference after learning than context fear paradigms. This corroborates experimental outcomes in the literature showing the same effect in other forms of place navigation and context fear memory (Broadbent et al., 2006; Gulbrandsen et al., 2013; Scott et al., 2016; McDonald et al., 2010; Sparks et al., 2011).

In Chapter 3, we found that pre-training hippocampal damage did not prevent formation of place navigation memories in a concurrent cue-place water task, but delayed

its acquisition. In our discussion, we reviewed findings in human and animal models suggesting hippocampal damage does not prevent spatial memory, but might prevent precise spatial localization. In addition, reports on both contextual fear conditioning and place navigation tasks show that rats with hippocampal damage might learn these tasks more slowly than rats with an intact hippocampus (Hales et al., 2014; McDonald & Hong, 2000; Wiltgen et al., 2006; Zelikowsky et al., 2012). We propose that these results may be explained in terms of relative reliance on pattern separation and pattern completion functions performed by the hippocampus on incoming and outgoing information from distributed cortical regions.

Precisely locating a goal in place navigation tasks, and linking the sequence of places traversed to reach that location, can be viewed as pattern separation and completion operations. Multiple studies have shown that rats express "fast-forward" patterned activity in place cell sequences during traversal of a familiar track, and likely before approaching a target location or at a choice point (Johnson & Redish, 2007; Lisman & Redish, 2009; Redish, 2016). This pattern of activation may be a neural basis for vicarious trial and error (VTE) behavior. Although place cell sequences exist in other cortical regions, such as the retrosplenial cortex, post-training hippocampal damage disrupts spatial sequence coding and tuning in in the retrosplenial cortex (Mao et al., 2017, 2018). More recently, the same effect has been observed in distributed cortical areas (Esteves et al., 2018). Although not a focus of the present thesis, temporal sequence coding identified in hippocampal ensembles can also be interpreted as a pattern completion function (Middleton & McHugh, 2016; Sanders et al., 2019). Specifically,

ensembles in the hippocampus generate temporal firing patterns that are not correlated with sensory features of a task while animals either running on a treadmill prior to a choice point (Howard & Eichenbaum, 2015), or during a trace interval between a CS and US (McEchron et al., 2003; Solomon et al., 1986). Consistent with these electrophysiological observations, hippocampal damage impairs multiple types of temporal and sequence memory tasks (Fortin et al., 2002; Moyer et al., 2015; Ocampo et al., 2017; Solomon et al., 1986).

The circuitry of the hippocampus is well-equipped for pattern separation and pattern completion: the densely packed, large cell population in the dentate gyrus serves to make similar incoming patterns more different, while the auto-associative connectivity of CA3 completes previously learned information from partial inputs (Leutgeb and Leutgeb, 2007; O'Reilly & Rudy, 2001; Rolls, 2010). Although pattern separation and pattern completion are performed in multiple brain networks, the hippocampus is well equipped to carry out these computations with large amount of input form many brain regions due to its distributed connectivity with cortical and subcortical areas, as discussed in Chapter 1. Experiments using tasks that have particular dependence on pattern separation and pattern completion also show that partial hippocampal damage or inactivation causes impairment. In a context fear conditioning task where animals learn to pattern complete a context based on transport cues, small amounts of damage, temporary inactivation, or plasticity blockade cause memory impairment (Matus-Amat et al., 2004, 2007; Rudy et al., 2002). However, when pattern completion based on transport cues is not required to associate the context with a foot shock, animals show no impairment

(Matus-Amat et al., 2004). Recently we also found that partial hippocampal damage causes AA for context discrimination when the contexts are made identical except for olfactory cues introduced into each chamber (McDonald et al., 2018). However, in Chapter 4 we found that when contexts differ in several sensory modalities complete, pre-training hippocampal damage does not cause AA. Finally, it is possible that the observed learning rate differences in some tasks may be due to a pattern completion deficit. The ability to integrate and reinstate information in distributed cortical modules from the top-down (i.e. hippocampus back to cortical regions) is more efficient to learn and retrieve information than through hetero-associative, bottom-up processes (Rudy and O'Reilly, 1999). Accurate memory performance in some tasks requires the association of features that have distributed representation cortical regions. With repeated, bottom-up activation, cortico-cortical associations develop that can retrieve one feature from activation of another (Lee et al., 2016). Supporting this notion, recent experiments have shown that distributed, repeated training or reactivation might drive bottom up retrieval to support memory unaffected by hippocampal disruption, but massed training causes memory to remain hippocampus-dependent (Lehmann et al., 2009; Lehmann & McNamara, 2011).

In an important study examining the differences between massed versus distributed learning on hippocampus-independent memory, Lehmann et al. (2009) conditioned rats to fear a context with a series of foot shocks administered in a single, massed training session, or across 10 training sessions over a 5-day period. They discovered that complete hippocampal lesions caused RA for the massed-trained rats, but

not when training was distributed. Further, rats with distributed conditioning and post-training hippocampal damage were able to discriminate between the shock paired context and a novel context that was not paired with foot shock. Thus, the non-hippocampal memory must contain at least some contextual details. A similar experiment that used temporary inactivation to completely turn off the hippocampus found a similar outcome (Gulbrandsen et al., 2013). One study on the effects of reactivations by briefly placing animals in their shock paired context after fear conditioning also found that complete hippocampal damage did not cause RA following repeated, distributed memory reactivation (Lehmann & McNamara, 2011). To more clearly delineate how bottom-up activation can support memory retrieval independent of the hippocampus, it will be valuable to examine the effects of repeated and distributed learning episodes in multiple memory tasks.

We maintain that the existing evidence forces the view that no single category accurately describes which type of memory depends on the hippocampus, but several relationships have emerged in this discussion between the extent of hippocampal damage, order of learning episode and hippocampal interference, memory task, and repetition of training that predict memory loss vs memory sparing. Interactions between key parameters likely determine the amount of memory retrieved following hippocampal damage or inactivation.

Hippocampal Amnesia in a Multi-Dimensional Parameter Space

In a paper published several months after his death, David Olton proposed, "a quantitative, parametric examination of mnemonic variables may provide a powerful approach to identify precisely the mnemonic processes that require the hippocampal formation in addition to the taxonomic systems" (Wan, Pang, & Olton, 1994. p. 880). Although Olton's characterization of hippocampus-dependent memory processes differs from ours, the notion that memory deficits should be described in a multidimensional parameter space is highly useful and we argue, necessary, for expanding and clarifying causes of amnesia. The relationships we have discussed between hippocampal damage and several parameters can be used to construct a multi-dimensional space that accounts for our observations we have made and generates new predictions on the relationship between the hippocampus and memory.

The first parameter is often the dependent variable in any memory task, that is *memory performance* as inferred through a measure of behavior. Following aversive conditioning this could be the amount of freezing, or in spatial navigation the time spent near or number of traverses across a target location during a probe trial. A treatment that causes memory loss decreases the outcome for this parameter, while improved memory would cause an increase.

As discussed and demonstrated experimentally throughout this thesis, the extent of hippocampal damage after learning predicts memory impairment in several paradigms, such as contextual fear (Sutherland et al., 2008), simple discriminations (Epp et al., 2008), and associations often referred to as cue memories (Lehmann et al., 2007). Specifically, in these tasks we find that partial (~50%) hippocampal damage or

inactivation causes little observable memory impairment, whereas more complete (>70%) damage or inactivation causes severe RA but not AA (Lee et al., 2017; Scott et al., 2016). Thus, our second parameter in this space will be the *amount damaged*, or specifically the extent of hippocampal lesion or inactivation. If we limit our space to three dimensions, *memory performance* is represented by a plane bisecting a cube.

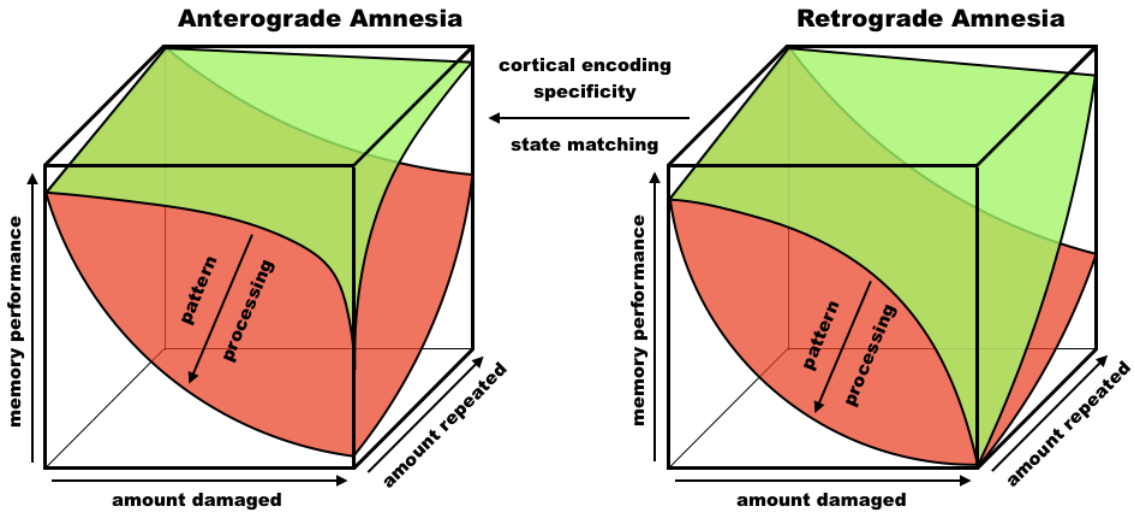
We also presented evidence that different amounts of disruption have different amnestic effects when accurate memory retrieval requires pattern separation or pattern completion. Although these computations can be performed in other circuits, the hippocampus is well-equipped to operate these functions on large amounts of information from distributed cortical areas (Leutgeb & Leutgeb, 2007; Yassa & Stark, 2011). In tasks that require these processes, smaller amounts of hippocampal damage are likely to cause memory impairment (Kesner et al., 2004). If these functions are not required for accurate retrieval, larger amounts of damage or inactivation are necessary to observe amnesia (Kent et al., 2016). Although pattern separation and completion are orthogonal directions in a pattern similarity parameter space, we will refer to them collectively as *pattern processing* within a single dimension.

The HR concept suggests that the broad range of RA following hippocampal damage is due to a failure to achieve a similar pattern of activity in distributed cortical areas compared to a previous learning event. By contrast, the absence of AA in many of the same tasks is due to a similar cortical activity state that is a reinstated when the hippocampus is absent during both learning and memory testing epochs. Although not explicitly stated in our original articulation of this concept, this idea is analogous to the

effect of encoding specificity with cortical activity (Godden & Baddeley, 1975), wherein more similar cortical activity states to initial learning are more likely to promote accurate memory retrieval, and less similar states produce amnesia. The amount of similarity in cortical activity between a learning event and later memory retrieval, or the degree of *state matching* is therefore the fourth parameter that we suggest determines *memory performance*. In addition, this parameter space maintains the HR prediction that a memory encoded in the absence of hippocampal activity can later be interfered with if the hippocampus is online. The parameterization of cortical encoding specificity, or *state matching*, also suggests that this type of interference would be related to the amount of hippocampus inactivated during encoding that is later active during memory testing. The more of the hippocampus is offline during learning, but online during retrieval, the more amnesia that will be observed.

We have also discussed that repeated, distributed training or reactivation can make memories independent of the hippocampus. This type of bottom-up reinstatement of cortical activity may be sufficient for cortical-cortical associations to form that need not be reinstated through top-down pattern completion for accurate retrieval. Another parameter we therefore define is the *amount repeated* in a distributed manner of the learning event or its reactivation. More overtraining may be necessary to support hippocampus-independent memory in tasks requiring more *pattern processing* than tasks that do not require these functions.

Based on these parameters and the empirical relationships we have described between them, we propose a Memory Manifold Theory™ (MMT; Figure 6)[4] that predicts *memory performance* based on several dimensions: 1) *amount damaged*; 2) *pattern processing*; 3) *amount repeated*; 4) *cortical encoding specificity* or *state matching*. For representation simplicity, Figure 1 illustrates these relationships in two 3-dimensional cubes (rather than more accurately as a manifold in a 5-D hypercube) that show patterns of AA and RA with hippocampal damage or inactivation, and the surfaces in each space depict *memory performance* based on values of other parameters. The MMT suggests that relationships between variables predicting memory performance and hippocampal function can be described as a manifold in five-dimensional hyperspace.



[4] In its current usage, a manifold is a contour that bisects a 5-D hypercube into two volumes based on the *amount retrieved*, analogous to the previous mentioned 3-D cube. We proposed this concept in an article recently submitted to *Hippocampus*, "Has Multiple Trace Theory been refuted?" Manuscript Number: HIPO-19-034.

Figure 6. The Memory Manifold Theory (MMT) offers an alternative to contemporary accounts of memory organization, and suggests that no single category, or verbal dichotomy, accurately describes whether a memory depends on the hippocampus. Instead, relationships between parameters in a 5-dimensional space create a manifold that predicts *memory performance*. These parameters at least include the *amount damaged* or inactivated, the *amount repeated* in a distributed manner, either through brief reactivation or overtraining, *pattern processing*, and *state matching*. The amount of *pattern processing* encompasses both pattern separation and pattern completion functions that the HPC is well-equipped and shown to operate on distributed cortical activity. Although pattern separation and completion are orthogonal directions in a similarity state space, experimental and computational modeling literature suggests the HPC is superior at operating both of these parameters. Although other brain regions can also support basic *pattern processing*, tasks which demand these functions will be more sensitive to smaller amounts of hippocampal damage than task that do not. Finally, we suggest that the differences in effects of hippocampal damage on AA and RA are due to an effect of cortical encoding specificity. When the HPC is intact during learning it provides information to distributed cortical areas that is an essential component to the target memory code. Following post-training hippocampal damage a different state of activity is present in distributed cortical areas, and a low amount of activity *state matching* occurs, resulting in RA. By contrast, if the HPC is absent during both learning and memory testing, the same activity pattern is present among distributed cortical networks, and greater *state matching* is achieved, resulting in little to no memory impairment for tasks

that do not require great pattern processing or that have been highly reactivated. However, for tasks that require a greater degree of *pattern processing* AA will occur.

Applying the Memory Manifold Theory

Rather than making categorical predictions or predictions based upon a single conceptual dichotomy, the MMT predicts relationships between parameters within as a manifold. The relationships between parameters in this space also make a coherent account of experimental observations we describe in the present thesis. In Chapter 2 and 3 we presented a novel task to examine cue- and place-based navigation in parallel. We observed that changes to cue locations induced remapping in CA1 related to cue choice accuracy, and that greater similarity resulted in more accurate cue choice. The MMT accounts for this observation by suggesting greater *state matching*, measured as similarity in CA1 population activity, is related to discrimination behavior. Although we presented evidence for this relationship in the hippocampus, preliminary data also suggests the same relationship is exists in cortical areas (LeDuke et al., 2017). In addition, we found RA for cue-based navigation was related to lesion size, and that pre-training lesions cause a delay in cue-place behavior, but similar spatial navigation strategies emerge with continued training in damaged rats to control animals. The MMT predicts that both small and large lesions would result in a learning delay since pattern completion aids in rapid acquisition. An observation supporting this prediction is that lesion size did not predict the number of days to an 80% correct cue choice criterion, with our smallest lesion being approximately 30% hippocampal damage.

In Chapter 4, we described how extensive hippocampal damage resulted in RA but not AA in fear discrimination between distinct contexts. The MMT accounts for this difference in outcomes following pre- or post-training hippocampal damage as a result of differences in *state matching* in a task requiring low *pattern processing*. RA with post-training hippocampal damage is due to a loss of information from the hippocampus provided to the cortex during learning, and therefore low *state matching* results in a lower *memory performance*. By contrast, with pre-training lesions the cortex does not have hippocampal information provided during either learning or memory testing, and therefore greater *state matching* is achieved and consequently greater *memory performance*. Our hippocampal infusion of ROP to induce temporary inactivation caused an ~50% reduction in CA1 activity. For the distinct contextual discrimination that we used, which we suggest requires less *pattern processing*, this *amount damage* or inactivation does not severely decrease *memory performance*. During spatial navigation testing, however, we observed that ROP infusion caused RA. The MMT suggests this is due to a higher demand for *pattern processing* in the MWT, which is more sensitive to detect memory impairment following partial hippocampal inactivation or damage, corroborating similar findings in current research literature (Broadbent et al., 2006; Scott et al., 2016; McDonald et al., 2010).

The novel theoretical framework we have presented provides a coherent account of the experimental observations made in this thesis. MMT is based on a breadth of data about which other, categorical models of memory organization are silent. Similar to the HR concept, the MMT represents a departure from categorical frameworks of memory

organization. This view also generates several novel and testable predictions on the hippocampus and memory that can be examined using advanced neuroscience methods, including *in vivo* imaging of wide-scale neural activity dynamics and various cellular inactivation techniques.

MMT Predictions

1) Hippocampal inactivation after learning causes a reduction in cortical *state matching* that that is negatively correlated with *memory performance*.
2) Changes in CA1 *state matching* positively correlate with *state matching* in distributed cortical and subcortical regions.
3) Less damage or inactivation causes amnesia when greater *pattern processing* is required.
4) *Pattern processing* will be reduced in the absence of the hippocampus.
5) Greater *amount repeated* causes bottom-up associations to form in distributed networks supporting memory retrieval independent of the hippocampus.

Conclusions and Future Directions

Memory is a distributed process involving many brain areas that were not the focus of this thesis, such as the amygdala, striatum, cerebellum, and frontal cortex. The hippocampus has been a particular focus due to its unique connectivity with many brain areas and rich history of study in memory research literature. We discussed similarities between contemporary models of memory organization, current challenges to these

views, and some possible solutions. Based on experimental observations made within and beyond this work, we conclude that the MMT captures the essential parameters predicting memory performance in relation to the hippocampus. Although some other parameters might also predict the *amount retrieved* and require further empirical characterization. These dimensions might include differences in function of the dorsal and ventral hippocampal axes (Strange et al., 2014), the transverse axes (Danielson et al., 2016; Nakazawa et al., 2016), and differences in sub-circuits such as the temporal-ammonic and tri-synaptic pathways during memory retrieval (Albasser et al., 2009; Poirier et al., 2008). Other regions have their own manifolds that also interact with these hippocampal memory parameters.

Additional brain regions and their functions that contribute to LTM can be described within a manifold, but would likely have different parametric relationships or dimensions to consider. For example, the amygdala is usually described as being an emotional processing region in memory tasks, such as contextual fear (LeDoux, 2003). However, strong overtraining parameters also enable animals with amygdala damage to acquire fear memory (Maren, 1999). Amygdalar efferent connectivity also differs from the hippocampus, such as direct connectivity to midbrain and mesencephalic regions but fewer areas in posterior cortex (Price & Amaral, 1981; Stefanacci et al., 1996), and likely has different relationships to parameters such as cortical *state matching* and *amount repeated*. Experiments aiming to capture these relationships could be used to generate an amygdalar manifold within the MMT. Our view for the future of MMT stretches beyond the contributions of one brain area to LTM.

Further experimental validation of the parameters discussed here will also allow fine-tuning of each manifold, from which we can generate functions to describe these relationships. In the current form, our model can be applied to make predictions on the relative expected outcomes in within each parameter. Though further experimental validation is needed, it is likely that the manifold will be defined by 5-dimensional polynomial equation. The predicted *memory performance* for a subject could be determined by adding the values of each parameter to determine its expected location in 5-D hyperspace. Differences in outcomes can also be calculated by subtracting the sum of each subject or group's location in hyperspace, and important features extracted possibly with the application of principal component analysis.

We propose the MMT as a path forward in contemporary memory research that moves beyond verbal dichotomies, which continue to produce more categories when one model fails and could lead the field into an incoherent account of memory organization. The MMT frames memory as a heterarchic, distributed process that can be described in multi-dimensional parameter spaces based on empirical observation, rather than categorical absolutes.

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