# INTERACTIONS OF THE HIPPOCAMPUS AND NON-HIPPOCAMPAL LONG-TERM MEMORY SYSTEMS DURING LEARNING, REMEMBERING, AND OVER TIME

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## Abstract

The hippocampus and non-hippocampal long-term memory systems each have the capacity to learn and express contextual fear memory. How these systems interact during learning and remembering revolves around hippocampal mediated interference, where the hippocampus dominates for both the acquisition and expression of long-term memory. Hippocampal interference during learning can be overcome by modifying learning parameters such that learning is distributed across multiple independent sessions. The standard view of the role of the hippocampus in long-term memory retrieval is that it is temporally limited, where recently acquired memory is dependent on hippocampal function though as a memory ages, dependency is transferred to other memory systems by a process called systems consolidation. Distributed training demonstrates that learning parameters create a memory that is resistant to hippocampal damage. We find little evidence to support temporally based systems consolidation, and present data that supports the view that if the hippocampus is initially involved in learning a memory, it will always be necessary for accurate retrieval of that memory. A critical assessment of the rat literature revealed that initial memory strength, and/or lesion techniques might be responsible for the few studies that report temporally graded retrograde amnesia using contextual fear conditioning. Our experiments designed to directly test these possibilities resulted in flat gradients, providing further evidence that the hippocampus plays a permanent role in long-term memory retrieval. We propose and assess alternatives to the standard model and conclude that a dual store model is most parsimonious within the presented experiments and related literature. Interactions of the hippocampus and non-hippocampal systems take place at the time of learning and remembering, and are persistent over time.

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## Abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| CA1, CA3 | Cornu Ammonis regions 1 and 3 |
| DRT | Distributed Reinstatement Theory |
| fEPSP | Field excitatory post-synaptic potential |
| GABA | Gamma-aminobutyric acid |
| HPC | Hippocampus |
| M | Muscimol infused group |
| mth | Month |
| MTT | Multiple Trace Theory |
| NMDA | *N*-methyl-D-aspartic acid |
| PBS | Phosphate buffered saline |
| PFA | Paraformaldehyde |
| RA | Retrograde amnesia |
| S | Saline infused group |
| SMSC | Standard Model of Systems Consolidation |
| TTX | Tetrodotoxin |
| wk | Week |

# Chapter 1

## The Role of the Hippocampus in Retrieval of Long-Term Memory

The brain is us, who we are, what we do, how we feel. How this extremely complex system can take in and record our vast range of experiences, has intrigued scientists for decades. Memories in large part allow us to interact with our world in a productive and effective way, though when the brain systems that produce memory go wrong, our whole world can quickly fall apart. A relatively small structure called the hippocampus resides within the medial temporal lobe, and acts to orchestrate aspects of memory processes. Without the hippocampus, the record of our personal past disappears.

The focus of this thesis is on the interaction of the hippocampus with other memory systems and how this interaction affects contextual fear memory retrieval. Contextual fear conditioning provides a robust behavioural learning and memory paradigm that involves multiple memory systems. Fear memories are quickly learned and are maintained over a prolonged period, and therefore provide opportunity to assess how memory systems interact over time. Because of the involvement of the hippocampus in context memory, we can use this task to test the involvement of the hippocampus in the retrieval of memories at different time intervals after the learning episode. The experiments described in this thesis were designed to investigate the short- and long-term role of the hippocampus in contextual fear memory. In addition, between-systems interaction was investigated at the time of learning and at the time of remembering. These experiments combine to support the idea that the hippocampus is permanently involved in context memory retrieval. This claim is not part of the modal view on hippocampus and memory. Much of the motivation for the experimental work of this thesis is to extend the empirical evaluation of the claim.

Chapter 1 of this thesis provides an overview of hippocampal anatomy, hippocampus relevant memory and amnesia, and introduces some of the theoretical concepts necessary

to place the following experimental chapters in context. Considering the current state of the field of learning and memory, the pitfalls in the traditional view of the role of the hippocampus will be discussed. Chapter 2 presents data that detail how learning parameters can overcome hippocampal interference during learning to create a hippocampal-independent contextual fear memory, and discusses the finding in light of traditional views of hippocampal function. Chapter 3 provides data that support the concept of hippocampal interference (overshadowing) during learning and remembering. Evidence is presented that supports the idea that hippocampal interference of memory acquisition in non-hippocampal systems can also extend to the time of memory retrieval.

Chapter 4 presents data that reveal that memory strength at the time of learning does not influence hippocampal dependence at the time of remembering. These data, along with the following chapter, add support to current trends in hippocampal research. Chapter 5 presents data that show that hippocampal lesion-induced seizure activity does not differentially disrupt recently acquired contextual fear memory when compared to much older memory. A flat gradient of retrograde amnesia is described. Lastly, Chapter 6 concludes by placing these experimental results within the context of the current state of hippocampal memory research, and discusses the implications for current leading theories of memory.

### *Hippocampus Neuroanatomy*

Within the medial temporal lobe resides a group of highly organized structures that combine to form the hippocampal formation. This formation is unique in that it features primarily unidirectional information flow through a set of anatomically distinct subregions. Simply stated, neocortical input arrives at the hippocampal formation via input from the perirhinal and parahippocampal cortices into the entorhinal cortex (Amaral & Lavenex, 2007). Figure 1.1 illustrates the hippocampal formation connectivity. Entorhinal cortex layer II neurons are the major input into a subset of regions that compose the tri-synaptic pathway.

This input comprises the perforant path, and synapses on the cells of the dentate gyrus subfield. From the dentate gyrus the mossy fibre pathway projects to and terminates in the CA3 subfield. The principal cells of CA3 in turn project via the Schaffer collaterals to the CA1 subfield. The information flow from the entorhinal cortex to dentate gyrus to CA3 to CA1 comprises the tri-synaptic circuit. Up to this point, information flow has maintained a unidirectional, nonreciprocal nature, and continues such with CA1 projecting to the subiculum as well as back to the entorhinal cortex. In addition, the subiculum also projects back to the entorhinal cortex, thus completing the hippocampal formation processing loop.

For the purpose of terminology within this thesis, the term *hippocampus* refers to the subfields composing the tri-synaptic circuit, being the dentate gyrus, CA3, and CA1 (see Figure 1.2 for an illustrated description of the rat hippocampus and subregions). Information received by the hippocampus is highly processed and multimodal. The perirhinal and parahippocampal cortices can be considered the initial parts of the medial temporal lobe that receive, integrate and process information from multiple neocortical sites (Lavenex & Amaral, 2000), and then project this information to the entorhinal cortex where it is processed further. From here, information is divided and sent into the hippocampus via the dentate gyrus as described previously, as well as directly to the CA3 and CA1 regions (Amaral & Lavenex, 2007). At this point, the hippocampus is receiving highly processed information terminating in the dentate gyrus and CA subfields, and performs further processing before sending information back to the entorhinal cortex. Because of this processing architecture, the hippocampus can be thought to be sitting atop a hierarchical recurrent processing loop.

### *Memory and Amnesia*

The privileged location of the hippocampus in information processing has produced profound interest in the circuitry and role of the hippocampus. This interest was amplified by

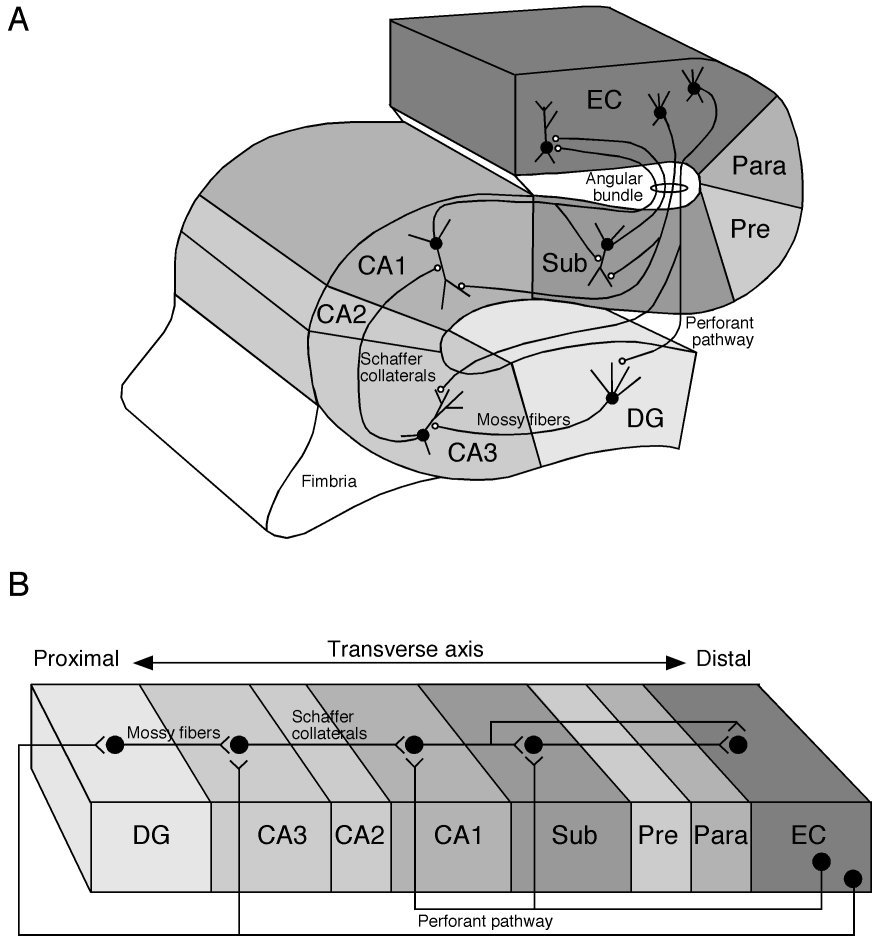


Figure 1.1: **The Hippocampal Formation.** (A) Output from entorhinal cortex layer II projects to the dentate gyrus and CA3 hippocampal subregions via the perforant path. Layer III neurons in the entorhinal cortex project to CA1 and subiculum via the perforant and alvear path. The tri-synaptic circuit of the hippocampus begins with the perforant path synapse on the dentate gyrus (synapse one), from there mossy fibre projections synapse on CA3 neurons, and finally from there via the Schaffer collaterals projections synapse on CA1 neurons. CA1 projects to subiculum and they both project back to deep layers of entorhinal cortex. (B) An alternative illustration of the hippocampal formation projections and connectivity along the transverse axis. Figures with permissions from Amaral & Lavenex, 2007.

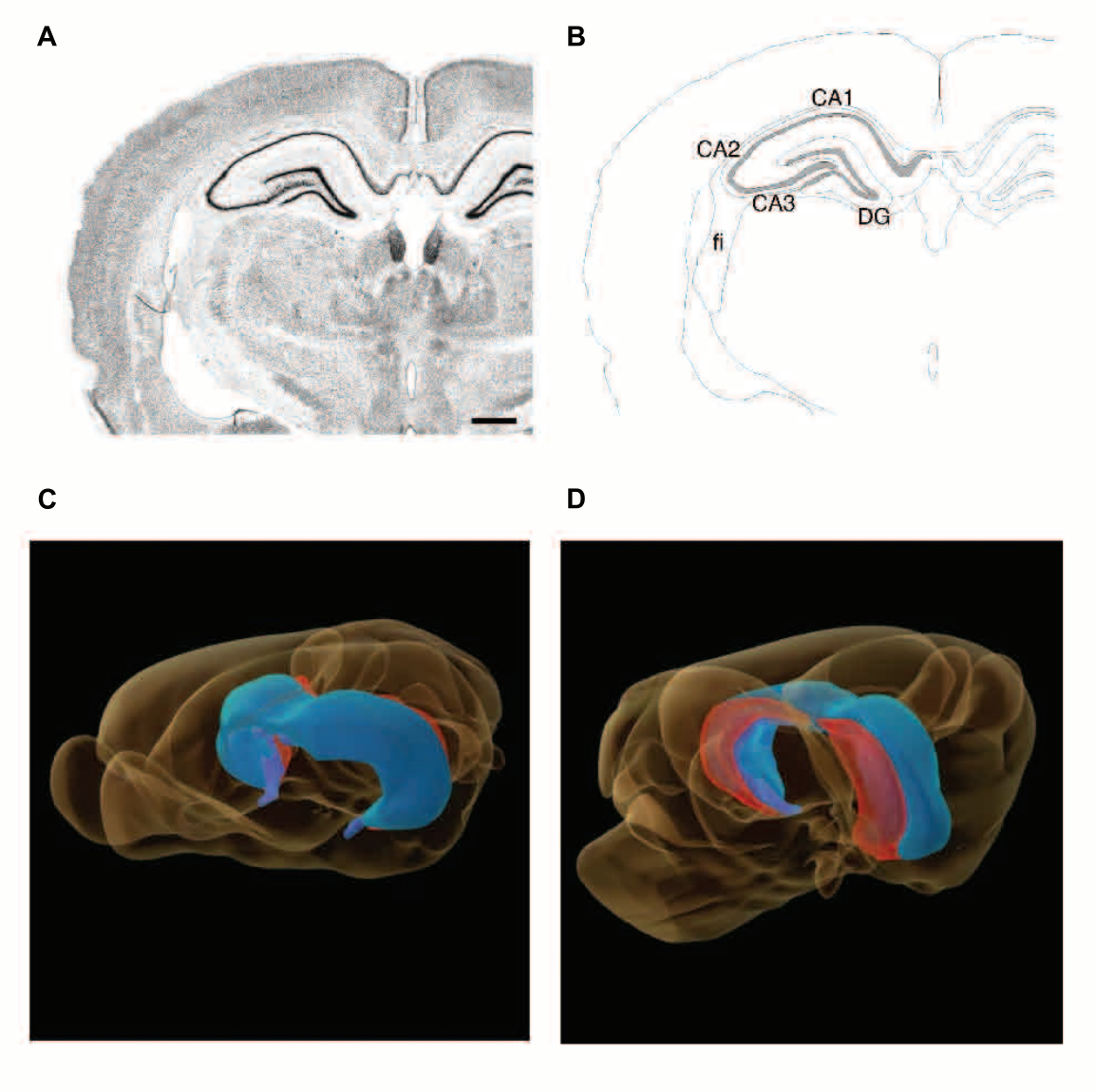


Figure 1.2: **Rat Hippocampus Reconstructed** (A,B) Nissl-stained section and line drawing illustrating the dorsal hippocampal subregions in the coronal plane of the rat brain (adapted with permissions from Amaral & Lavenex, 2007). (C,D) 3-dimensional reconstruction of the rat brain taken from Nissl-stained sections (front-side and back-side views respectively). The hippocampus is detailed in blue and purple, and the subiculum is in red (created by Daniel Poda, 2007).

seminal observations in humans in which damage was directed to the hippocampal region of the medial temporal lobe. In 1955, at the Transactions of the American Neurological Association, Brenda Milner and Wilder Penfield of McGill University in Montréal, were the first to present two cases (patients PB and FC) from patients that had undergone selective hippocampal resection in an attempt to alleviate debilitating seizure activity (Milner & Penfield, 1955). Aside from the data detailing the effects of hippocampectomy on seizures, these patients experienced unexpected memory loss extending up to four years prior to their surgery, and in addition could not form new autobiographical memories (Milner, 2005). These accounts of temporal lobe amnesia following hippocampal damage seemed to point to the fact that the hippocampus is critical for the maintenance of memory.

These initial observations were soon followed up with description of medial temporal lobe amnesia in what has become the most famous and widely studied neuropsychological patient in neuroscience history. The patient's name is now known to be Henry Gustav Molaison, though for over fifty years of study he was known only by the initials HM. HM had suffered from intractable epilepsy thought to stem from a bicycle accident as a child, that left him incapable of functioning normally as an adult. At the time, the neurosurgeon William Scoville at Hartford Hospital in Connecticut was experimenting with treatments to alleviate epileptic symptoms, and HM was referred to him for treatment. Scoville determined that HM's epilepsy was localized to the left and right medial temporal lobes, and it was decided to remove them. Although HM's seizures were controlled following the surgery, he was left with profound memory impairments, and Brenda Milner was requested to examine HM (Scoville & Milner, 1957). The initial work by Scoville & Milner (1957) on HM set the course for the field of learning and memory and research on the hippocampus in general.

Further studies of HM along with other medial temporal lobe patients have shown patterns of memory impairment that reveal selective roles the hippocampus and other medial

temporal lobe structures play in specific kinds of memories. Medial temporal lobe amnesic syndrome is classified by a dissociation between two classes of memory that had been recognized at the time, following damage to the medial temporal lobe (Cohen & Squire, 1980); memories that depend on the hippocampus to be learned as well as remembered, and memories that do not. In this conception of hippocampal mediated memory, retrograde (memory for the past) and anterograde (forming new memory) amnesia present together for hippocampally dependent memory. The syndrome is marked by a disability in remembering recent facts and events (declarative memory), while the ability to learn and remember skilled performance tasks is left intact (procedural memory). For example, patient HM could not remember personal episodes for a period of time leading up to the surgery, and could not form new memories of events that had happened since the surgery. Even in the presence of severe declarative amnesia, HM could readily learn new skills such as mirror drawing—increased performance in tracing an object on a piece of paper while viewing the drawing through a mirror—displaying preserved procedural memory. The dissociation between declarative and procedural memory in temporal lobe amnesics sparked a revolution in memory research, driving the development of appropriate animal models of temporal lobe amnesia and the elaboration of the concept of multiple memory systems in the brain.

Much research has used non-human primate and rodent models to investigate more specific and task dependent properties of temporal lobe amnesia. The main advantages of using animal models is the anatomical and functional specificity of the region investigated, as well as increased control over information and experiences learned by the subject prior to incurred amnesia (Eichenbaum, 2002). Scoville & Milner's (1957) assessment suggested that the memory impairments seen in HM are most likely due to the damage of the hippocampus although many other structures were affected in the surgery. Following from these initial observations, research on hippocampal function in particular has been the main focus in rat models of temporal lobe amnesia.

One of the most significant observations of HM was that his retrograde amnesia appeared to be limited to a period of years prior to the surgery (Scoville & Milner, 1957). Memories during this period were completely gone, while memories from his early life seemed spared from disruption. This pattern of retrograde amnesia is termed to be *temporally graded*. A pattern of equivalent amnesia for recent and remote memories is termed to exhibit a *flat gradient*. The observation of temporally graded retrograde amnesia within some human cases, as well as selective disruption of memories for facts and events, provided impetus for researchers to find animal models that could be used to investigate how the structures of the medial temporal lobe process memory. Because of the distinctive pathology presented in HM and others like him, developing appropriate animal models of temporal lobe amnesia had to meet the following criteria: *a*) sensory, motor, and motivational processes had to be intact; *b*) memory impairments for events leading up to medial temporal lobe damage had to be temporally limited—remote memory spared; *c*) memory impairments had to be global—spanning from memories of past events to the formation and expression of new learning memory, and; *d*) procedural memory had to remain intact.

Using rodent models, impairments in memory for past information following hippocampal damage has been shown in many memory tasks, for example contextual fear conditioning (Sutherland & McDonald, 1990; Good & Honey, 1991; Kim & Fanselow, 1992; R. Phillips & LeDoux, 1992, 1994), fear-potentiated startle (Sparks, O'Brien, Lehmann, & Sutherland, 2005), socially transmitted food preference (Winocur, 1990), spatial navigation (Astur, Mumby, Weisend, & Sutherland, 1994; Bolhuis, Stewart, & Forrest, 1994; Cho, Beracochea, & Jaffard, 1993) and negative patterning discrimination (Rudy & Sutherland, 1989) to name a few. Unfortunately, it has been difficult for researchers to attain an accurate animal model of the human temporal lobe amnesia described initially by Scoville and Milner (1957). The difficulty resides in satisfying two of the conditions: *a*) temporally graded retrograde amnesia; and *b*) that memory impairments had to be global, i.e., com-

bined retrograde and anterograde amnesia. The majority of studies designed to directly assess the function of the hippocampus over time have resulted in amnesia for recently acquired memory as well as more remote memory, termed *flat gradient*. In fact, of the 40 studies performed using rats, less than 30% found temporally graded retrograde amnesia following hippocampal damage (see Sutherland, Sparks, and Lehmann (2010); Sutherland and Lehmann (2011) for comprehensive reviews of this literature). Temporal gradients have been found in tasks using context fear (Kim & Fanselow, 1992; Anagnostaras, Maren, & Fanselow, 1999; Maren, Aharonov, & Fanselow, 1997; Winocur, Frankland, Sekeres, Fogel, & Moscovitch, 2009), trace eye blink (Takehara, Kawahara, & Kirino, 2003), trace fear (Quinn, Ma, Tinsley, Koch, & Fanselow, 2008), and flavour/odour memory (Winocur, 1990; Winocur, McDonald, & Moscovitch, 2001; Clark, Broadbent, Zola, & Squire, 2002; Ross & Eichenbaum, 2006; Tse et al., 2007). Table 1.1 provides a comprehensive list of the appropriate studies conducted in rats. The division between studies that show *flat gradients* versus studies that show *temporal gradients* following damage to the hippocampus has fuelled discord within the field of learning and memory, and is further discussed below.

Even though robust retrograde amnesia has been found in many tasks, when animals are trained after damage to the hippocampus, there is often no impairment in learning the information—a lack of anterograde amnesia (Sutherland, Lehmann, Spanswick, Sparks, & Melvin, 2006; Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006; Lehmann, Sparks, et al., 2009; Sparks, Lehmann, & Sutherland, 2011a). We can infer from these findings that non-hippocampal networks can readily acquire the necessary information to exhibit "normal" behaviour. The dissociation between retrograde and anterograde effects can be found in contextual fear conditioning (Lehmann, Sparks, Hadikin, & Sutherland, 2006; Lehmann, Sparks, et al., 2009; Sparks et al., 2011a), fear potentiated startle (Lehmann, Sparks, O'Brien, McDonald, & Sutherland, 2010), the visible platform version of the Morris water task (Sutherland et al., 2001) and picture discrimination in the visual water task

Table 1.1: Summary of the studies designed to directly examine the effects of HPC damage on remote memories in rodents.

| **Memory task** | **Damage** | **RA duration[1] (days)** | **Reference** |
|---|---|---|---|
| Context fear | 25% dorsal | 1 (27) | Kim & Fanselow, 1992 |
| Context fear | 25% dorsal | 1 (49) | Anagnostaras et al., 1999 |
| Context fear | 40% dorsal | 28 (71) | Maren et al., 1997 |
| Context fear | 70% d+v | 1 (28) | Winocur et al., 2009 |
| Context fear | 40% dorsal | Flat (180) | Lehmann et al., 2007 |
| Context fear | 85% d+v | Flat (180) | Lehmann et al., 2007 |
| Context fear | 40% dorsal | Flat (84) | Sutherland et al., 2008 |
| Context fear | 40% ventral | Flat (84) | Sutherland et al., 2008 |
| Context fear | 85% d+v | Flat (84) | Sutherland et al., 2008 |
| Context fear | 85% d+v | Flat (35) | Sparks et al., 2011 |
| Tone fear | 25% dorsal | None (28) | Kim & Fanselow, 1992 |
| Tone fear | 25% dorsal | None (50) | Anagnostaras et al., 1999 |
| Tone fear | 40% dorsal | Flat (100) | Maren et al., 2009 |
| Tone fear | 40% dorsal | Flat (84) | Sutherland et al., 2008 |
| Tone fear | 40% ventral | Flat (84) | Sutherland et al., 2008 |
| Tone fear | 85% d+v | Flat (84) | Sutherland et al., 2008 |
| Light fear | 85% d+v | Flat (42) | Lehmann et al., 2010 |
| Spatial navigation | 80% d+v | Flat (98) | Bolhuis et al., 1994 |
| Spatial navigation | 80% d+v | Flat (90) | Mumby et al., 1999 |
| Spatial navigation | 75% d+v | Flat (105) | Sutherland et al., 2001 |
| Spatial navigation | 85% d+v | Flat (98) | Clark et al., 2005a, 2005b |
| Spatial navigation | 95% d+v | Flat (42) | Martin et al., 2005 |
| Spatial navigation | 40% dorsal | Flat (98) | Clark et al., 2005a, 2005b |
| Spatial navigation | 45% dorsal | Flat (42) | Martin et al., 2005 |
| Spatial navigation | 45% ventral | Flat (42) | Martin et al., 2005 |
| Object discrimination | 75% d+v | Flat (105) | Sutherland et al., 2001 |
| Object discrimination | 80% d+v | None (83) | Mumby et al., 1999 |
| Object discrimination | 75% d+v | None (3) | Lehmann, Lacanilao et al., 2007 |
| Object discrimination | 75% d+v | None (3) | Lehmann, Clark et al., 2007 |
| Object exploration | 75% d+v | Flat (35) | Gaskin et al., 2003 |
| Shock-probe | 75% d+v | Flat (14) | Lehmann et al., 2006 |
| Picture memory | 75% d+v | Flat (90) | Epp et al., 2008 |
| Trace eyeblink | 45% dorsal | 1 (6) | Takehara et al., 2003 |
| Trace fear | 45% dorsal | 1 (199) | Quinn et al., 2008 |
| Flavour/odour | 25% d+v | 2 (3) | Winocur et al., 1990 |
| Flavour/odour | 80% d+v | 2 (3) | Winocur et al., 2001 |
| Flavour/odour | 85% d+v | 1 (9) | Clark et al., 2002 |
| Flavour/odour | 75% d+v | 1 (20) | Ross & Eichenbaum, 2006 |
| Flavour/odour | 90% d+v | 1 (1) | Tse et al., 2007 |

(Epp, Keith, Prusky, Douglas, & Sutherland, 2004). These dissociations suggest that, when present, the hippocampus interferes with non-hippocampal networks acquiring these long-term memories. These results appear to contradict the initial criteria of rat models of temporal lobe amnesia. Though it is apparent that our picture of what comprises declarative and procedural memory is incomplete and in need of revision, evidence might be emerging for a third type of memory—memory that is not dependent on the hippocampal structure, however highly influenced by the hippocampus.

### *Concept of Multiple Memory Systems*

Memory systems in the mammalian brain are defined as containing a collection of anatomically distinct regions of connectivity that interact in such as way as to perform a distinct physiological function (Sharp, 2006; Sherry & Schacter, 1987; Squire, 1992). An appreciation for the idea of multiple memory systems began to be realized through the work of Scoville and Milner (1957) on patients with damage to the medial temporal lobes. These patients exhibited profound amnesia for certain types of information, either things learned prior to the onset of medial temporal lobe damage (retrograde amnesia) or things learned after (anterograde amnesia). As mentioned above, HM suffered from an inability to form lasting memory for certain types of information, while other information was learned normally. From these observations, it was concluded that the medial temporal lobe contained a memory system that differed from other memory systems in the type of information stored. Researchers have continued to investigate the region specific information in which various memory systems are involved.

Through work with patient HM, as well as other human amnesics and experimental work on non-human animals, we have gained insight into the concept that there are different kinds of memory that are processed and stored in different parts of the brain (Kesner, 1998; Squire, 1992; Squire, Knowlton, & Musen, 1993; A. Phillips & Carr, 1987; Kesner

& DiMattia, 1987; Butters, Martone, White, Granholm, & Wolfe, 1986; Cohen, 1984; Mishkin, Malamut, & Bachevalier, 1984; O'Keefe & Nadel, 1978; Hirsh, 1974; Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957). The Multiple Memory System Theory (MMST) (Squire, 1992) represents the traditional view that different brain regions contain circuitry necessary to accomplish different learning and memory tasks. For instance, MMST is supported by the finding that HM lost declarative memory after temporal lobe resection, while non-declarative memory remained intact (Squire et al., 1993).

The concept that there are multiple long-term memory systems in the mammalian forebrain is supported by evidence stemming from neuropsychological research on humans, non-human primates, and other mammals (Tulving, 1972; Squire, 1987; Cohen & Squire, 1980; Mishkin et al., 1984; Gaffan, 1974; Winocur, 1980; Sutherland & Rudy, 1989). Most of the body of work assessing multiple long-term memory systems have focused on characterizing the function of the hippocampus and its relationship interacting with other memory systems. Multiple long-term memory systems interact in such as way as to provide appropriate behavioural output. This interaction is thought to happen through cooperation, competition, or facilitation. Although these three types of interaction have been investigated independently, it it generally assumed that memory processes act in parallel (McDonald, Devan, & Hong, 2004; White & McDonald, 2002). The manner in which the systems interact is thought to be dependent on the performance requirements of the memory task (Hirsh & Krajden, 1982). Because information is being processed in parallel, the memory system as a whole can adapt to various situations that place greater demand on a certain type of information processing.

The majority of experiments investigating the concept of multiple memory systems in the mammalian brain have shown functional dissociations between distinct brain regions. These experiments utilize techniques that disable specific regions either permanently or temporarily (e.g., Sutherland, O'Brien, & Lehmann, 2008; Sparks et al., 2011a; McDon-

ald & White, 1994, 1993). A series of clever experiments in rats have added insight into differential processing within multiple memory systems i.e., McDonald & White, 1993, 1994, 1995; White & McDonald, 1993. These experiments culminated with the description of the Multiple Parallel Memory Systems theory by White and McDonald (2002) stating how these systems can interact in parallel. In addition to describing how the hippocampus can interact with non-hippocampal systems, White and McDonald (2002) go on to discuss evidence of information processing in two other regions of the rat brain (striatum and amygdala), and how the three systems together interact in specific tasks.

### *Memory Systems Interaction—Overshadowing and Interference*

Memory systems can act competitively, where one system acquires greater associative strength if information processing in the other system is reduced or removed. This type of interaction has been strongly supported by studies designed to dissociate the processing functions of the hippocampus, amygdala and dorsal striatum regions of the rat brain (McDonald & White, 1993; Packard, Hirsh, & White, 1989; McDonald & White, 1994; White & McDonald, 1993; O'Keefe, Nadel, Keightley, & Kill, 1975). These studies exploit the distinction between discrete cue and general context learning, and the potential interactions between the systems that process this type of information in forming associations. In every case presented, the removal of one type of information processing enabled the other systems to acquire greater associative strength as observed through behavioural output.

The hippocampus can act to retard the formation of associations within and between other memory systems (White & McDonald, 2002). In experiments performed by McDonald and White (1993) and Packard et al. (1989), rats were trained in a win-stay task to assess information processing and interactions between the hippocampus and dorsal striatum. Performed on a radial arm maze, a light stimulus located on the end of select arms of the maze

indicated the presence of a food reward. Once the food reward was retrieved from the baited arms, the associated light was turned off. Rats learn the association between the light stimulus and location of food reward, and the number of visits to non-baited arms decreases. Removal of the dorsal striatum prior to training impaired acquisition of the task, though removal of either the fimbria-fornix or amygdala did not. Processing done by the dorsal striatum is necessary for the acquisition of this simple stimulus-response behaviour. What is interesting to note is that the group of rats that received fimbria-fornix lesions acquired the task faster than the normal control group. Output from the hippocampus competitively interacts with non-hippocampal processing in the acquisition of the stimulus-response association.

A number of studies show that the context (spatial) information that is processed by the hippocampal system interacts competitively with other systems that process more direct stimulus–response associations using discrete cues. Some examples of evidence for hippocampal competition has come from the visual platform version of the Morris water task (McDonald & White, 1994), conditioned place preference on the radial arm maze (McDonald & White, 1993; White & McDonald, 1993), and food finding along a circular track (O'Keefe et al., 1975). In each of these examples, removing hippocampal processing prior to training, accelerated learning of an association with a discrete cue under certain conditions.

The hippocampus can also interfere with the storage and retrieval of contextual fear memory stored in non-hippocampal regions (Sparks, Lehmann, & Sutherland, 2011b; Lehmann, Sparks, et al., 2009; Biedenkapp & Rudy, 2009; Wang, Teixeira, Wheeler, & Frankland, 2009; Sparks, Lehmann, & Sutherland, 2006; Wiltgen et al., 2006; Maren et al., 1997). Contextual fear involves the learned association between a context paired with a foot shock. In rodents, the behavioural expression of this memory is manifest in freezing behaviour when the subject is returned to the fearful context. This behaviour can be learned and ex-

pressed with or without the hippocampus (Sparks et al., 2011b; Lehmann, Sparks, et al., 2009; Biedenkapp & Rudy, 2009; Wang et al., 2009; Sparks et al., 2006; Wiltgen et al., 2006; Maren et al., 1997). Interference by the hippocampus on non-hippocampal systems in this task is dependent on when the learning takes place in relation to inactivation of the hippocampal system.

Removing the hippocampal system following, or prior to, contextual fear conditioning results in differential freezing when subjects are later tested for fear memory (Lehmann, Sparks, et al., 2009; Wang et al., 2009; Wiltgen et al., 2006; Maren et al., 1997; Sparks et al., 2011b). In these experiments, rats or mice were placed into a novel environment (conditioning chamber) and permitted to explore and learn about the context. Following a prescribed amount of time, a series of mild foot shocks were administered, after which the subject was removed from the environment. A number of days later, lesions were performed to impair the function of the hippocampal system. Following a period of recovery the subjects were returned to the conditioning environment to assess memory of the foot shock. Those that had received hippocampal damage exhibited profound retrograde amnesia (significantly less freezing than the intact control group). Conversely, when the same damage to the hippocampal system is induced prior to conditioning, no differences are observed in the levels of freezing—there is an absence of anterograde amnesia.

From these studies (Wiltgen et al., 2006; Maren et al., 1997; Lehmann, Sparks, et al., 2009; Sparks et al., 2011b), it is clear that the context-foot shock association can be learned and expressed by either of the hippocampal or non-hippocampal systems. Though, when the hippocampal system is present during learning, and removed before testing, the non-hippocampal system cannot support task appropriate behaviour. The dissociation between retrograde and anterograde amnesia in contextual fear conditioning is considered to be a result of interference of one memory system (hippocampus) over another (non-hippocampal) in acquiring the conditioned stimulus–unconditioned stimulus association that supports fear

behaviour.

Hippocampal interference of non-hippocampal systems at the time of learning may prevent these systems from expressing contextual fear memory when the hippocampus is removed, but this interference can be overcome (Lehmann, Sparks, et al., 2009). Training rats to fear a context by presenting multiple foot shocks within one conditioning session produces a robust contextual fear memory, though this memory does not survive damage to the hippocampus. By distributing the same number of foot shocks across multiple conditioning sessions over days, we were able to establish contextual fear memory in non-hippocampal systems that did survive hippocampal damage. Hippocampal interference during a single conditioning session inhibited non-hippocampal systems from learning the context-shock association, though when the conditioning was distributed, the non-hippocampal systems were able to incrementally gain appropriate associative strength. These data are detailed in Chapter 2, and provide evidence of how a contextual fear memory can become independent of the hippocampus for retrieval.

The interfering interaction between these systems can also occur at the time of contextual fear memory retrieval (Sparks et al., 2011a). In these experiments, we used an inactivation technique that allowed modulation of hippocampal processing during conditioning and/or testing using the same task described above. When the hippocampus was "turned off" during the conditioning session, freezing behaviour during testing depended on the hippocampus being "on" or "off". If the hippocampus was turned off once again during testing, then the non-hippocampal systems were able to express fear behaviour. Though, if the hippocampus was on during testing, it interfered with the expression of the fear memories represented in the non-hippocampal systems and produced retrograde amnesia. Evidence that the non-hippocampal system was capable of expressing the memory was provided by turning off the hippocampus during a second test which resulted in recovered freezing behaviour. Chapter 3 describes how the interference effects of the hippocampus

extend to the time of contextual memory retrieval.

Traditional views of how multiple memory systems operate cannot accommodate the described dissociation between retrograde and anterograde amnesia. Traditional theories are founded upon the premise that each memory system contains circuitry that performs a hard-wired style of processing essential in carrying out specific forms of learning (Squire, 1992; White & McDonald, 2002). Therefore, removing a system before or after learning is predicted to result in equivalent retrograde and anterograde amnesia—removing a system disables learning a certain memory, or it does not. The situation of multiple memory systems capable of learning and supporting similar task behaviour requires a more dynamic view of multiple-systems interaction, and how such a model might operate has been suggested by Fanselow (2010). According to this framework, memory systems can be viewed as primary and alternate pathways able to mediate similar behaviours. These pathways are distinct in their efficiency, primary pathways being more efficient than alternates. During learning, primary pathways may compete and interfere with (or inhibit) learning within alternate pathways. This dynamic systems interaction view is discussed in Chapter 6 in relation to the data presented in this thesis.

Together, these studies provide evidence of multiple memory systems that can interact independently or competitively. The manner in which these systems interact is dependent on the task demands placed on the rat. Together, these systems contribute to appropriate behavioural output within certain tasks. It is evident that there are types of memories that depend on the hippocampus for learning and/or retrieval, and those types that do not. For the purpose of simplicity within this thesis, the systems supporting these memories will be classified as hippocampal or non-hippocampal.

### *Context Representations*

Associative learning theories stemming from Ivan P. Pavlov (1927) and his work on the association learned between a conditional stimulus (CS) and an unconditional stimulus (US) were traditionally used to explain learning behaviour in nonhuman animals. Experiments were designed to assess the performance of animals following repeated exposure to a cue or set of cues. In formal associative learning theory, contextual information is considered the environment where learning takes place (Balsam, 1985). For example, in a simple Pavlovian conditioning experiment, the conditioning chamber itself serves as providing context information for the learning that takes place within the chamber. This chamber may be composed of many individual elements such as plexiglas walls, a dim light, metal floor, and food hopper, all of which combine to form the environment for learning. By definition, context does not need to be limited to the composition of external physical attributes of the environment, but can also be represented by internal physiological states (such as drug states (Overton, 1985; Cunningham, 1979), or even mental states (i.e., abstract concepts, episodic memory recall). One common feature within all of these examples of contexts is that they provide a *stable* environment for learning to take place.

*Context* is a word often used to describe the environment (or setting) within which something happens. Within a context, anything from specific events, statements, concepts, or ideas can be set. Often when we try to assess a particular outcome or result, we use the phrase "putting it in context," as a way of describing the process of getting an accurate picture of the situation. Doing this helps in gaining an understanding of what is being assessed. This process of contextualization seems quite natural and necessary for our understanding of the world around us. Context appears to have a strong influence in our decisions, even though most often it is not noticed.

It is not surprising that learning theorists recognized the importance of context and began investigating its influence on learning and performance (Balsam, 1985). As the interest

in the influence of context on learning grew, it was clear that traditional associative learning theories did not adequately account for its role (Hull, 1943; Skinner, 1938; Guthrie, 1935; Thorndike, 1931; Pavlov, 1927). Traditional theories needed revision because they were focused solely on the association between two elements or units, and also because of the difficulty in deciding what the elements are, even in a simple learning experiment (Balsam, 1985). Though it was not until the latter half of the twentieth century that traditional association theories were adapted to account for context, the concepts were presented as early as work by Kohler (1929). Tulving and Thomson (1973) theorized the context is encoded as part of the memory representation, and that the context is necessary for accurate subsequent memory retrieval. Similarly, Spear (1973, 1978) emphasized the importance of context on successful memory retrieval. Some of the specific examples that will be presented here of the influence context has on associative learning comes from the work of Bouton (1993, 2004) and his explanations of interference paradigms. From these theorists, it is concluded that context plays a pivotal role in accurate memory retrieval by being encoded in the memory representation. Therefore, it is often the case that context facilitates appropriate memory performance.

The stability of elements within an environment is an essential component in defining a context (Rudy, 2009; Nadel, 2008). This stability can be contrasted with the action of punctate cues—elements in the environment that occur, though not in a stable pattern. To extrapolate from an example used by Rudy (2009) in describing these features, consider the context of your office. While sitting at your desk, the features of your office are most likely stable and blend into the background (being the place that you work). The phone suddenly ringing is a punctate cue that is not stable within this environment, therefore this cue is not part of the context *per se*, rather something that happens within your office. Conversely, should for some annoying reason your phone be incessantly ringing without end, it is no longer a punctate cue but a stable feature to be added to contextual information. Therefore,

any element that remains stable within an environment is considered part of the context (i.e., be it a ring, tone, light, odour, etc.).

The nature of a context is one of stability and unsurprisingness. These features stand in stark contrast to those of punctate cues which can demand attention. Within a context, a unique CS can be associated with a US, and for all intents and purposes this association is all an animal is required to learn in a classic Pavlovian conditioning experiment (i.e., associating a tone (CS) with a foot shock (US) (Kim & Fanselow, 1992; R. Phillips & LeDoux, 1994). Though the CS-US association is often what is assessed in classic conditioning studies, the context is also involved in *incidental learning* whereby the subject learns about where the CS-US association was formed (Good, Hoz, & Morris, 1998; Honey & Good, 1993; Penick & Solomon, 1991).

As the following examples illustrate, appropriate behavioural performance can be dependent upon context. The context may act in ways to disambiguate CSs with multiple meanings, or offer a backdrop that acts to modify the expression of the CS-US association. Context can play a *facilitating* as well as *inhibiting* role in behavioural performance. Though only a few examples of how this can happen are detailed below, there is a rich literature that offers much more description of the influence context can have on conditioning.

Retrieval of certain CS-US associations has been found to be modulated by the learning and remembering contexts. Altering the internal context (physiological) can affect the retrieval of associations, causing decrements in retrieval. This is the hallmark state-dependent retrieval. A classic example of this phenomenon involved humans trained one day on memory tasks either sober or under the influence of alcohol. Tests of memory the following day showed that retrieval of those things learned the previous day was aided by the subjects being in a state congruent with that during learning (i.e., sober-sober, intoxicated-intoxicated) (Goodwin, Powell, Bremer, Hoine, & Stern, 1969). The same dependency has been observed by altering external contexts, such as learning under water or on dry land (Godden

& Baddeley, 1975). Although memory retrieval was possible in the incongruent contexts, what was common in all of these cases was the context facilitation of memory retrieval.

Contexts are stable features, or sets of features, that can facilitate/inhibit the formation of CS-US associations, as well as act to disambiguate CSs that are involved in multiple associations. These roles enable a level of expectancy within different environments, i.e., what cues can be expected in an environment, and if they are encountered, what specific meaning do they have there? Expectancy is critical in choosing appropriate behaviours given certain occasions. The role of context and expectancy in associative learning might be more complicated than the simple examples provided explain, though we now have an idea of how this can occur.

The manner in which contextual elements are represented in neural networks is theorized to occur in two orthogonal ways (Rudy, Huff, & Matus-Amat, 2004). According to the features view, the context is represented by encoding the independent features of the environment, and each of these encoded features has the capacity to be associated with an US during learning (e.g., contextual fear conditioning). Conversely, according to the conjunctive view, the independent features of the environment are bound into a unitary representation, and it is this conjunction of features that is associated with the US during learning. The neural systems responsible for encoding the elemental and conjunctive representations are thought to be neocortical systems and the hippocampus respectively (Rudy & Sutherland, 1989; Rudy & O'Reilly, 1999; O'Reilly & Rudy, 2001; Rudy & O'Reilly, 2001).

Following from this idea, contextual fear conditioning would require either one of the elemental or conjunctive representations to be associated with the foot shock. Experiments have determined that the basolateral amygdala is involved in cue-shock association (Maren & Fanselow, 1996), and both the non-hippocampal and hippocampal systems have inputs into this region. The basolateral amygdala has connections with the central amygdala,

which is responsible for driving the conditioned freezing response via the periaquaductal grey. According to this model, contextual fear conditioning can be supported by two different memory representations, the hippocampus and non-hippocampal systems.

### *Between Systems Memory Consolidation*

A crucial aspect of the initial observations made regarding HM's (as well as other human amnesics at the time) amnesia for events in the past was that it seemed to be time limited, or temporally graded. This means that memories for events that happened within the range of months to years preceding his surgery could not be recalled, though older memories such as those from his childhood seemed intact (Milner, 1959; Penfield & Milner, 1958). The pioneering work of on human amnesics following removal of the hippocampal system (Scoville & Milner, 1957; Milner, 1959; Penfield & Milner, 1958) formed a foundation for the following 50+ years of memory research. This foundation was built upon finding a physiological correlate to initial observations made by Ribot (1881). Ribot recognized that amnesic patients often exhibited a temporally graded pattern of retrograde amnesia. The temporal gradient was defined by amnesia being most severe for recently acquired memories, while remote memories seemed to be spared. Damage to the hippocampal system was formally recognized by Scoville and Milner (1957) as being responsible for this pattern of retrograde amnesia.

Patterns of temporally graded retrograde amnesia suggest that the hippocampal system has a time-limited role in the expression of certain long-term memories. The mechanism proposed to account for this pattern of amnesia is called systems consolidation (Bayley, Gold, Hopkins, & Squire, 2005; Squire & Alvarez, 1995; Moscovitch et al., 2005; Nadel & Moscovitch, 1997). Simply put, memories that are initially dependent on information processing by the hippocampal system are consolidated in non-hippocampal systems through an interaction that is dependent on the passage of time. Using this concept, theories of

long-term memory were established to account for the data. Of these theories, the Standard Model of Systems Consolidation (SMSC) (Squire & Spanis, 1984; Bayley et al., 2005; Squire & Alvarez, 1995) and Multiple Trace Theory (MTT) (Moscovitch et al., 2005; Nadel & Moscovitch, 1997) are most prominent in related literature.

Mechanisms underlying the process of systems consolidation have been speculated by numerous theorists (Squire & Spanis, 1984; Murre, 1996; McClelland, McNaughton, & O'Reilly, 1995; Alvarez & Squire, 1994), though the details of how this process happens remain unclear. The basic assumption underlying the idea of systems consolidation is that memory is stored in a distributed fashion throughout multiple memory systems, though quickly becoming established in the hippocampal system, and relatively slower in the non-hippocampal system. A set of connections within the non-hippocampal system are strengthened by a process of hippocampus-dependent reactivation, so that over a protracted amount of time these connections are strengthened to a point of no longer requiring the hippocampal system for retrieval (Meeter & Murre, 2004). This independence of non-hippocampal memories relies on a time-dependent interaction between multiple memory systems, where the hippocampus promotes memory storage in other systems.

Many labs have used animal models to directly investigate hippocampal-dependent systems consolidation processes in rats (Sutherland, O'Brien, & Lehmann, 2008; Kim & Fanselow, 1992; Anagnostaras et al., 1999; Martin, Hoz, & Morris, 2005; Lehmann, Lacanilao, & Sutherland, 2007; Maren et al., 1997; Clark, Broadbent, & Squire, 2005), and see Sutherland et al. (2010); Sutherland and Lehmann (2011) for a comprehensive review of all experiments also listed in Table 1.1. To do so, rats were trained on tasks that are known to involve hippocampal processing. Following training, the hippocampal system was removed at various training-to-surgery intervals (i.e., 1, 2, 14, 28, 180 days). If, with the passage of time, the hippocampus interacted in such a way with non-hippocampal systems to consolidate memory, then task performance at longer intervals is predicted to be better

when compared with shorter intervals (temporally graded retrograde amnesia). Rather than confirming this prediction, the vast majority of these studies observed comparable levels of amnesia at all surgery-lesion intervals (flat gradient). These results do not support the idea that a systems consolidation interaction, dependent on time, exists in the rodent brain. It is clear that not all experiments present data that show flat gradients. Table 1.1 outlines the experiments that have been performed as a direct test of long-term dependence of certain memories on the function of the hippocampus. Some of these studies show temporal gradients, as one would expect from initial observations of patients with temporal lobe amnesia (Scoville & Milner, 1957; Milner, 1959; Penfield & Milner, 1958). So, why then is there a discrepancy among these studies?

Two possibilities for the discrepancy are critically examined as part of this thesis using a contextual fear conditioning task: *a*) the systems consolidation process depends upon the strength of the initial conditioning (Chapter 4); and *b*) lesion-induced seizure activity disrupts context fear memories stored in non-hippocampal systems, particularly at remote time points (Chapter 5).

The strength of initial conditioning may affect interactions between memory systems that happen over a period of weeks to months. Examination of the contextual fear studies listed in Table 1.1 reveals that three out of four papers showing temporally graded retrograde amnesia used from 10–15 context-shock pairings during the single conditioning session (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Winocur et al., 2009). With the exception of the Maren et al. (1997) study, the remainder of the studies used a much weaker conditioning procedure with between 2–5 shocks and found a flat gradient (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008; Sparks et al., 2011b). We designed an experiment to test the possibility that conditioning strength is the factor responsible for the different patterns of retrograde amnesia. In Chapter 4, weak or strong contextual fear memory was examined at multiple surgery-to-lesion intervals allowing us to analyze any

effects of systems consolidation processes over time.

Damage to the hippocampus can cause a disruption to remote network circuitry (Jarrard & Meldrum, 1993). This has been a concern using most permanent lesion techniques including oft used selective neurotoxic lesions (McClelland et al., 1995; Liang, Ho, & Patel, 2000; Anagnostaras & Gale, 2002). Because the seizure activity can be very severe, it is difficult to determine whether retrograde amnesia found after hippocampal lesions (Kim & Fanselow, 1992; Anagnostaras, Gale, & Fanselow, 2001) is due to disruptive seizure activity propagating to non-hippocampal regions during/after lesion, or due to removal of the associated memory network established within the hippocampus. Direct evidence elucidating this possibility in retrograde amnesia studies has not been provided. Chapter 5 critically examines this issue in relation to temporally graded retrograde amnesia.

### *Hippocampal Temporary Inactivation*

While permanent lesion techniques are the norm, an alternative tool used to investigate hippocampal function is a temporary lesion. Temporary inactivation involves turning off neural transmission in discrete regions of circuitry for a prescribed period of time from as little as 15 min to as long as 2 weeks (Lomber, 1999; Riedel et al., 1999). While the tissue is inactivated, basic cellular mechanisms continue to function maintaining the health of the network so that when the network turns on, normal function may resume (Riedel et al., 1999). Though maintenance processes persist during inactivation, the overall ability of the network to encode or retrieve stored information is lost or significantly diminished. Temporary inactivations of discrete neural networks provide powerful advantages that are not possible with permanent lesion techniques, such as: (a) avoiding recovery of function, (b) flexibility in research design, and (c) control over temporal parameters (Lomber, 1999). With these techniques, the role of the hippocampus during memory acquisition, consolidation, and retrieval can be systematically investigated within specific learning and memory

tasks. It is important in considering the diverse results with temporary inactivation that in most cases the extent of hippocampal inactivation has not been established.

The use of temporary inactivations to investigate hippocampal function has a long history stretching back to pioneering work by Avis and P.L.Carlton (1968) and Hughes (1969). In these studies, temporary hippocampal spreading depression, induced by intra-hippocampal infusions of potassium chloride, was used to determine hippocampal involvement in various components of a conditioned emotional response task. Inactivating the hippocampus 24 h following training disrupted performance during a retention test 4 days later. Hughes (1969) tested this finding further by inactivating the hippocampus at multiple time points following learning (i.e., 1, 3, 7, and 21 days) and found similar results—hippocampal inactivation at recent or remote time points produced retrograde amnesia.

Since this time, a range of inactivation techniques have been used to investigate the role of the hippocampus in memory, including infusions of local anaesthetics (e.g., tetrodotoxin and lidocaine) (Atkins, Mashhoon, & Kantak, 2008; Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999), the GABAa agonist muscimol (Holt & Maren, 1999; Matus-Amat, Higgins, Barrientos, & Rudy, 2004), and AMPA/kainate receptor antagonists (Micheau, Riedel, Roloff, Inglis, & Morris, 2004; Riedel et al., 1999). In general studies using temporary inactivations of the hippocampus to investigate retrograde effects show comparable results to those found with permanent lesions. Retrograde amnesia induced by hippocampal inactivation during memory retention has been reported in the Morris water task (Broadbent, Squire, & Clark, 2006; Micheau et al., 2004; Riedel et al., 1999; Teixeira, Pomedli, Maei, Kee, & Frankland, 2006), a working memory variant of the Morris water task (Bohbot, Otahal, Liu, Nadel, & Bures, 1996), radial arm maze (Maviel, Durkin, Menzaghi, & Bontempi, 2004), conditioned emotional response (Avis & P.L.Carlton, 1968; Hughes, 1969), auditory fear conditioning (Corcoran, Desmond, Frey, & Maren, 2005; Corcoran & Maren, 2001; Hobin, Ji, & Maren, 2006; Holt & Maren, 1999; Maren &

Holt, 2000), and the Fanselow immediate shock variant of contextual fear conditioning (Biedenkapp & Rudy, 2009; Matus-Amat et al., 2004). It is important to note that most of the work done with temporary inactivation of the hippocampus has targeted, with a single bilateral infusion site, the dorsal portion of the hippocampus. The exceptions to this trend have focused on a single bilateral infusion site in the ventral hippocampus (Hobin et al., 2006), ventral subiculum (Biedenkapp & Rudy, 2009), and posterior hippocampus (Avis & P.L.Carlton, 1968; Hughes, 1969). Because hippocampal tissue spared from inactivation can maintain and express contextual fear memories (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008), it is important to inactivate as much of the structure as possible when assessing the role of the hippocampus in a specific task. To increase the degree of temporary inactivation, we target the dorsal as well as ventral hippocampus simultaneously (Sparks et al., 2011a; Gulbrandsen, Sparks, & Sutherland, 2011).

Although comparable retrograde effects are found when using permanent lesions and temporary inactivation techniques, there are a number of findings that could be interpreted as inconsistent. Most recently, Resstel, Joca, Correa, and Guimaraes (2008) found that inactivation of the dorsal hippocampus (using cobalt chloride) during testing for contextual fear memory did not cause a memory impairment. Similarly, Holt and Maren (1999) did not find an effect of dorsal hippocampal inactivation (using muscimol) during retention testing in the standard context fear paradigm. These results are not surprising considering that permanent lesions of the dorsal hippocampus need to be very extensive in order to impair expression of fear memory in a similar task. With a similarly short learning-lesion interval Lehmann, Lacanilao, and Sutherland (2007) and Lehmann, Glenn, and Mumby (2007) found only a very small effect of bilateral dorsal hippocampus damage. Apparent contradictions arise in the results of lesion methods (permanent vs. temporary) prompt the question of whether comparable amounts of the hippocampal network are affected. It is quite possible that the inactivation parameters used in the contradictory studies did

not inactivate enough of the hippocampal network to produce retrograde amnesia. Alternatively, our inactivations did not extend to include the ventral-most portion of CA1 and subiculum that have been shown, using temporary inactivations, to mediate contextual fear (Biedenkapp & Rudy, 2009).

Given the advantages of temporary inactivation of the hippocampus, it is interesting that its application has been quite limited, not widely applied to diverse memory tasks at multiple time points after a learning episode. This limit could result from inadequate characterization of the temporal, spatial, and physiological effects of inactivations (i.e., duration of inactivation, extent and spread of network inactivation, and possible long-term physiological modifications due to inactivation). It will be necessary to elucidate optimal parameters of inactivating the hippocampus, possibly through electrophysiological recording and/or imaging of immediate early gene activity (Kubik, Miyashita, & Guzowski, 2008). There is currently good agreement in the results of retrograde amnesia experiments using permanent or temporary inactivation, but the range of parameters for comparison is too small to be definitive. Chapter **??** details a set of temporary inactivation techniques and discusses the use of the GABA agonist muscimol in inhibiting hippocampal activity.

### *Conclusions and Thesis Organization*

The focus of this thesis is on the interaction of the hippocampus with non-hippocampal memory systems and how this interaction affects contextual fear memory retrieval. Contextual fear behavioural tasks provide a robust learning and memory paradigm, that can instantiate memories that can be recalled weeks if not months after learning. Because of the involvement of the hippocampus in context memory, we can use the contextual fear conditioning task to test the involvement of the hippocampus in the retrieval of different ages of context memory. The experiments described in this thesis were designed to investigate two things: *a*) the effects of hippocampal interference at the time of learning as

well as the time of remembering; and *b*) the time limited role of the hippocampus in the retrieval of contextual fear memory. These experiments combine to support the idea that the hippocampus is permanently involved in context memory processes.

With the exclusion of Chapter 6, the remainder of this thesis presents completed experimental projects that are written in manuscript format. Each experimental chapter is organized to stand alone for a reader interested in one set of experiments and results; therefore, some information between chapters will be redundant. Data, figures, and text that have already been published elsewhere are included in this thesis under the explicit written consent of the copyright holder.

To reiterate the chapter contents, Chapter 2 presents data that detail how learning parameters can overcome hippocampal interference during learning to create a hippocampal-independent contextual fear memory, and discusses the finding in light of traditional views of hippocampal function. Chapter 3 provides data that support the concept of hippocampal interference (overshadowing) during learning and remembering. Evidence is presented that supports the idea that hippocampal interference of memory acquisition in non-hippocampal systems can also extend to the time of memory retrieval. Appendix 1 provides both a quantitative assessment of the effects of using muscimol to temporarily block hippocampal activity, and the methods developed to utilize this technique. These techniques were developed and assessed to perform the experiments in Chapter 3.

Chapter 4 presents data that reveal that memory strength at the time of learning does not influence hippocampal dependence at the time of remembering. These data, along with the following chapter, add support to current trends in hippocampal research. Chapter 5 presents data that show that hippocampal lesion-induced seizure activity does not differentially disrupt recently acquired contextual fear memory when compared to much older memory. A flat gradient of retrograde amnesia is described. Last, Chapter 6 concludes by placing these experimental results within the context of the current state of hippocampal

memory research, and the implications for leading theories of memory.

# Chapter 2

## Making Context Memories Independent of the Hippocampus


## Abstract

We present evidence that certain learning parameters can make a memory, even a very recent one, become independent of the hippocampus. We confirm earlier findings that damage to the hippocampus causes severe retrograde amnesia for context memories, but we show that repeated learning sessions create a context memory that is not vulnerable to the damage. The findings demonstrate that memories normally dependent on the hippocampus are incrementally strengthened in other memory networks with additional learning. The latter provides a new account for patterns of hippocampal retrograde amnesia and how memories may become independent of the hippocampus.[1]


## Introduction

Contextual fear conditioning can be supported by two neural systems, one that contains the hippocampus, and one that does not. Evidence for this assertion comes from studies in which the hippocampus, in rats, is damaged either before or after the contextual fear conditioning. Extensive damage to the hippocampus before conditioning has little effect on contextual fear conditioning (Maren et al., 1997; Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998; Wiltgen et al., 2006). This result can only mean that there is a non-hippocampal memory system that can support fear of context. In contrast, there is unequivocal evidence that moderate to extensive damage to the hippocampus soon after learning severely impairs the ability of the conditioning context to evoke fear, suggesting that the hippocampus normally makes a major contribution to this type of memory (Kim

[1] Chapter published as: Lehmann, H., Sparks, F.T., Spanswick, S.C., Hadikin, C., McDonald, R.J., & Sutherland, R.J. (2009). Making context memories independent of the hippocampus. *Learning & Memory, 16:417–420.*

& Fanselow, 1992; Maren et al., 1997; Frankland et al., 1998; Anagnostaras et al., 1999; Debiec, LeDoux, & Nader, 2002; Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008; Wang et al., 2009).

The dissociable effects of pre- and post-training hippocampus damage on contextual fear conditioning have been interpreted as suggesting that: (1) When the hippocampus is intact during learning it interferes with other systems and prevents them from acquiring an independent contextual fear conditioning memory, and (2) when the hippocampus is absent, these other systems are released from this interference and are able to rapidly acquire an independent memory (Maren et al., 1997; Frankland et al., 1998; Fanselow & Poulos, 2004; Driscoll, Howard, Prusky, Rudy, & Sutherland, 2005; Lehmann, Sparks, et al., 2006; Sutherland et al., 2006). The latter interference from the hippocampus on the other memory systems has been termed overshadowing. Figure 2.1 depicts data from our laboratory demonstrating the overshadowing phenomenon and the dissociable effects of hippocampus damage induced before and after contextual fear conditioning.

Very little, however, is known about the parameters determining the extent to which the hippocampal system interferes with the non-hippocampal system for control over contextual fear. The purpose of the current study is to provide some insight into this issue. Typically, contextual fear conditioning in rats is conducted in a single conditioning session in which a configuration of static background cues is paired with several foot shocks. When returned to the conditioning context, rats display several species specific defensive responses including freezing (i.e., absence of movement except for breathing). Several theorists have proposed that non-hippocampal systems are more likely to be recruited when there are multiple experiences with similar events, which, in turn, would mitigate the necessity of the hippocampus for memory expression (O'Keefe & Nadel, 1978; Sherry & Schacter, 1987; McClelland et al., 1995; O'Reilly & Rudy, 2001; White & McDonald, 2002). Accordingly, we hypothesized that repeated contextual fear conditioning sessions

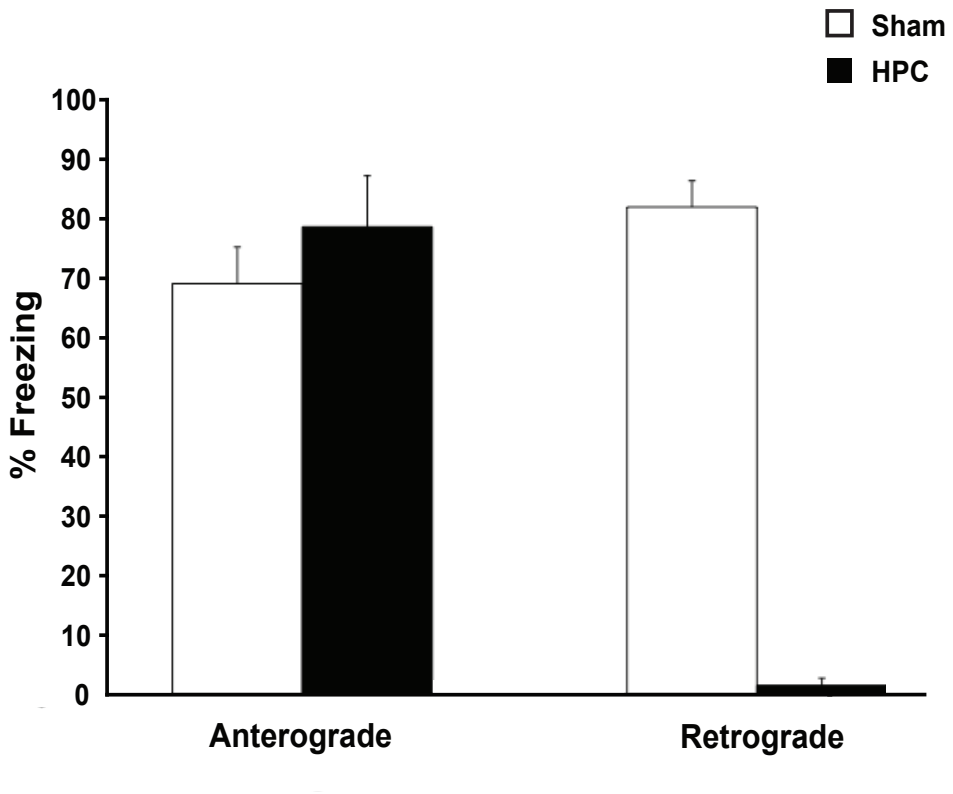


Figure 2.1: **Evidence of Overshadowing.** Complete hippocampus damage inflicted 1–3 days after a single contextual fear conditioning session (post-training / Retrograde) caused profound freezing deficits during the retention test ($p < 0.05$), suggesting that hippocampus damage causes retrograde amnesia. In contrast, complete hippocampus damage inflicted before (pre-training / Anterograde) a single contextual fear conditioning session did not impair freezing on the retention test ($p > 0.05$), suggesting that despite the absence of the hippocampus the rats are able to acquire and retain the memory (absence of anterograde amnesia). Importantly, the single conditioning session in both instances involved the same procedures and learning parameters (2 shocks over a 7 min session). Combined, these findings imply that non-hippocampal systems can rapidly acquire and retain a contextual fear conditioning memory, but that normally an intact hippocampus acquires and retains the memory by overshadowing or interfering with the non-hippocampal systems.

separated by hours and days would overcome the hippocampal interference or overshadowing effect. In other words, with repeated learning sessions, enough information would be incrementally captured by the non-hippocampal system to support a contextual fear memory that would survive complete damage to the hippocampus.

## Materials and Methods

### *Subjects*

The University of Lethbridge Animal Care Committee approved all procedures in accord with the guidelines set by the Canadian Council on Animal Care. Subjects were male Long-Evans rats (350 g) obtained from a commercial supplier (Charles River Laboratories, Inc., QC, Canada). Rats were housed in standard laboratory cages in a room with an ambient temperature of 21°C, 35% relative humidity, 12/12 hr light/dark cycle (lights on at 07:00), and were provided with food and water ad libitum. Behavioural testing was conducted during the light phase of the cycle.

### *Surgery*

Rats were anesthetized by isoflurane inhalation (Janssen, Toronto, ON, Canada) (3.5% with 1 litre/min oxygen, reduced to 1% after a surgical plane was established) and administered an analgesic (buprenorphine, 0.07 cc., 0.3 mg/ml i.p.; Reckitt & Colman, Richmond, VA, USA). They were then placed in a stereotaxic frame (Kopf instruments, Tujunga, CA, USA), a midline scalp incision was made, and periosteum excised to expose the top of the skull. Small burr holes were drilled through the skull using anterior/posterior and medial/lateral coordinates relative to Bregma as detailed in Table 4.1. The HPC lesions were made by intra-HPC infusions of N-methyl-D-aspartic acid (NMDA; 7.5 $\mu$g/$\mu$l in 0.9% saline; Sigma Chemical Co., St. Louis, MO, USA) at 10 sites bilaterally (see Table 4.1 for

coordinates). The infusions were done sequentially through a 30-guage injection cannula attached to a 10 $\mu$l Hamilton syringe (Hamilton Co., Reno, NV, USA) via polyethylene tubing (PE-50; Small Parts Inc., Lexington, KY, USA), which were attached to a micro-infusion pump (Harvard Apparatus, South Natick, MA, USA). A total volume of 0.4 $\mu$l was infused at a flow rate of 0.15 $\mu$l per minute. The injection needle was left in place for an additional 2.5 min following the injection to facilitate diffusion. Following the infusions, the scalp incision was closed using sutures. As the rats recovered from the anaesthetic, a prophylaxis against seizures was administered (diazepam; 0.2cc; 10mg/ml, i.p.; Sabex, Boucherville, QC, Canada). The same surgical procedures were used for the Sham rats except that no damage was done to the skull or brain. The rats were allowed to recover for a minimum of 10 days before subsequent behavioural procedures.

Table 2.1: Coordinates used for 10-site hippocampus lesion in adult male rat (measurements in millimetres relative to bregma).

| **Site** | **Anteriorposterior** | **Mediolateral** | **Dorsoventral** |
|---|---|---|---|
| 1 | –3.0 | ±1.0 | –3.6 |
| 2 | –3.0 | ±2.0 | –3.6 |
| 3 | –4.0 | ±2.0 | –4.0 |
| 4 | –4.0 | ±3.5 | –4.0 |
| 5 | –4.9 | ±3.0 | –4.1 |
| 6 | –4.9 | ±5.2 | –5.0 |
| 7 | –4.9 | ±5.2 | –7.2 |
| 8 | –5.7 | ±4.4 | –4.4 |
| 9 | –5.7 | ±5.1 | –6.0 |
| 10 | –5.7 | ±5.1 | –7.3 |

### *Apparatus*

Conditioning and testing were carried out in four identical observation chambers (30 x 24 x 21 cm; MED-Associates, Burlington, VT, USA). The chambers were constructed from aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door) and were situated in cabinets located in a brightly lit and isolated room. The floor of each chamber

consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (centre to centre). Rods were wired to a shock source and solid-state grid scrambler (MED-Associates) for the delivery of footshock USs. The chambers were wiped with dilute cleaner disinfectant (to which the rats were nave) and stainless steel trays cleaned with the same solution were placed underneath the grid floors. Ventilation fans in each cabinet supplied background noise (65 dB, A scale).

### *Behavioural Procedures*

Rats received 11 fear conditioning sessions across 6 d. In each session, they were placed in a context and received mild foot shocks (Shock Context). Concurrently, the rats were exposed 10 times to another context in which they never received shock (No-Shock Context). The No-Shock Context served as a control condition to measure whether the rats simply showed generalized fear or could show context-specific memory. Within 72 h following the last conditioning session, rats either received sham surgery or complete lesions of the hippocampus using the neurotoxin N-methyl-D-aspartic acid (NMDA) (Lehmann, Clark, & Whishaw, 2007). Rats were then tested for retention in both the Shock and No-Shock Contexts in a counterbalanced order. In addition, in a single learning episode, another group of rats received a matching number of shocks (i.e., 12 shocks) and context exposure (i.e., 17 min), and then received surgery 7–10 d after conditioning. The latter interval is identical to the interval between the initial conditioning session and surgery in the repeated learning condition. Behaviour while in the conditioning context was digitally recorded using FreezeFrame Video-Based Conditioned Fear System and analyzed by Actimetrics Software (Coulbourn Instruments, Wilmette, IL, USA) for average freezing times. Freezing was defined as the absence of movement except for that due to respiration. Also, the amount of time spent freezing during the tests for each rat was converted to a percent freezing score ([time freezing/test time]X100). These procedures were used for all experiments.

Figure 2.2 illustrates and describes the design of the experiments.

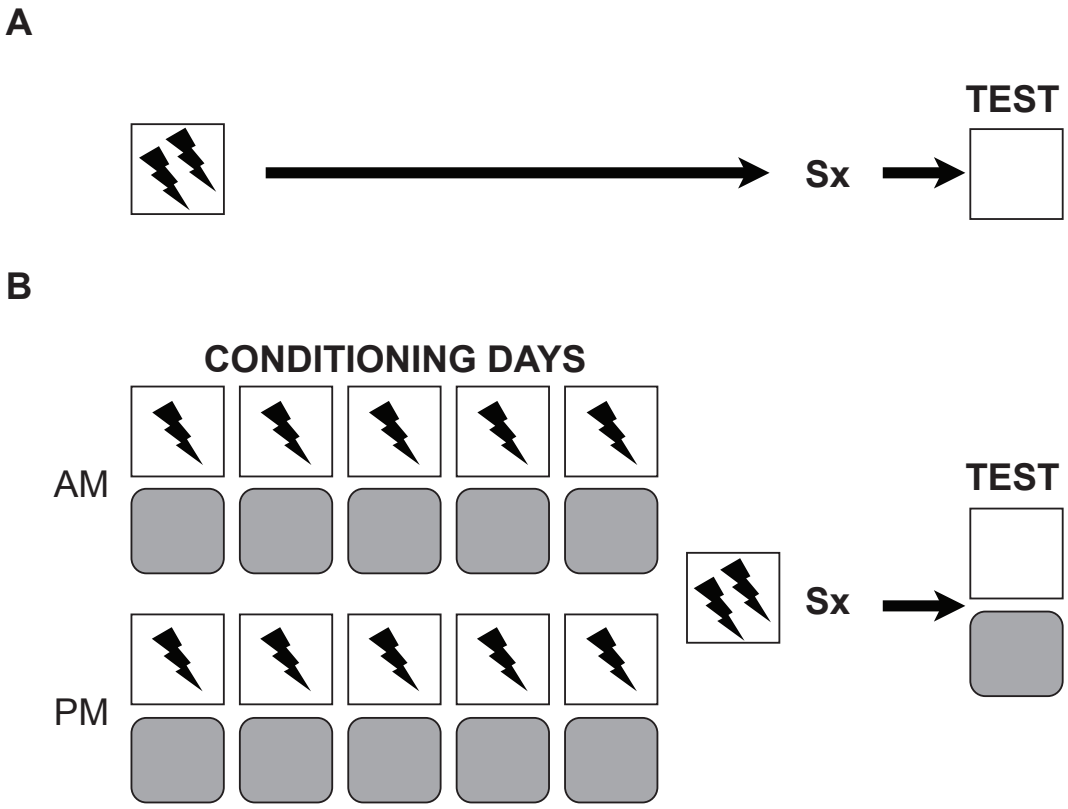


Figure 2.2: **Experimental Design.** Illustration of the experimental design used in (A) the single conditioning session and (B) repeated conditioning session experiments. In A the rats were initially placed in the conditioning chamber for 17 min and received the first of 12 foot shocks (1 mA/2 sec) at the 300-sec mark, and then one following every 58 sec after shock offset. Seven to 10 d later, the rats were returned to the chamber to assess freezing over a 5-min retention test. In B the rats were placed initially in the conditioning chamber for 1 min and received a shock at the 45-sec mark (Shock Context). Approximately 45 in later, the rats were placed in a different chamber for 1 min and did not receive shock (No-Shock Context). The procedure was repeated twice daily for five consecutive days, and the Shock and No-Shock chamber order was counterbalanced according to the principles of a Latin Square design. The rats then received sham or hippocampal damage 1–3 d later. The rats' retention was assessed in both contexts 10 d after surgery in both the Shock and No-Shock Context in a counterbalanced order with a 24-h span between tests. Importantly, the number of shocks, context exposure time, and interval between initial learning and surgery were matched between both experiments.

### *Histology*

After completion of behavioural testing, all animals were sacrificed by administering an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.9% phosphate buffered saline, followed by 4% paraformaldehyde in 0.9% phosphate buffered saline. The brains were removed and post-fixed for 24 hr in paraformaldehyde, then transferred and stored in 30% sucrose and 0.9% phosphate buffered saline with sodium azide (0.02%) for at least 48 hr before sectioning. The brains were sectioned in the coronal plane 40 $\mu$m thick using a cryostat microtome (-19°C); every fourth section taken throughout hippocampus in the permanent lesion groups, and every section taken proximal to cannula tracks in temporary lesion groups. Sections were wet-mounted on glass microscope slides and later stained with cresyl violet for visualization of hippocampal lesion induced damage, or cannula and injector placement. The amount of damage to the hippocampus in Experiment 1 was assessed using the Cavalieri method (Schmitz & Hof, 2005) to calculate the volume of spared principal sub-fields (CA fields and dentate gyrus). The volume of spared sub-fields was then divided by volume estimates from an intact control group to calculate percent lesion.

## Results

### *Behaviour*

#### *Distributed Conditioning Sessions Spared Memory Following Hippocampal Damage*

When all shocks were delivered in a single session, hippocampus damage caused profound retrograde amnesia. As illustrated in Figure 2.3A, the hippocampal rats displayed significantly less freezing than control rats during the retention test ($t_8 = 23.895, p < 0.001$). This result replicates all previous studies in which the hippocampus was damaged days after a

single contextual fear conditioning training session (Kim & Fanselow, 1992; Maren et al., 1997; Frankland et al., 1998; Anagnostaras et al., 1999; Debiec et al., 2002; Lehmann, Clark, & Whishaw, 2007; Sutherland et al., 2008).

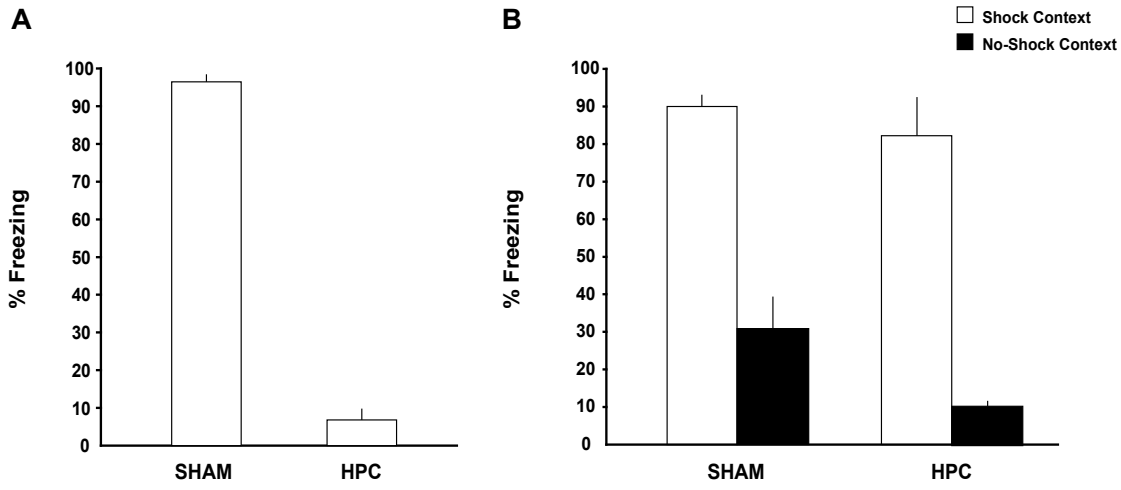


Figure 2.3: **Retention Test.** Mean (± SEM) percent time freezing by Sham and Hippocampal rats during the retention test of the (A) single conditioning (12 shocks) experiment and (B) repeated conditioning session experiment. In A the Hippocampal rats showed significantly less freezing ($p < 0.001$) that the Sham rats, suggesting that the damage caused profound retrograde amnesia for contextual fear conditioning learned in a single session 7–10 d before surgery. In B the performance of the Hippocampal rats did not significantly differ from the Sham rats, and they exhibited significantly more freezing in the Shock Context than the No-Shock Context ($p < 0.001$). Consequently, repeated conditioning sessions prevented the retrograde amnesic effects normally observed in contextual fear conditioning following hippocampal damage, suggesting that other neural networks were now able to support the memory.

In striking contrast, memory for contextual fear conditioning was spared when the hippocampus was damaged after repeated conditioning sessions. Figure 2.3B shows the percent time spent freezing during the retention test in the Shock and No-Shock Contexts. An ANOVA with between-group factor (Lesion: Sham and Hippocampal) and within-group factor (Context: Shock and No-Shock) revealed a significant main effect of Context ($F_{1,14} = 84.731, p < 0.001$), indicating that the rats displayed higher levels of freezing in the Shock than in the No-Shock Context. The effect of Lesion ($F_{1,14} = 4.280, p = 0.058$)

was not significant, nor was the Lesion x Context interaction ($F_{1,14} = 0.877, p = 0.369$), suggesting that extensive hippocampus damage did not impair memory. The tendency for an effect of Lesion is due to the Hippocampal rats freezing less than the Sham rats in the No-Shock Context ($p = 0.06$) rather than freezing less in the Shock Context ($p = 0.457$).

### *Hippocampal Damage Does Not Impair Ability to Discriminate Contexts*

The repeated conditioning sessions clearly enabled a contextual fear representation to be established in non-hippocampal memory systems. However, it is surprising that the hippocampus damage did not impair the ability to discriminate between the Shock and No-Shock Context, because evidence suggests that context discrimination is dependent on the hippocampus (see Moscovitch, Nadel, Winocur, Gilboa, and Rosenbaum (2006)). Indeed, studies of rats with hippocampus damage induced before learning have shown that contextual fear conditioning is acquired quickly by non-hippocampal systems in a single session, but the ability to discriminate between the training context and a new context is lost (Frankland et al., 1998; Antoniadis & McDonald, 2000; Winocur, Moscovitch, & Sekeres, 2007). Hence, it is significant in the present study that the hippocampus damage did not impair context discrimination abilities in the rats that received repeated learning episodes. The latter appear to have established a context representation, outside of the hippocampus, that was not bereft of details. Yet, one should consider that the rats in the repeated sessions experiment received experience in both the Shock and No-Shock Contexts prior to surgery, and this discrimination training procedure may have established two different non-hippocampal representations. It remains possible that hippocampus damage would impair the ability to discriminate the Shock Context from a new context, which is what is found in anterograde amnesia studies (Frankland et al., 1998; Antoniadis & McDonald, 2000; Winocur et al., 2007). To address this possibility, a new experiment examined whether hippocampus damaged rats could discriminate the Shock Context from a Novel Context.

Rats were trained with the same repeated learning protocol as described earlier, with the exception that the rats were never placed in the No-Shock Context prior to surgery. One to 3 d following learning, the rats either received Sham or complete hippocampus damage. They were then tested for retention in the Shock and the Novel (i.e., No-Shock) Context in a counterbalanced order. Figure 2.4 shows the percent time spent freezing during the retention test in the Shock and Novel Contexts. An ANOVA with between-group factor (Lesion: Sham and Hippocampal) and within-group factor (Context: Shock and Novel) revealed that the rats froze significantly more in the Shock than the Novel Context ($F_{1,10} = 57.393, p < 0.001$). However, no significant difference was found between the Hippocampal and Sham groups ($F_{1,10} = 0.597, p = 0.458$) and the Lesion x Context interaction did not reach significance ($F_{1,10} = 0.123, p = 0.733$). Thus, as in the previous repeated sessions experiment, the hippocampus damage did not cause retrograde amnesia for contextual fear conditioning and, more importantly, the hippocampus damage did not impair the ability to discriminate between the original context and new context.

### *Histology*

The absence of amnesia for contextual fear conditioning in the current study is not due to insufficient damage to the hippocampus. We calculated (see Lehmann, Lacanilao, and Sutherland, 2007) that an average of 83% of the hippocampus was damaged across rats (smallest: 64%; largest: 90%) in the repeated learning experiments (see Table 2.2 for histological details). The amount of hippocampus damage is substantially more than that found in most studies reporting impairments for contextual fear conditioning following hippocampus damage (Kim & Fanselow, 1992; Maren et al., 1997; Frankland et al., 1998; Anagnostaras et al., 1999; Debiec et al., 2002) and more than for the single-session experiment (average 76%) in which we currently report amnesia. Therefore, the amount of hippocampus damage inflicted in the rats in this study is certainly sufficient to disrupt

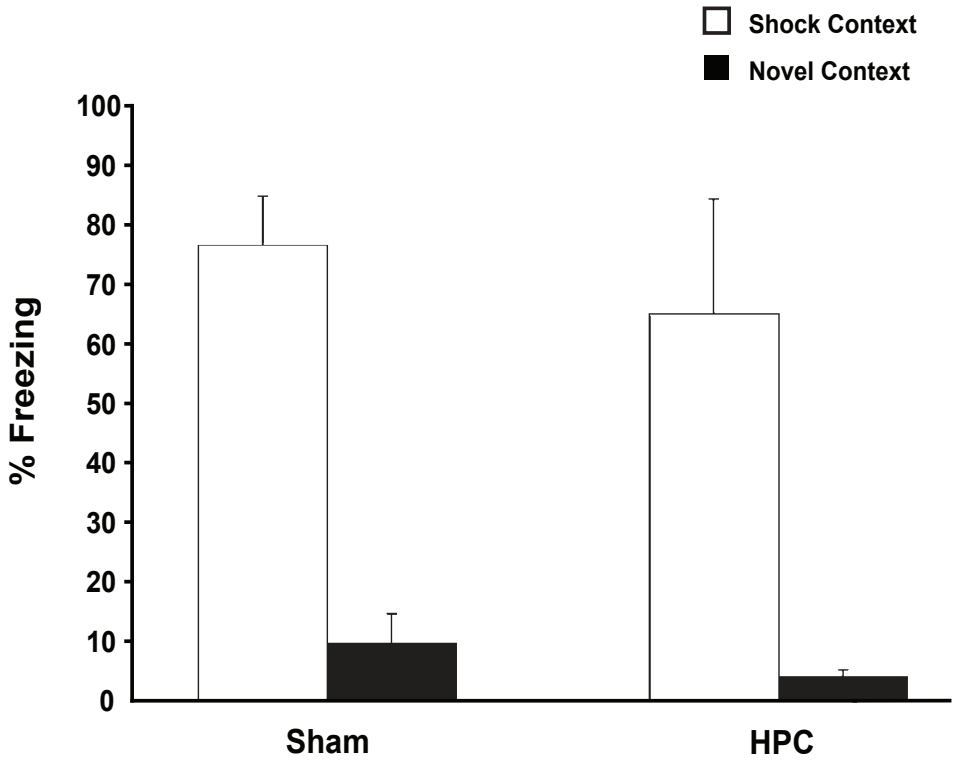


Figure 2.4: **Discrimination Retention Test.** Mean ($\pm$ SEM) percent time freezing by Sham and Hippocampal rats in the Shock and Novel Contexts during the retention tests of the discrimination experiment. The rats exhibited significantly more freezing in the Shock than the Novel Context ($p < 0.001$), and the Hippocampal rats did not significantly differ from the Sham rats, suggesting that the Hippocampus damaged rats remembered the specific meaning of the Shock Context as well as control rats. Hence, repeated conditioning sessions established a context-rich representation in non-hippocampal systems, which supports successful context discriminations.

hippocampus-dependent memories.

Figure 2.5 illustrates the smallest and largest amount of HPC damage for the rats across the four experiments and Table S1 depicts the HPC damage estimates in each experiment. In sum, the NMDA produced significant cell loss in all principal subfields of the HPC, as well as in the dentate gyrus and the most anterior part of the ventral subiculum in each HPC rat. Across rats, the damage was consistent throughout the entire septo-temporal extent of the HPC. Minor sparing was most often found in of the most ventro-temporal part of the HPC. All rats sustained minor damage to the cortex overlying the HPC at the points where the injection cannulae were inserted. Some rats also sustained minor damage to fimbria/fornix. No damage was noticed in the thalamus, amygdala, or rhinal cortex.

Table 2.2: Estimate of the percent hippocampus damage in each experiment.

| **Experiment** | **Smallest** | **Largest** | **Average** |
|---|---|---|---|
| 1 - Repeated sessions | 63.65 | 88.34 | 81.65 |
| 2 - Single session | 58.97 | 89.95 | 76.41 |
| 3 - Discrimination | 64.95 | 90.22 | 83.70 |

## Discussion

Like others (Kim & Fanselow, 1992; Maren et al., 1997; Frankland et al., 1998; Anagnostaras et al., 1999; Debiec et al., 2002; Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008), we found that damage to the hippocampus after a single contextual fear-conditioning session involving multiple shocks produces profound retrograde amnesia for contextual fear conditioning. However, in two separate experiments, distributing shock across multiple conditioning sessions prevented this amnesia. In one case, the rats experienced ContextShock pairings in one context and no shock in another context. Following this training, rats with damage to the hippocampus did not differ from control rats in the absolute amount of freezing in the training context nor in their ability to discriminate between

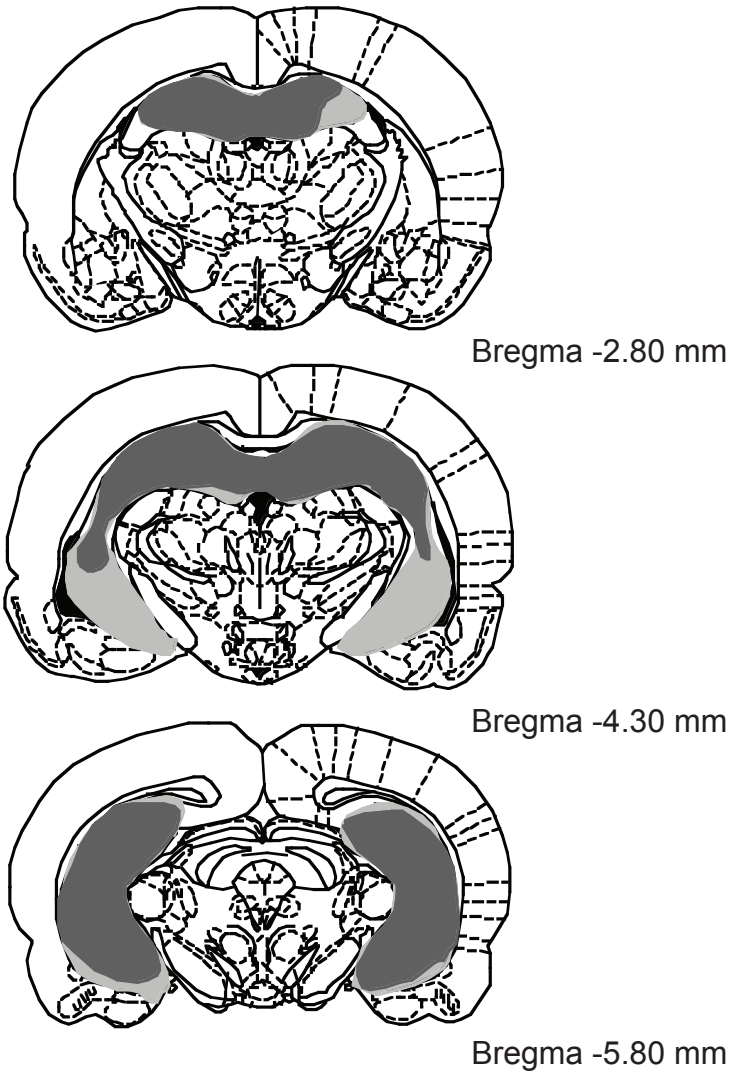


Figure 2.5: **Histology.** Illustrations of the smallest (dark grey) and largest (light grey) lesion observed bilaterally through the rostral and caudal extent of the HPC across experiments in the current study.

the two contexts. In the second case, rats only received the multiple ContextShock sessions. Rats with damage to the hippocampus could not be distinguished from control rats during the test in the training context or in their responses to a novel context. These findings provide new support for the general idea that contextual fear conditioning can be supported by both hippocampal and non-hippocampal systems. This conclusion is supported by (1) the finding that damage to the hippocampus following a single conditioning session virtually eliminates freezing during the test, implying the importance of the hippocampal system, and (2) that following multiple conditioning sessions, damage to the hippocampus has no effect on either contextual fear displayed in the training context or their ability to discriminate the training context from other contexts, suggesting the existence of non-hippocampal systems that can support contextual fear. The findings also reveal that the overshadowing or interference by the hippocampus over the non-hippocampal memory systems for control over contextual fear is not absolute. Following a single conditioning session, removal of the hippocampus produced a devastating retrograde amnesia, illustrating substantial overshadowing. However, distributing conditioning across several sessions completely attenuated the effects of damage to the hippocampus, revealing that non-hippocampal systems can support contextual fear conditioning despite the hippocampus, and revealed the importance of multiple sessions for this to occur.

The overshadowing by the hippocampus is based on the familiar idea in associative learning at the behavioural level, where through a competitive process some of the cues that redundantly predict a reinforcer acquire the ability to generate strong conditioned responding, while other equally predictive, but less salient cues do not (Sout, Arcediano, Escobar, & Miller, 2003). Conditioning to the less potent cues proceeds more effectively if the more potent competitors are absent. Following the same principle, if the hippocampal representation is active, then learning in the non-hippocampal systems suffers strong interference. In contrast, in the absence of the hippocampal representation, learning in non-

hippocampal systems is released from this interfering effect of the hippocampus. Thus, the learning rate in non-hippocampal networks is potently lowered by the activity of the hippocampus. However, with repeated learning, other structures, which are overshadowed by the hippocampus, may cumulatively build a representation that achieves hippocampal independence. The current findings clearly support this hypothesis, whereby repeated learning episodes incrementally established a contextual fear-conditioning representation outside of the hippocampus that mitigated the usual retrograde amnesic effects of hippocampus damage.

One important question is where does the hippocampus interference occur? Biedenkapp and Rudy (2009) recently reported that the hippocampus competes with the basolateral region of the amygdala during fear conditioning. Previously, Guarraci, Frohardt, and Kapp (1999) found that the amount of conditioned fear produced by training could be increased if the dopamine D1 receptor agonist SKF82958 was injected into the basolateral region. Biedenkapp and Rudy (2009) reasoned that if this is the area where the hippocampus interferes with non-hippocampal systems for the association with shock, then a local infusion of SKF82958 before a single session of contextual fear conditioning should attenuate the interference and allow the non-hippocampal system to gain more control over contextual fear. Their data supported this hypothesis, which leads to the possibility that with multiple conditioning sessions, the non-hippocampal system gradually gains association with these fear-supporting neurons in this region of the brain.

Patients with bilateral damage to the hippocampus often exhibit temporally graded retrograde amnesia, such that recently acquired memories are lost, whereas remote memories, especially those acquired years before the damage, are more likely to be spared (Scoville & Milner, 1957; Rempel-Clower, Zola, Squire, & Amaral, 1996). This pattern of amnesia is taken as evidence for temporally based systems consolidation, whereby over time the essential support for memories is "switched" from dependence on the hippocampus to

neocortical networks (McClelland et al., 1995; Squire & Alvarez, 1995; Anagnostaras et al., 2001; Meeter & Murre, 2004; Squire, Stark, & Clark, 2004; Wiltgen, Brown, Talton, & Silva, 2004; Frankland & Bontempi, 2005). Our research, however, points to another process for becoming independent of the hippocampus, a change in the strength of the representation in non-hippocampal systems during learning rather than a consolidation process linked to the passage of time since the learning episode. A study of a former London taxi driver with bilateral hippocampus damage alludes to this possibility (Maguire, Nannery, & Spiers, 2006). This amnesic patient showed greater retrograde amnesia for roads that he used less commonly than the major arteries that he used regularly. Hence, greater exposure to the major arteries established memories in non-hippocampal systems, whereas roads with less exposure remained dependent on the hippocampus regardless of the age of the memory. Our findings add support to this view, because studies examining the effects of complete hippocampus damage after a single conditioning episode suggest that the hippocampus is permanently involved in contextual fear conditioning (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008); yet, with repeated learning episodes we clearly demonstrated that the memory rapidly becomes independent of the hippocampus. The latter is important because the process for memories becoming independent of the hippocampus need not require systems consolidation.

In conclusion, this is the first example of intact contextual fear memories following complete hippocampus damage induced soon after learning. Importantly, repetition of the learning episode underlies the change in memory from hippocampus dependent to hippocampus independent. We argue that each learning episode incrementally establishes a representation in non-hippocampal memory systems—a representation that ultimately becomes sufficiently strong to support memory expression without the hippocampus. The current findings also demonstrate the critical need to consider learning parameters when discussing patterns of retrograde amnesia and the role of the hippocampus in memory.

# Chapter 3

## Between-Systems Memory Interference During Retrieval


## Abstract

Context memories normally depend on the hippocampus (HPC), but, in absence of the HPC, other memory systems are capable of acquiring and supporting these memories. This suggests that the HPC can interfere with other systems during memory acquisition. Here we ask whether the HPC can also interfere with the retrieval of a context memory that was independently acquired by a non-HPC system. Specifically, we assess whether the HPC can impair the retrieval of a contextual fear conditioning memory that was acquired while the HPC was temporarily inactive. Rats were infused with the GABAA receptor agonist muscimol in the dorsal and ventral HPC either before acquisition, retrieval, or prior to both acquisition and retrieval. Consistent with the effects of permanent HPC lesions on contextual fear conditioning, if the HPC was inactive at both the time of acquisition and retention, memory was intact. Thus, non-HPC systems acquired and supported this memory in absence of the HPC. However, if the HPC was inactive during acquisition but active thereafter, rats displayed severe deficits during the retention test. Moreover, when the same rats received a second retention test but with the HPC inactive at this time, the memory was recovered, suggesting that removal of a form of interference allowed the memory to be expressed. Combined, these findings imply that the HPC competes and/or interferes with retrieval of a long-term memory that was established in non-HPC systems.[1]


[1]Chapter published as: Sparks, F.T., Lehmann, H., & Sutherland, R.J. (2011). Between-systems memory interference during retrieval. *European Journal of Neuroscience, 34:780-786*.

## Introduction

Recent experiments suggest that the hippocampus can interfere with mnemonic processes in non-hippocampal systems (Maren et al., 1997; Sutherland et al., 2006; Wiltgen et al., 2006; Driscoll et al., 2005; Frankland et al., 1998; Lehmann, Sparks, et al., 2009). In several tasks, hippocampus damage causes severe retrograde amnesia, but not anterograde amnesia (Sutherland et al., 2010). For instance, damage to the hippocampus typically impairs contextual fear conditioning when the damage is caused after, but not before training. This dissociation suggests that non-hippocampal systems can readily acquire and express context fear if the hippocampus is permanently damaged prior to training, even in tasks that normally require the hippocampus. Furthermore, the lack of anterograde amnesia despite severe retrograde amnesia implies that the hippocampus can prevent the non-hippocampal systems from acquiring and maintaining an independent memory that supports task performance.

If the hippocampus can interfere with acquisition of an independent memory in non-hippocampal systems, then it is reasonable to ask whether the hippocampus may also interfere with retrieval of a normally hippocampus-dependent memory that has been established in non-hippocampal systems. Is it possible that retrieval of a context memory, established in non-hippocampal systems at a time when the hippocampus is inactive, would be disrupted when the hippocampus resumes normal functioning? Evidence from temporary hippocampal inactivation experiments suggests that it may be the case, but the issue has not been fully examined. For instance, disruption or inactivation of the ventral hippocampus prior to fear conditioning can cause anterograde amnesia (Zhang, Bast, & Feldon, 2001; Bast, Zhang, & Feldon, 2001). Though the main conclusion from these findings is that the amnesia is a result of a failure to establish a lasting fear memory, the possibility that non-hippocampal regions may have formed fear memory that is interfered with at the time of retrieval is not addressed.

Interestingly, permanent lesions of the hippocampus, contrary to inactivations, do not cause anterograde amnesia for context fear memories and the discrepant pattern of amnesia may be accounted for by an hippocampus interference account. Unlike in the permanent lesions studies, the hippocampus is back to normal at the time of test in these inactivation studies and may be interfering with memory retrieval from the non-hippocampal systems. This possibility was examined in the current study by reversibly inactivating the hippocampus of rats prior to acquisition, retention, or both acquisition and retention in a contextual fear-conditioning task. The key manipulation to demonstrate the retrieval interference effect, however, was made by directly assessing, in a second retention test, whether removing the hippocampal-retrieval interference restored the memory.

## Materials and Methods

### *Subjects*

The University of Lethbridge Animal Care Committee approved all procedures in accord with the guidelines set by the Canadian Council on Animal Care. Subjects were 17 female Long-Evans rats (250-300 g) (Experiment 1) obtained from the Canadian Centre for Behavioural Neuroscience vivarium (University of Lethbridge, Lethbridge, AB, Canada) and 46 male Long-Evans rats (325-350 g) (Experiments 2) obtained from a commercial supplier (Charles River Laboratories, Inc., QC, Canada). Rats were housed in standard laboratory cages in a room with an ambient temperature of 21°C, 35% relative humidity, 12/12 hr light/dark cycle (lights on at 07:00), and were provided with food and water ad libitum. Behavioural testing was conducted during the light phase of the cycle.

### *Surgery*

#### *HPC Lesions*

Rats were anesthetized by isoflurane inhalation (Janssen, Toronto, ON, Canada) (3.5% with 1 litre/min oxygen, reduced to 1% after a surgical plane was established) and administered an analgesic (buprenorphine, 0.017 mg/kg, s.c.; Reckitt & Colman, Richmond, VA, USA). They were then placed in a stereotaxic frame (Kopf instruments, Tujunga, CA, USA), a midline scalp incision was made, and periosteum excised to expose the top of the skull. Small burr holes were drilled through the skull using the anterior/posterior and medial/lateral coordinates in Table 3.1. The HPC lesions were made by intra-HPC infusions of N-methyl-D-aspartic acid (NMDA; 7.5 $\mu$g/$\mu$l in 0.9% saline; Sigma Chemical Co., St. Louis, MO, USA) at 7 sites bilaterally (see Table 3.1 for coordinates). The infusions were done sequentially through a 30-guage injection cannula attached to a 10 $\mu$l Hamilton syringe (Hamilton Co., Reno, NV, USA) via polyethylene tubing (PE-50; Small Parts Inc., Lexington, KY, USA), which were attached to a micro-infusion pump (Harvard Apparatus, South Natick, MA, USA). At both of the most ventral sites, a total volume of 0.5 $\mu$l was infused at a flow rate of 0.15 $\mu$l per minute. At the remaining 5 sites, a volume of 0.4 $\mu$l was infused using the same flow rate. The injection needle was left in place for an additional 3.5 min following the injection to facilitate diffusion. Following the infusions, the scalp incision was closed using sutures. As the rats recovered from the anaesthetic, a prophylaxis against seizures was administered (diazepam; 0.2cc; 10mg/ml, i.p.; Sabex, Boucherville, QC, Canada). The same surgical procedures were used for the Sham rats except that no damage was done to the skull or brain. The rats were allowed to recover for a minimum of 10 days before subsequent behavioural procedures.

### *Cannula Implantation*

Surgical procedures followed those described for the hippocampal lesions. For placement of guide cannulae and jewellers screws, small burr holes were drilled through the skull with care not to damage the underlying tissues. Stainless steel guide cannulae (23 gauge; 10 mm in length for dorsal hippocampus, 13 mm for ventral hippocampus) were bilaterally implanted in to the dorsal (Anterior/Posterior -3.5 mm, Medial/Lateral +/-2mm, Dorsal/Ventral -3 mm on the basis of the Paxinos and Watson (1998) rat brain atlas) and ventral (Anterior/Posterior -5.8 mm, Medial/Lateral +/-5 mm, Dorsal/Ventral -5 mm) hippocampus. Cannulae were fixed to the skull with three jeweller's screws and dental acrylic. After surgery, stainless steel stylets (dorsal -10 mm and ventral -13 mm) were placed in the guide cannula to prevent clogging. Rats were allowed to recover for 10 days before commencement of the behavioural procedures. For a detailed description of all cannula implantation procedures refer to Appendix 1.

## ***Intracranial Drug Infusions***

One hour before conditioning, rats were transported in squads of two from their home cage to an infusion room where they remained for 30 min. Rats received a bilateral infu-

Table 3.1: Coordinates used for 7-site hippocampal lesion in adult female rat (measurements in millimetres relative to bregma).

| **Site** | **Anterior** | **Lateral** | **Ventral** |
|---|---|---|---|
| 1 | –3.1 | ±1.5 | –3.6 |
| 2 | –4.1 | ±3.0 | –4.0 |
| 3 | –5.0 | ±3.0 | –4.0 |
| 4 | –5.0 | ±5.2 | –7.3 |
| 5 | –5.8 | ±4.4 | –4.4 |
| 6 | –5.8 | ±5.1 | –7.5 |
| 7 | –5.8 | ±5.1 | –6.2 |

sion of either sterile physiological saline (0.9%; S group) or muscimol (5-Aminomethyl-3-hydroxyisoxazole hydrobromide dissolved in 0.9% sterile saline, 1 $\mu$g/$\mu$l; Sigma-Aldrich Canada, Oakville, ON, Canada; M group) at a rate of 0.32 $\mu$l/min for 94-s. A 0.5 $\mu$l infusion was made in each of the dorsal and ventral sites, for a total hemispheric infusion of 1 $\mu$l (i.e., 1 $\mu$g muscimol per hemisphere). Injection cannulae (30 gauge; stainless-steel) attached to polyethylene tubing (PE-50; Small Parts Inc., Lexington, KY, USA) were placed in, and extended 1 mm beyond (11 mm dorsal, 14 mm ventral) the indwelling guide cannulae. The distal ends of the PE-50 tubing were attached to 10 $\mu$l Hamilton syringes (Hamilton Co., Reno, NV, USA), which were attached to a micro-infusion pump (Harvard Apparatus, South Natick, MA, USA). After the infusion pumps were turned off, the injection cannulae were left in place for 1 min to allow for diffusion of the drug. After infusions were completed, rats were transported back to their home cage where they remained until conditioning. The time from infusion to conditioning was 30 minutes. The infusion squads were counterbalanced for both conditioning chamber and infusion. The testing phases of the experiment followed the same infusion protocol. Infusion treatment was balanced across the conditioning and two testing phases.

### *Apparatus*

Conditioning and testing were carried out in two identical observation chambers (30 x 24 x 21 cm; MED-Associates, Burlington, VT, USA). The chambers were constructed from aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door) and were situated in cabinets located in a brightly lit and isolated room. The floor of each chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (centre to centre). Rods were wired to a shock source and solid-state grid scrambler (MED-Associates) for the delivery of footshock USs. The chambers were wiped with dilute cleaner disinfectant (to which the rats were nave) and stainless steel trays cleaned with the same solution

were placed underneath the grid floors. Ventilation fans in each cabinet supplied background noise (65 dB, A scale).

### *Behavioural Procedures*

For contextual fear conditioning, rats were transported to the conditioning room two at a time in separate plastic transport tubs, placed in the conditioning chambers, and allowed to explore for 3 min before five foot shocks (2-s; 1-mA) were administered with an inter-shock interval of 60-s. The duration of the conditioning session for each rat was 8 min. The first retention session was conducted 24 hr after the conditioning session (the exception being Experiment 1 where retention was tested 11 days after conditioning). Rats were transported to the conditioning room in the same manner as on the conditioning day; each animal was placed into the conditioning chamber for a 3 min retention session. Behaviour while in the conditioning context was digitally recorded using FreezeFrame Video-Based Conditioned Fear System and analyzed by Actimetrics Software (Coulbourn Instruments, Wilmette, IL, USA) for average freezing times. Freezing was defined as the absence of movement except for that due to respiration. Also, the amount of time spent freezing during the tests for each rat was converted to a percent freezing score ([time freezing/test time]X100). These procedures were used for all experiments.

### *Histology*

After completion of behavioural testing, all animals were sacrificed by administering an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.9% phosphate buffered saline, followed by 4% paraformaldehyde in 0.9% phosphate buffered saline. The brains were removed and post-fixed for 24 hr in paraformaldehyde, then transferred and stored in 30% sucrose and 0.9% phosphate buffered saline with sodium azide

(0.02%) for at least 48 hr before sectioning. The brains were sectioned in the coronal plane 40 $\mu$m thick using a cryostat microtome (-19°C); every fourth section taken throughout hippocampus in the permanent lesion groups, and every section taken proximal to cannula tracks in temporary lesion groups. Sections were wet-mounted on glass microscope slides and later stained with cresyl violet for visualization of hippocampal lesion induced damage, or cannula and injector placement. The amount of damage to the hippocampus in Experiment 1 was assessed using the Cavalieri method (Schmitz & Hof, 2005) to calculate the volume of spared principal sub-fields (CA fields and dentate gyrus). The volume of spared sub-fields was then divided by volume estimates from an intact control group to calculate percent lesion.

## Results

### *Evidence of Interference: Hippocampal Lesions Cause Retrograde but not Anterograde Amnesia*

The aim of this experiment was to replicate the dissociable effects of pre- and post-learning hippocampus damage on contextual fear conditioning, in order to convincingly show that this type of memory is normally dependent on the hippocampus, but can be readily acquired and retained by at least one other network in its absence. In Experiment 1a, rats either received sham (n = 4) or complete hippocampus damage (n = 5) 24-48 hr after a contextual fear conditioning session. Following a 10 d recovery period, the rats were given a 5-min a retention test. In Experiment 1b, the rats initially received sham (n = 4) or complete hippocampal (n = 4) lesions. Following 10 d recovery, the rats were then conditioned and tested for retention another 11 d later. Thus, the conditioning to testing intervals for Experiments 1a and 1b were equivalent, the only difference was whether the rats received the damage before or after conditioning.

Injections of NMDA produced extensive damage to the hippocampus, including all principal subfields (CA1-CA3) and the dentate gyrus. Using unbiased stereology (Schmitz & Hof, 2005), it is estimated that 87.2% of the hippocampus was damaged across all the lesion rats (SD = 7.95; Min = 72.45; Max = 97.33) and Figure 3.1 depicts a schematic reconstruction of the extent of brain damage caused by the NMDA infusions. Briefly, all lesion rats had hippocampus damage extending throughout the dorsal and ventral hippocampus. Very little damage occurred in the overlying cortex or subiculum and the latter damage was restricted to the most anterior region of the subiculum.

Importantly, the hippocampus damage caused retrograde but not anterograde amnesia (Figure 3.2). The rats that received hippocampus damage after conditioning showed significantly less freezing than shams during the retention test ($t_7 = 4.00, p = 0.005$). In contrast, when the hippocampus was damaged before conditioning freezing did not significantly differ between hippocampal and Sham rats ($t_6 = -0.03, p = 0.98$). Thus, these findings are consistent with other lesion studies demonstrating that complete hippocampus damage causes retrograde, but not anterograde amnesia for contextual fear conditioning (Maren et al., 1997; Frankland et al., 1998; Wiltgen et al., 2006; Lehmann, Sparks, et al., 2009). These findings also demonstrate that the learning parameters used in the current study lead to a memory that is vulnerable to hippocampal interference.

### *The Hippocampus Can Interfere With Memory Retrieval*

We then examined our main hypothesis that the hippocampus can interfere with retrieval from non-hippocampal memory networks. The design of the experiment is depicted in Figure 3.3 which indicates whether the injection of saline or muscimol was made before acquisition or prior to retention testing. Four different groups were tested in this experiment. First, the control group (S-S) received saline injections into the hippocampus immediately prior to acquisition and saline injections again immediately before the retention test. Sec-

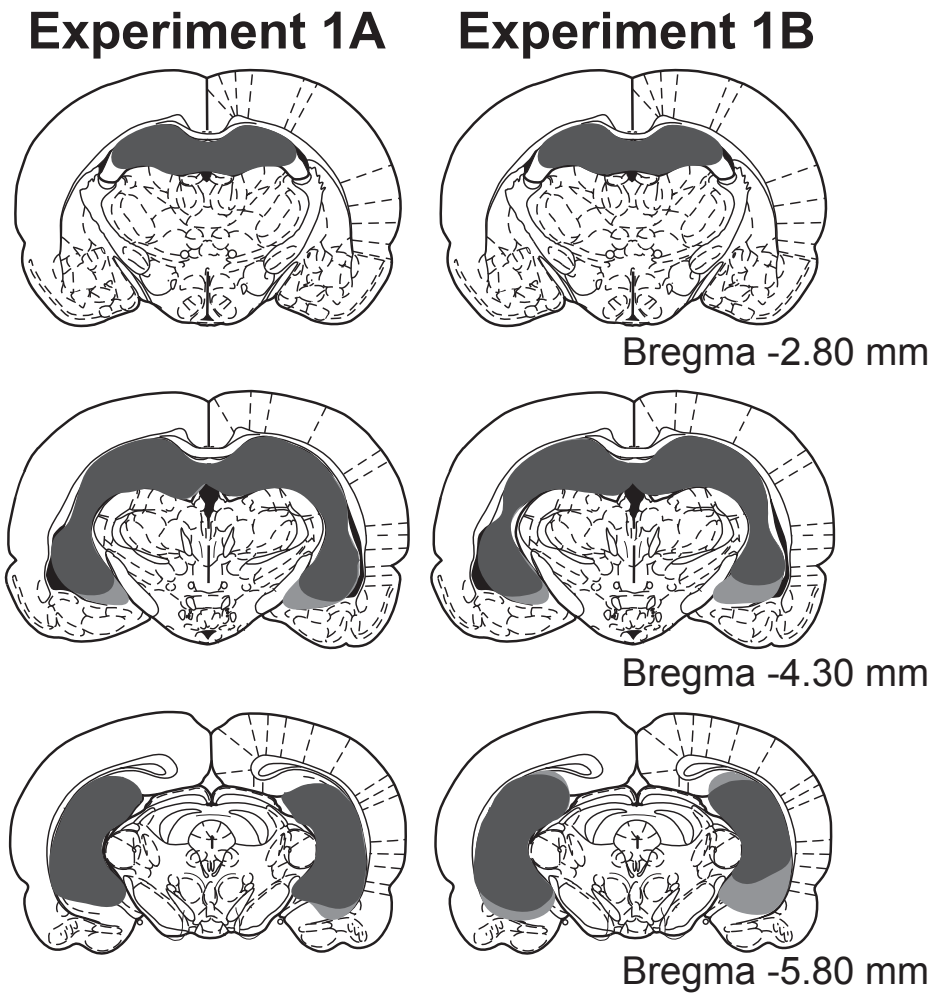


Figure 3.1: **Schematic Reconstruction.** Graphic illustrations depicting the smallest (dark grey) and largest (light grey) lesion observed bilaterally through the rostral and caudal extent of the hippocampus for lesion groups in Experiments 1A and 1B. The brain sections are adapted from Paxinos & Watson, 1997.

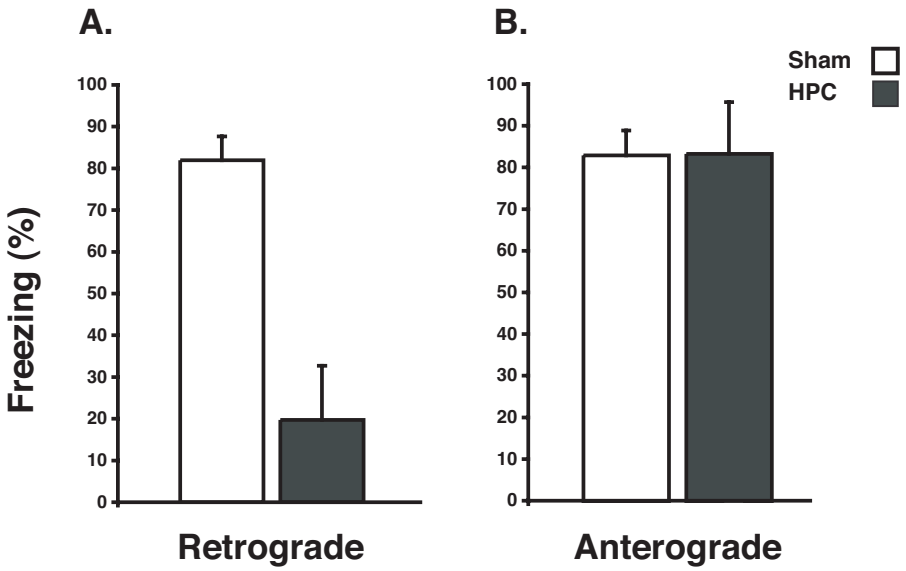


Figure 3.2: **Retention Testing.** Mean $\pm$ SEM percentage freezing during the context memory retention session for rats that underwent contextual fear conditioning either before (retrograde) or after (anterograde) damage to the hippocampus. Rats that received no damage to the hippocampus are represented by Sham, while rats that received hippocampal lesions are represented by HPC. (A) A significant decrease in freezing in rats that had hippocampus damage after training. (B) Training after hippocampus damage results in normal freezing behaviour.

ond, we had a group that should produce results that resemble the effects of pre-training hippocampal lesions. In this group the hippocampus was inactive during acquisition and retention testing (M-M). Given that pre-training lesions do not cause amnesia in this task, we predicted that the M-M group would perform similar to the control group. This would also confirm that contextual fear conditioning could be acquired and expressed by non-hippocampal systems as the hippocampus would be inactivated at both times. Third, and critical for this study, we had a group that received muscimol before acquisition and saline prior to test (M-S). Therefore, the hippocampus was inactivated at the time of learning and the memory would necessarily be acquired by non-hippocampal systems. Yet, at the time of test, the hippocampus would be back to "normal" and in a position to interfere with the retrieval of the memory from the non-hippocampal system. Accordingly, we predicted that this group would be impaired compared to the control group. Fourth, we included a group mimicking the effect of post-training hippocampus damage, which received saline prior to acquisition and muscimol prior to test.

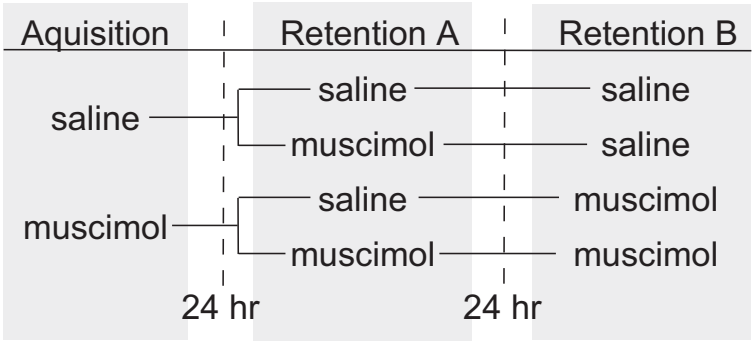


Figure 3.3: **Experimental Design.** Division of groups in Experiment 2 per acquisition, retention A and retention B phases—within subjects A-B-A experimental design. Saline groups have an active hippocampus and muscimol groups have an inactive hippocampus prior to respective phases.

Rats with cannula histologically determined to be outside the target region of the dorsal and ventral hippocampus (total = 5) were excluded from the study, yielding an n = 6-9 rats

per group. The injection tip placements for Experiment 2 are depicted in Figure 3.4.

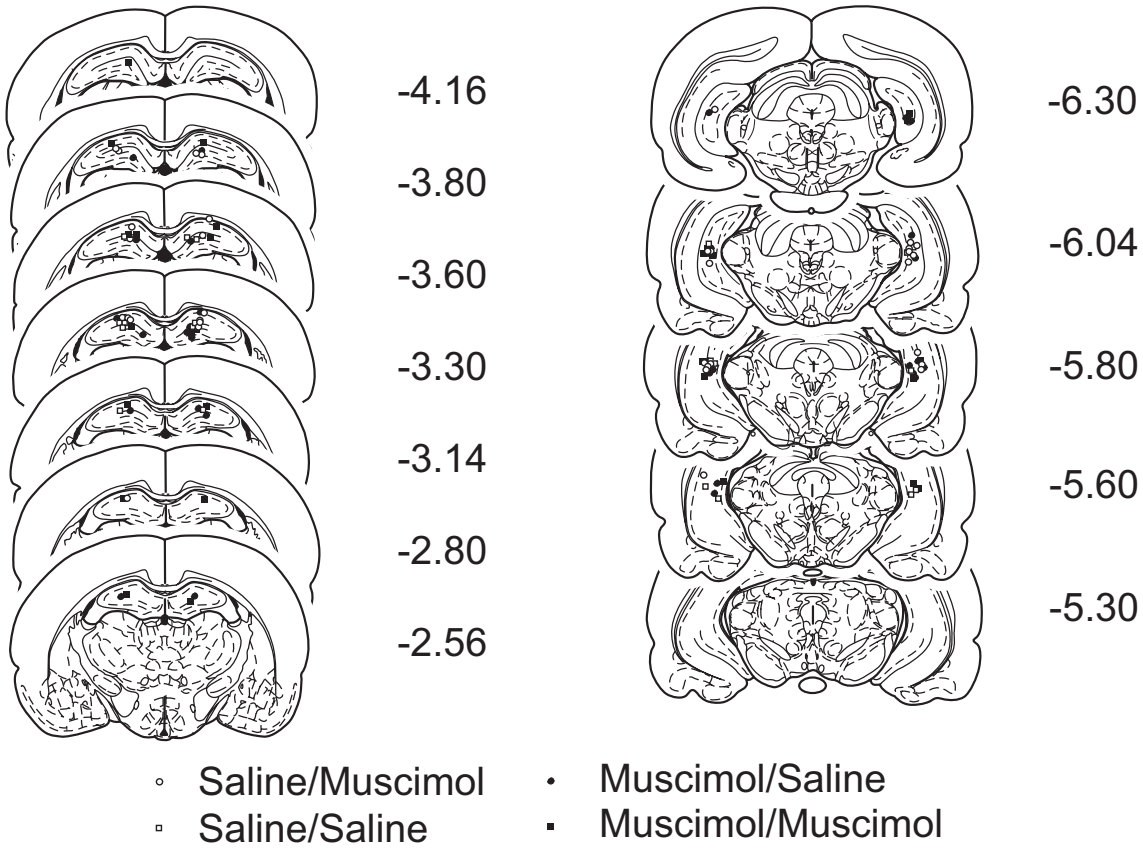


Figure 3.4: **Cannulae Placement Histology.** Coronal schematic representation of injection cannula tip placements in the dorsal (left) and ventral (right) hippocampus for those rats included in the analysis for Experiment 2. The values to the right of the graphics indicate the position of each section relative to bregma (mm caudal to bregma). The section graphics are adapted from Paxinos & Watson, 1998.

*Bilateral Infusions of Muscimol into Dorsal and Ventral Hippocampus do not Increase Baseline-Freezing Levels*

Freezing was measured during the pre-shock interval of the conditioning phase (3 min). Baseline freezing levels of rats infused with muscimol ($MEAN = 2.34; SD = 1.10$) did not statistically differ from the saline infused control rats ($MEAN = 2.62; SD = 2.31$),

($t_{30} = 0.37, p = 0.71$). This result demonstrates that infusions of muscimol do not increase baseline-freezing levels when rats are placed into a novel context.

### *Reinstating Hippocampal Function After Conditioning Disrupts the Expression of Contextual Fear Memories*

Figure 3.5 illustrates freezing performance for each group during a first retention test (Retention A). A one-way ANOVA revealed significant group differences ($F_{3,29} = 4.37, p = 0.012$). Post hoc comparisons then revealed that only the M–S froze significantly less than the S-S control group ($p < 0.05$). Thus, contextual fear conditioning can be normally acquired and expressed without the hippocampus (M–M), but the return of normal hippocampal function disrupted this memory (M–S).

To further corroborate the memory impairment of the M–S group, freezing levels during the retention test were compared to pre-shock levels from the acquisition session. Specifically, pre-shock freezing levels of all rats from the first 3 min of the conditioning session were combined to establish a baseline freezing level for a novel context (saline and muscimol infused groups showed no difference during the pre-shock interval ($t_{30} = 0.37, p = 0.71$) so were combined for analysis). A one-sample *t*-test revealed that the S–S (control group) froze significantly more during the retention test than the pre-shock baseline ($t_7 = 4.270, p = 0.004$), but that the M–S group did not ($t_8 = 1.98, p = 0.083$). Thus, the M–S group behaved as if they were shock naïve and exhibited no memory for the context-shock association.

#### *Inactivating the Hippocampus Reveals Contextual Fear Memory Previously not Expressed*

To confirm that the memory impairment was caused by interference of the hippocampus due to restoration of its normal functioning, the rats were tested a second time and now

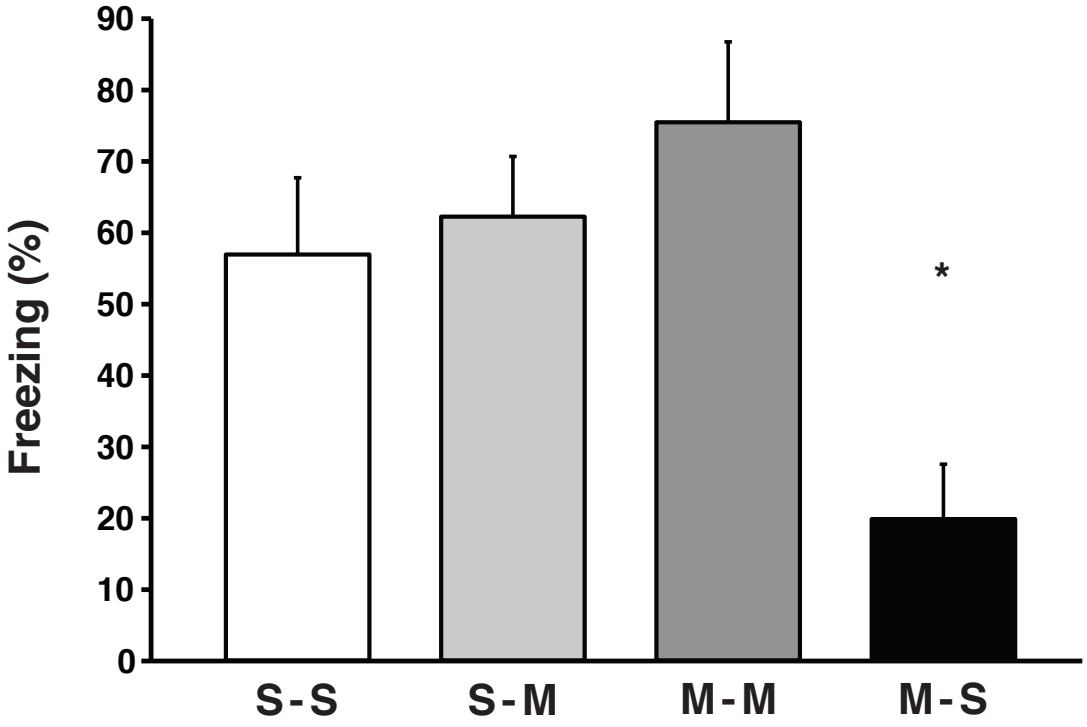


Figure 3.5: **Retention Session A.** Mean $\pm$ SEM percent freezing during the first context retention session for rats that underwent contextual fear conditioning with an active hippocampus 'S' or inactive hippocampus 'M' (Experiment 2). The rats with an active hippocampus during the retention session are S–S and M–S, the rats with an inactive hippocampus during the retention session are S–M and M–M. $*p < 0.05$, significant group difference.

given the exact same treatment they received prior to acquisition. In other words, the M–S group that was impaired during the first retention test was now given M prior to the second retention test with the expectation that freezing would now reach control performance as the hippocampus would no longer be able to interfere. Figure 3.6 illustrates performance of each group on this second test (Retention B). A one-way ANOVA failed to reveal any significant group differences ($F_{3,29} = 0.23, p = 0.878$) suggesting that M–S–M was now freezing to the same extent as the control group. Moreover, the M–S–M group froze significantly more during the second retention test compared to pre-shock baseline ($t_8 = 3.33, p = 0.010$), suggesting that the context-shock association was now remembered. Therefore, removal of hippocampal interference, during the second retention session permitted the contextual fear memories within non-hippocampal networks to be expressed.

### *Inactivation of the Hippocampus does not Cause Retrograde Amnesia*

An unexpected finding was the failure to cause retrograde amnesia for contextual fear conditioning with pre-test inactivation of the hippocampus using muscimol (see Figure 3.5; S–M). Indeed, permanent lesions of the hippocampus following learning, as shown in Experiment 1a, reliably cause retrograde amnesia, yet inactivating the dorsal and ventral hippocampus with muscimol did not. Other laboratories have reported similar failures with muscimol injections into the dorsal hippocampus (Holt & Maren, 1999), but greater success using sodium channel blockers (Bast et al., 2001; Broadbent et al., 2006). We thus examined whether the lack of retrograde amnesia is a result of the specific compound used to inactivate the hippocampus. Here we used bupivacaine to inactivate the hippocampus under the same learning parameters. Bupivacaine is a local anaesthetic (sodium channel blocker) that inhibits cell body activity, similar to muscimol, but also disrupts fibres of passage activity, which increases the likelihood of causing amnesia.

Cannulated rats were randomly assigned to one of two groups before entering two ex-

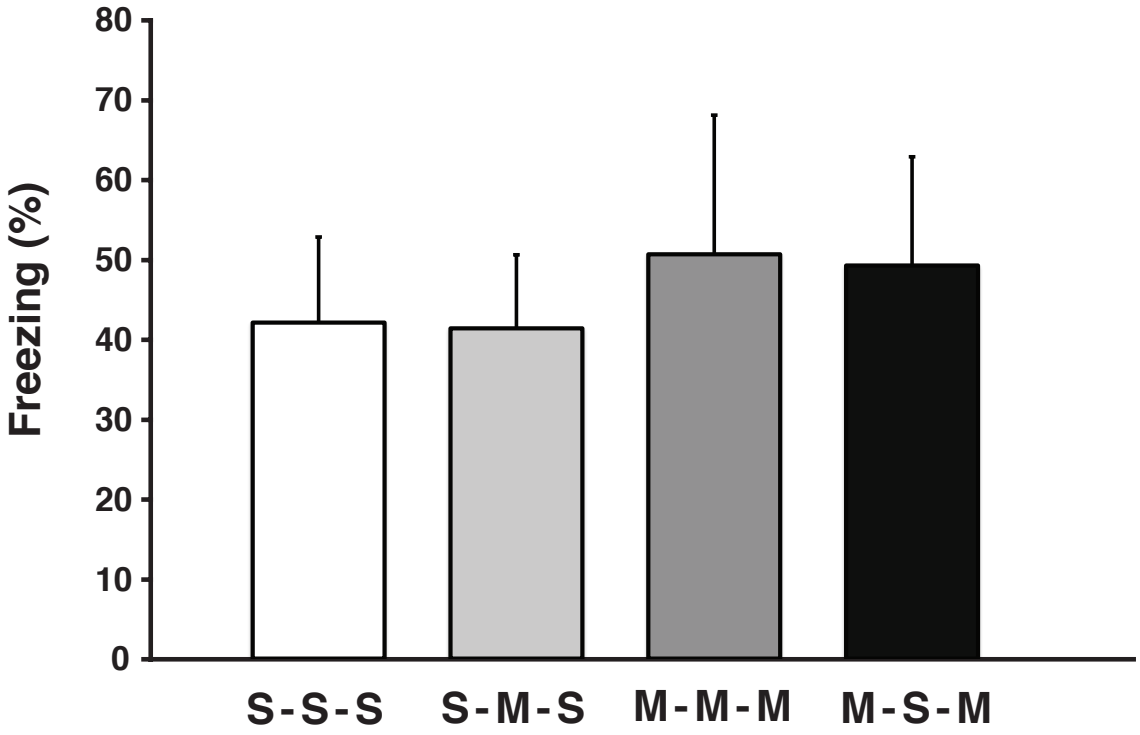


Figure 3.6: **Retention Session B.** Mean ± SEM percent freezing during the second context retention session for rats that underwent contextual fear conditioning with an active hippocampus 'S' or inactive hippocampus 'M' (Experiment 2). The rats with an active hippocampus during the retention session are S–S–S and S–M–S, the rats with an inactive hippocampus during the retention session are M–S–M and M–M–M.

perimental phases: fear conditioning and test. Rats in both groups received infusion of saline (S) prior to the conditioning session, and either S or bupivacaine (B) prior to the test session and the behavioural results are illustrated in Figure 3.7. The control group (S–S; n = 8) showed significant level of freezing during the test session compared to baseline ($t_7 = 6.75, p < 0.001$). Inactivating the hippocampus using the local anesthetic bupivacaine (S–B; n = 8) produced results that did not statistically differ from that of the control group ($t_{14} = 0.19, p = 0.852$). Therefore, similar to the results found using muscimol, bupivacaine infusions did not cause retrograde amnesia for contextual fear memory. The injection tip placements for Experiment 3 are depicted in Figure 3.8.

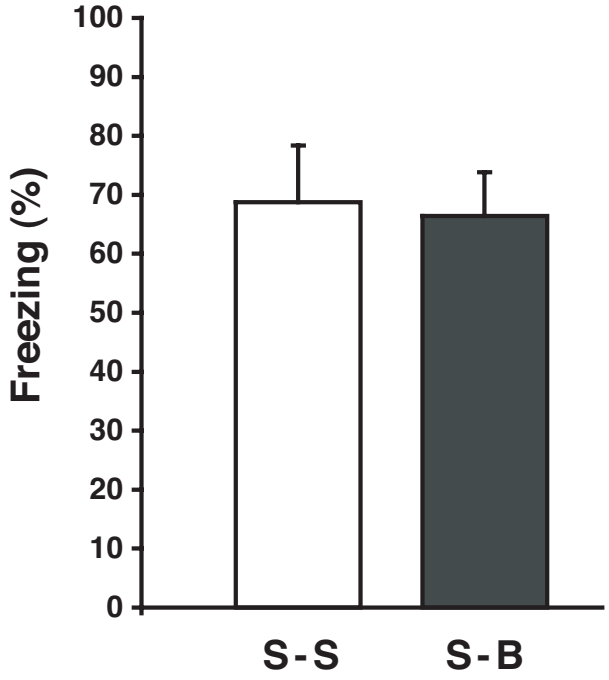


Figure 3.7: **Retention Session.** Mean ± SEM percent freezing during the first context retention session for rats that underwent contextual fear conditioning with an active hippocampus 'S' in Experiment 3. The rats with an active hippocampus during the retention session are S–S, and the rats with an inactive hippocampus during the retention session (bupivacaine infused) are S–B.

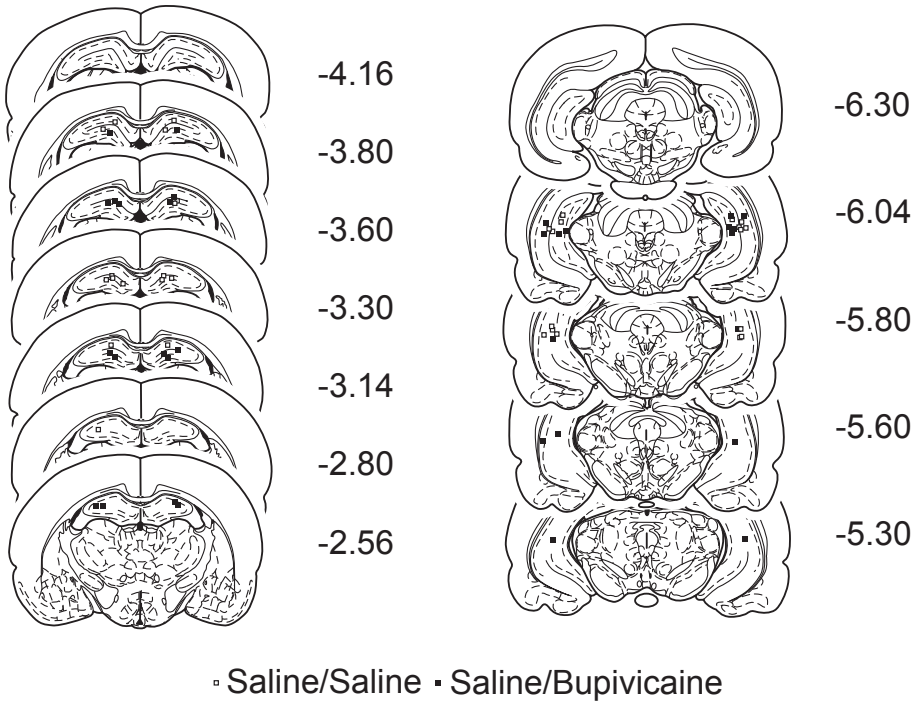


Figure 3.8: **Cannula Placement Histology.** Coronal schematic representation of the injection cannula tip placements in the dorsal (left) and ventral (right) hippocampus for those rats included in the analysis for Experiment 3. The values to the right of the graphics indicate the position relative to bregma (mm caudal to bregma). The brain section graphics are adapted from Paxinos & Watson, 1998.

## Discussion

Damage to the hippocampus after contextual fear conditioning causes retrograde amnesia, suggesting that the hippocampus is normally essential in this type of memory. In contrast, damage to the hippocampus prior to contextual fear conditioning does not cause anterograde amnesia, suggesting that in the absence of the hippocampus other systems can acquire and retain this memory. This dissociation was confirmed in Experiment 1 and supports the claim that the hippocampus interferes with or overshadows other systems during acquisition of contextual fear conditioning. An unanswered question, however, was whether the hippocampus could also interfere with the retrieval of a contextual fear memory that was established in the other systems? This was the main focus of the current study and indeed hippocampal function can be detrimental to the retrieval of a context fear memory that was independently acquired by non-hippocampal systems. Specifically, we found that combined inactivation of the dorsal and ventral hippocampus with muscimol prior to learning and retention testing (M-M group) is not associated with amnesia for contextual fear conditioning. Similar to the permanent lesion findings, that non-hippocampal systems can acquire and support this memory in absence of the hippocampus. If, however, the hippocampus is inactivated at the time of learning and its function restored at the time of test (M-S group), then the context fear memory is impaired; performance of these rats was worse than that of control rats (S-S) and no better than shock naïve rats (pre-shock data). Thus, a memory that was acquired and supported by non-hippocampal systems could no longer be retrieved because the hippocampus was now interfering with the process. Strongly confirming this interpretation, removing the inferred hippocampus-interference during a second retention test (M-S-M) fully reinstated the expression of the fear memory.

Substantial work has been conducted on the interaction between hippocampus and non-hippocampal systems with evidence suggesting competitive interactions between systems

(Maren et al., 1997; Frankland et al., 1998; Driscoll et al., 2005; Epp et al., 2008; Lehmann, Sparks, et al., 2009; Lehmann et al., 2010; Travis, Sparks, Arnold, Sutherland, & Whishaw, 2010). Amongst the evidence suggesting competitive interactions, most studies allude to a release of non-hippocampal systems once the hippocampus is damaged. Thus, the hippocampus would interfere with the acquisition of information in other systems by overshadowing them (Lehmann, Sparks, et al., 2009; Fanselow, 2010). This is the type of competition or interference that accounts for how permanent hippocampus damage causes retrograde, but not anterograde, amnesia for several types of memories (Driscoll et al., 2005; Epp et al., 2008; Travis et al., 2010; Lehmann et al., 2010), including contextual fear conditioning in rats (Maren et al., 1997; Wiltgen et al., 2006; Lehmann, Sparks, et al., 2009; Wang et al., 2009). Consistent with the findings of the current study, we propose that the competitive/interfering effect of the hippocampus on non-hippocampal systems extends beyond acquisition to also include retrieval. The hippocampal interference could result from the hippocampus output being a significant modulator of the representations in neocortical association areas at the time of memory retrieval. When the hippocampus is inactive at the time of learning, there is a lack of hippocampal output that can modulate or be incorporated into the non-hippocampal representation—the non-hippocampal systems form a hippocampal independent representation. If the hippocampus is then active at the time of memory retrieval, an output that is incongruent to that previously established in the non-hippocampal systems interferes with the original association of cues. The relevant representation is not reinstated and therefore does not contribute to appropriate behavioural output. As long as the hippocampus remains inactive at the time of memory retrieval, the non-hippocampal systems are capable of reinstating appropriate representations (see M-M and M-S-M groups).

An alternative account of our main finding would be a state-dependent learning/retrieval effect. Indeed, there are reports that memory performance is optimal when subjects are as-

sessed under the same conditions at the time of retrieval as at the time of learning (Ceretta, Camera, Mello, & Rubin, 2008; Goodwin et al., 1969). If the results were governed by state dependent learning/retrieval, one would predict that retrieval states consistent with that at the time of learning would lead to strong memory, whereas retrieval states inconsistent with that at the time of learning would cause memory deficits. Accordingly, inactivation of the hippocampus prior to conditioning and test (M-M) did not impair memory, whereas inactivating prior to learning but not prior to test (M-S) caused deficits. Looking at these data alone suggests that retrieval of the target memory may be dependent on the state of the memory systems being consistent with the time of conditioning. The state-dependent account, however, fails when taking into account the rats that acquired the context fear memory with an active hippocampus and tested with an inactive hippocampus (S-M group) as they did not suffer from retrograde amnesia. This rules out a pure or strong state-dependent learning/retrieval effect as the main explanation. Moreover, state-dependent learning implies incongruence between two conditions, which is consistent with our proposed view. The representation established in non-hippocampal networks when the hippocampus is inactive is disrupted by recovered function of the hippocampus causing an incongruent representation of the context.

The above-mentioned lack of retrograde amnesia with inactivation of the hippocampus only at the time of the retention (S-M) was unexpected and in contrast to the permanent lesion data (Experiment 1). Post-training neurotoxic lesions in Experiment 1 produced severe retrograde amnesia, whereas muscimol injections prior to the retention session did not. We thus examined whether the means by which the hippocampus was inactivated explained the discrepancy. Muscimol stimulates GABAa receptors that in turn inhibit neurons from firing action potentials. This method of inactivating the hippocampus allows for neural transmission through fibres of passage within the inactivated region. Permanent lesion techniques, such as neurotoxic infusions of NMDA, however, may disrupt these fibres of passage to a

greater extent, possibly leading to memory loss. To test this possibility, we performed the same behavioural procedures using the sodium channel blocker bupivacaine, which disrupts both neuronal activity and transmission along local fibres of passage. Similar to the muscimol effects, the bupivacaine infusions did not produce retrograde amnesia.

The discrepant effects of pre-test hippocampal inactivations vs. post-training permanent lesions are not unique to this study. For example, other laboratories have also failed to find evidence of retrograde amnesia after inactivating the dorsal hippocampus (Resstel et al., 2008; Holt & Maren, 1999), whereas there are numerous findings of retrograde amnesia following damage limited to the dorsal hippocampus (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008). Biedenkapp and Rudy (2009), however, report retrograde amnesia following temporary inactivations and permanent lesions of the hippocampus, but the area targeted was the ventral subiculum, which is not typically considered part of the hippocampus proper (dentate gyrus and CA fields). The ventral subiculum is a major output region for the hippocampus proper, sending projections to neocortical and subcortical regions (Naber & Witter, 1998; Naber, Witter, & Silva, 2000; Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000). Thus, the Biedenkapp and Rudy (2009) findings are not directly comparable the current study as it did not examine the contribution of the same neural areas to context fear memories and may be more disruptive than infusions in the dorsal and ventral hippocampus.

This is not to say that temporary inactivations of the hippocampus cannot produce retrograde amnesia for contextual fear conditioning, but it seems to be under narrower parameters than the ones used in the current study. Retrograde amnesia seems more reliably observed following inactivation of the hippocampus within the context pre-exposure facilitation of immediate shock version of contextual fear conditioning. In this conditioning version, rats are given a context pre-exposure session and a day later returned to the context to immediately receive a single foot shock and then removed from the context as soon

as the shock terminates. Thus, the conditioning involves two phases and the acquisition and retention of this type of context fear is extremely vulnerable to hippocampal disruption, including pre-test inactivations (Matus-Amat et al., 2004). Perhaps then the standard contextual fear procedure (i.e., single conditioning session and multiple shocks), as the one used in the current study, enables enough of a fear memory in non-hippocampal systems that it can be expressed even when the hippocampus is inactivated.

In conclusion, this is the first example of hippocampal interference of memories at the time of retrieval. Compelling evidence from recent studies has shown that the hippocampus interferes with other memory systems acquiring information. We now have evidence that this interference extends also to the time of memory retrieval. This effect can be viewed as a competition between memory systems for appropriate behavioural output. When the information stored in hippocampus and non-hippocampal networks is incongruent, the hippocampus dominates.

## Chapter 4

## Strength of Memory Representation and Systems Consolidation

### Abstract

Controversy surrounds the dependence of long-term context memories on the intact hippocampus. All experiments examining the effects of hippocampus damage on context memory for a single learning episode find that damage soon after learning results in robust retrograde amnesia. Some experiments find that if the learning-to-damage interval is extended, remote context memories are spared. In contrast, other experiments fail to find spared remote context memory. One possible explanation for inconsistency is the number of context-shock pairings used during the learning episode. The experiments showing spared remote memory used a greater number of context-shock pairings than the others. We designed an experiment to directly test this possibility: does increasing the number of context-shock pairings result in sparing of remote context memory after hippocampus damage? Rats were first divided into 6 experimental groups and trained using a standard contextual fear procedure. Rats received either 3 or 12 context-shock pairings during a single conditioning session and then either received complete hippocampus damage or Sham surgery at one of three time post-training time points: 1-wk, 2-months, or 4-months. 10 days post-surgery rats were tested for memory of the shock context. Consistent with all related studies, hippocampus damage 1-wk after training produced robust retrograde amnesia for both 3- and 12-shock groups whereas the Sham rats expressed significantly high levels of memory. At the longer learning-damage interval, rats that received hippocampus damage displayed similarly robust retrograde amnesia. This finding at the more remote time points was consistent for both the 3- and 12-shock groups. These results clearly demonstrate that increasing the number of context-shock pairings within a single learning session does not change the dependence of the memory on the hippocampus. Current evidence from our

group on retrograde amnesia has now shown that partial damage, dorsal vs. ventral damage, discrete cue + context conditioning, time after training, and number of context-shock pairings do not affect hippocampal dependence of context fear memories. When taken together, the evidence shows a flat gradient strongly supporting a permanent role of the hippocampus in context memory.

## Introduction

The function of the hippocampus is critical for remembering information about events that take place within a specific context. This role of the hippocampus has been shown repeatedly using Pavlovian conditioning of a foot shock with a context (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008, 2006, 2010). For instance, unequivocal evidence supports the fact that recently acquired contextual fear memory (from a single conditioning session) is disrupted following damage to the hippocampus. The same almost holds true for remote (old) contextual fear memories, where hippocampal damage disrupts memory expression for context fear learned up to 180 days prior to damage (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008, 2006, 2010; Sparks et al., 2011a). Equivalent retrograde amnesia for recent and remote memory is termed a *flat gradient*. Flat gradients are not only restricted to contextual fear memory, but have become the norm for most hippocampal dependent memory tasks including tone fear (Maren et al., 1997; Sutherland et al., 2008; Lehmann et al., 2010), spatial navigation (Bolhuis et al., 1994; Mumby, Astur, Weisend, & Sutherland, 1999; Sutherland et al., 2001; Clark, Broadbent, & Squire, 2005a, 2005b; Martin et al., 2005), object discrimination (Sutherland et al., 2001; Mumby et al., 1999; Lehmann, Lacanilao, & Sutherland, 2007; Lehmann, Glenn, & Mumby, 2007; Gaskin, Tremblay, & Mumby, 2003), shock-probe (Lehmann, Lecluse, Houle, & Mumby, 2006), and picture memory (Epp et al., 2008).

A prominent historical perspective on hippocampal function in memory is founded on a very different idea—that the hippocampus plays a temporary, or time-limited, role in memory retrieval (Scoville & Milner, 1957; Squire & Spanis, 1984; Kim & Fanselow, 1992). According to this view, memory is initially dependent on the hippocampus, and over time transitions to other structures (Marr, 1971; McClelland et al., 1995; Squire, 1992), a process termed *systems memory consolidation*. Controversy exists as to whether this perspective offers a generally applicable insight into hippocampal function (see Sutherland et al. (2010); Sutherland and Lehmann (2011) for review). Of the studies directly assessing the hippocampus role in recent and remote contextual fear memory, many do not offer support for a systems memory consolidation process (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008, 2006, 2010). A few show temporally graded retrograde amnesia (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997).

There are a number of possibilities as to why some studies of contextual fear memory after hippocampal damage find temporally graded retrograde amnesia while others find flat gradients. Studies have systematically addressed potential issues surrounding differences in lesion methods (Sparks et al., 2011a), extent and location of hippocampal damage (Sutherland et al., 2008), and pairing a discrete stimulus with the conditioning context (Lehmann et al., 2010; Sutherland et al., 2008). Another possible, and yet unaddressed explanation for discrepant results lies in the strength of the context-shock association during training. Two of the studies showing spared remote memory following hippocampal damage, used 10 (Anagnostaras et al., 1999) and 15 (Kim & Fanselow, 1992) context-shock pairings, while the studies showing flat gradients used from 2 to 5. Could it be that a stronger memory created with more context-shock pairings undergoes a process of systems memory consolidation, whereas a similar but weaker memory does not?

We examine this question directly by varying the number of context-shock pairings within a single conditioning session, and then damaging the hippocampus at three different

intervals. Rats receive either weak or strong conditioning, and hippocampal damage at an recent, mid, or remote time point. From these experiments we can determine whether or not increasing the strength of the conditioning association results in sparing of remote contextual fear memory following hippocampal damage.

## Materials and Methods

### *Subjects*

The University of Lethbridge Animal Care Committee approved all procedures in accord with the guidelines set by the Canadian Council on Animal Care. Subjects were 77 male Long-Evans rats (350 g) obtained from a commercial supplier (Charles River Laboratories, Inc., QC, Canada). Rats were housed in standard laboratory cages in a room with an ambient temperature of 21°C, 35% relative humidity, 12/12 hr light/dark cycle (lights on at 07:00), and were provided with food and water ad libitum. Behavioural testing was conducted during the light phase of the cycle.

### *Surgery*

Rats were anesthetized by isoflurane inhalation (Janssen, Toronto, ON, Canada) (3.5% with 1 litre/min oxygen, reduced to 1% after a surgical plane was established) and administered an analgesic (buprenorphine, 0.017 mg/kg, s.c.; Reckitt & Colman, Richmond, VA, USA). They were then placed in a stereotaxic frame (Kopf instruments, Tujunga, CA, USA), a midline scalp incision was made, and periosteum excised to expose the top of the skull. Small burr holes were drilled through the skull using anterior/posterior and medial/lateral coordinates relative to Bregma as detailed in Table 4.1. The HPC lesions were made by intra-HPC infusions of N-methyl-D-aspartic acid (NMDA; 7.5 $\mu$g/$\mu$l in 0.9% saline; Sigma Chemical Co., St. Louis, MO, USA) at 7 sites bilaterally (see Table 4.1 for coordinates).

The infusions were done sequentially through a 30-guage injection cannula attached to a 10 $\mu$l Hamilton syringe (Hamilton Co., Reno, NV, USA) via polyethylene tubing (PE-50; Small Parts Inc., Lexington, KY, USA), which were attached to a micro-infusion pump (Harvard Apparatus, South Natick, MA, USA). At both of the most ventral sites, a total volume of 0.5 $\mu$l was infused at a flow rate of 0.15 $\mu$l per minute. At the remaining 5 sites, a volume of 0.4 $\mu$l was infused using the same flow rate. The injection needle was left in place for an additional 3.5 min following the injection to facilitate diffusion. Following the infusions, the scalp incision was closed using sutures. As the rats recovered from the anaesthetic, a prophylaxis against seizures was administered (diazepam; 0.2cc; 10mg/ml, i.p.; Sabex, Boucherville, QC, Canada). The same surgical procedures were used for the Sham rats except that no damage was done to the skull or brain. The rats were allowed to recover for a minimum of 10 days before subsequent behavioural procedures.

Table 4.1: Coordinates used for 7-site hippocampus lesion in adult male rat (measurements in millimetres relative to bregma).

| **Site** | **Anteriorposterior** | **Mediolateral** | **Dorsoventral** | **Volume Infused ($\mu$l)** |
|---|---|---|---|---|
| 1 | –3.0 | $\pm$1.6 | –3.6 | 0.4 |
| 2 | –4.0 | $\pm$3.1 | –4.0 | 0.4 |
| 3 | –4.9 | $\pm$3.1 | –4.0 | 0.4 |
| 4 | –4.9 | $\pm$5.3 | –7.2 | 0.4 |
| 5 | –5.7 | $\pm$4.5 | –4.4 | 0.4 |
| 6 | –5.7 | $\pm$5.5 | –7.3 | 0.5 |
| 7 | –5.7 | $\pm$5.5 | –6.0 | 0.5 |

### *Apparatus*

Conditioning and testing were carried out in two identical observation chambers (30 x 24 x 21 cm; MED-Associates, Burlington, VT, USA). The chambers were constructed from aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door) and were situated in cabinets located in a brightly lit and isolated room. The floor of each chamber

consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (centre to centre). Rods were wired to a shock source and solid-state grid scrambler (MED-Associates) for the delivery of footshock USs. The chambers were wiped with dilute cleaner disinfectant (to which the rats were nave) and stainless steel trays cleaned with the same solution were placed underneath the grid floors. Ventilation fans in each cabinet supplied background noise (65 dB, A scale).

### *Behavioural Procedures*

For contextual fear conditioning, rats were transported to the conditioning room two at a time in separate plastic transport tubs, placed in the conditioning chambers, and allowed to explore for 3 min before either 3 or 10 foot shocks (2-s; 1-mA) were administered with an inter-shock interval of 60-s. The duration of the conditioning session for each rat was 13 min. The retention sessions were performed 10 days after surgery which was conducted either 1-week, 2-months, or 4-months after the conditioning session. Rats were transported to the conditioning room in the same manner as on the conditioning day; each animal was placed into the conditioning chamber for a 5 min retention session. Behaviour while in the conditioning context was digitally recorded using FreezeFrame Video-Based Conditioned Fear System and analyzed by Actimetrics Software (Coulbourn Instruments, Wilmette, IL, USA) for average freezing times. Freezing was defined as the absence of movement except for that due to respiration. Also, the amount of time spent freezing during the tests for each rat was converted to a percent freezing score ([time freezing/test time]X100). These procedures were used for all experiments. Figure 4.1 depicts the design of the experiment.

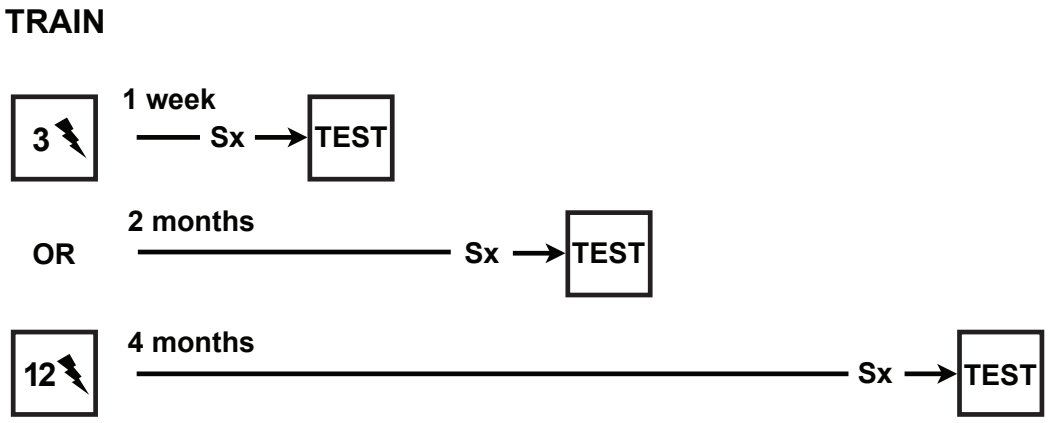


Figure 4.1: **Design.** Experimental design detailing training to surgery and testing intervals.

### *Histology*

After completion of behavioural testing, all animals were sacrificed by administering an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.9% phosphate buffered saline, followed by 4% paraformaldehyde in 0.9% phosphate buffered saline. The brains were removed and post-fixed for 24 hr in paraformaldehyde, then transferred and stored in 30% sucrose and 0.9% phosphate buffered saline with sodium azide (0.02%) for at least 48 hr before sectioning. The brains were sectioned in the coronal plane 40 $\mu$m thick using a cryostat microtome (-19°C); every fourth section taken throughout hippocampus in the permanent lesion groups, and every section taken proximal to cannula tracks in temporary lesion groups. Sections were wet-mounted on glass microscope slides and later stained with cresyl violet for visualization of hippocampal lesion induced damage, or cannula and injector placement. The amount of damage to the hippocampus in Experiment 1 was assessed using the Cavalieri method (Schmitz & Hof, 2005) to calculate the volume of spared principal sub-fields (CA fields and dentate gyrus). The volume of spared

sub-fields was then divided by volume estimates from an intact control group to calculate percent lesion.

## Results

### *Behaviour*

Figure 4.2 shows the percent time spent freezing during the contextual fear conditioning retention test. A three-way analysis of variance (ANOVA) with between-group factors lesion (Sham, Hippocampal), learning-surgery interval (1-wk, 2-mth, 4-mth), and number of context-shock pairings (3 and 12) revealed a significant main effect of lesion ($F_{1,75} = 172.395, p < 0.001$), indicating that damage to the hippocampus impaired freezing. No significant main effect was found for pairings ($F_{1,75} = .080, p = 0.778$) nor of interval ($F_{2,75} = .747, p = 0.478$). Neither the lesion X interval ($F_{2,75} = 0.344, p = 0.711$) or the lesion X pairings X interval ($F_{2,75} = 0.887, p = 0.417$) interactions were significant. The interaction between lesion and pairings did reach statistical significance ($F_{1,75} = 4.779, p = 0.032$) as the Sham rats from the 12 context-shock pairings group froze significantly more than the Sham rats from the 3 pairings group ($p = 0.047$), whereas this pattern was not found in the Hippocampal rats ($p = 0.099$).

### *Histology: NMDA Produced Damage to the Dorsal and Ventral Hippocampus*

The NMDA injections produced cell loss in all of the principal subfields (CA3-CA1) and dentate gyrus of the dorsal and ventral hippocampus, as well as the anterior portion of the subiculum for rats in each of the training-to-surgery intervals. Using unbiased stereology (Schmitz & Hof, 2005), it is estimated that, on average 73.1% of the hippocampus was damaged by infusions of NMDA. A two-way ANOVA with Shock (3- and 12-Shock) and training-to surgery Interval (Recent, Intermediate, and Remote) as factors

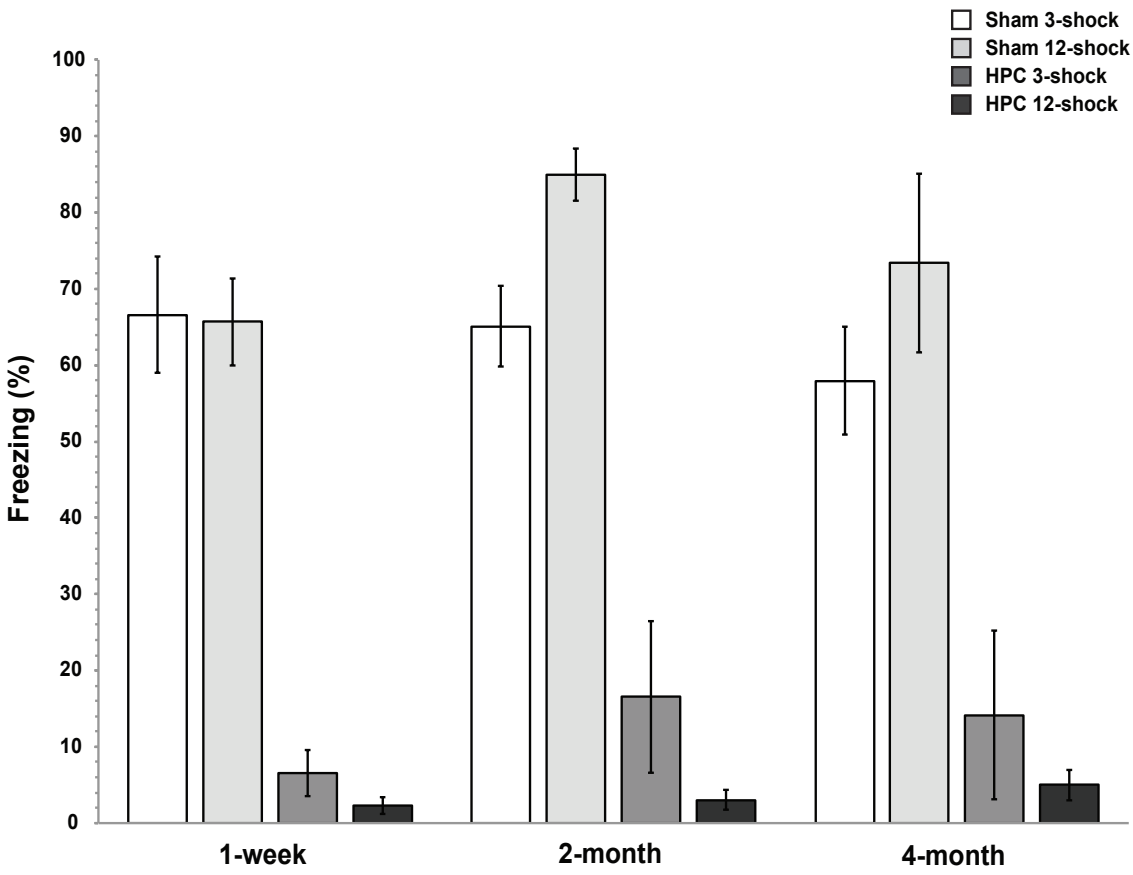


Figure 4.2: **Fear Conditioning Retention Sessions.** Mean ± SEM percentage freezing during the context memory retention session for rats that underwent contextual fear conditioning 1 week, 2 months, or 4 months prior to hippocampal surgery. Sham rats froze significantly more than the lesion groups.

was performed on amount of hippocampal damage, and revealed a main effect of interval ($F_{1,35} = 6.166, p < 0.05$). Pairwise comparisons showed that the Recent group had significantly more damage than the other two groups ($ps < 0.05$), and no significant difference was found between the Intermediate and Remote groups ($p = 0.51$). There was no main effect of Shock ($F_{1,35} = 2.681, p = 0.112$), and the interaction was not significant ($F_{1,35} = 0.827, p = 0.447$). Thus, the amount of damage among the lesion groups was similar for both shock conditions at each time point. The lowering and withdrawal of the

injection cannulae also caused minor damage in the parietal cortex. Rats that had extensive unilateral sparing combined with high memory performance were not included in the analyses. Figure 4.3 shows a schematic reconstruction of the extent of brain damage caused by the NMDA infusions.

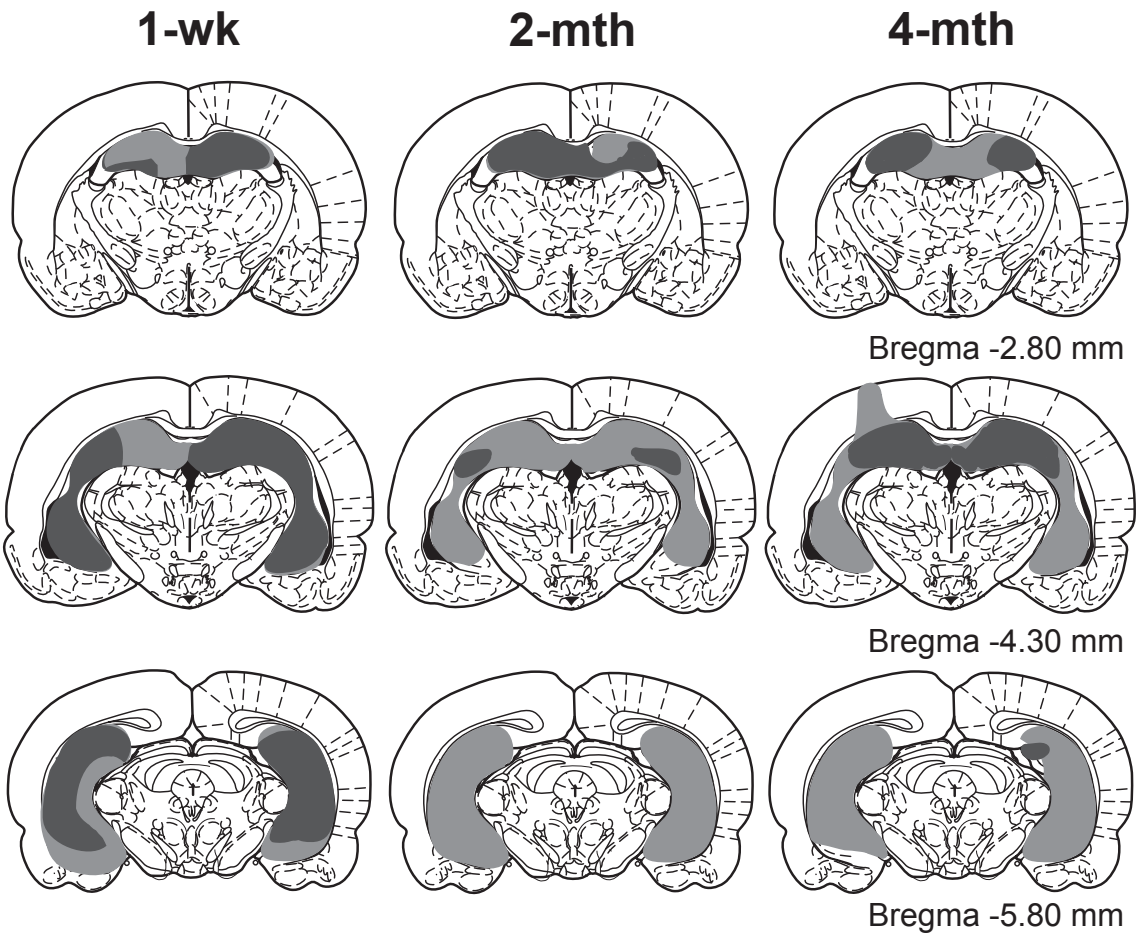


Figure 4.3: **Histology.** Illustration of the smallest (dark grey) and largest (light grey) lesion observed bilaterally through the rostral and caudal extent of the hippocampus for each of the 1-wk, 2-mth, and 4-mth lesion groups.

## Discussion

The present study is the first to demonstrate equivalent retrograde amnesia for recent and remote contextual fear memories with both weak and strong conditioning parameters. The primary motivation was to determine if varying the number of context-shock pairings during a single conditioning session would affect the hippocampus-dependence of a remote contextual fear memory. Rats were conditioned in a single session, with 3 or 12 context-shock pairings, after which complete hippocampal damage was performed at one of three training-to-surgery intervals (1-wk, 2-mth, 4-mth). To examine the effects of hippocampal damage, expression of contextual fear memory was assessed during a retention session following recovery from surgery. Freezing, a learned fear response, during the retention tests was used as an index of contextual fear memory recall. This experiment demonstrated three things: that rats with hippocampal damage had profound memory loss for a previously learned context-shock association; that this retrograde amnesia was equivalent for both the 3 and 12 shock training conditions; and most importantly, that this level of amnesia was equally observed for recent **and** remote contextual fear memories. These results extend previous findings that hippocampal damage produces amnesia for recent and remote contextual fear memories (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008; Quinn et al., 2008; Sparks et al., 2011b) by demonstrating a flat gradient for strong memories.

The design of the present experiment was similar to previous work that examined the role of the hippocampus in recent and remote memory recall. Taken together the experiments allow evaluation of several ideas concerning the role of the hippocampus in long-term contextual fear memory. First, we can evaluate predictions made by the standard model of systems consolidation. According to this model, contextual fear memories should initially depend on the hippocampus for retrieval, and with the passage of a sufficient amount of time (weeks to months) this dependency will shift to non-hippocampal structures

for the accurate retrieval of this memory. This means that damage to the hippocampus soon after learning the context-fear association (recent memory) will produce retrograde amnesia, whereas the same damage incurred long after learning (remote memory) will not have an affect on memory retrieval. This pattern of amnesia is called *temporally graded (or temporally limited) retrograde amnesia*. The present results do not offer support for the standard model of systems consolidation. In this study, control rats exhibit robust contextual fear retention for recent memories (acquired 1-wk prior to surgery) as well as for more remote memories (extending 2- and 4-mth from learning to surgery). The memory performance for the remote memory was just as robust as the recent memory, showing that control rats can retain the context fear memory for extended periods of time, and that retrieval of this remote memory produces high levels of freezing behaviour. Conversely, rats with hippocampal damage exhibited severe amnesia for the context, and this was observed for all of the learning-to-surgery intervals. In fact, none of the hippocampus damaged groups showed freezing levels above that expected from a rat having never been shocked in the context. It is clear that hippocampal damage produced complete retrograde amnesia for recent as well as remote context fear memory. This display of *flat graded retrograde amnesia* is consistent with recent experiments also designed to directly test the long-term dependence of contextual fear memories on the hippocampus (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008; Quinn et al., 2008; Sparks et al., 2011b), and supports recent evaluations of the permanent role of the hippocampus in memory retrieval over time (Sutherland et al., 2010; Sutherland & Lehmann, 2011).

Other studies examining the long-term role of the hippocampus in contextual fear memory retrieval present evidence for temporally graded retrograde amnesia, where recent memory is affected by hippocampal damage and remote memory is less affected (Kim & Fanselow, 1992; Maren et al., 1997; Anagnostaras et al., 1999; Winocur et al., 2009). These studies, along with studies finding flat gradients are listed in Table 4.2. All of the

studies detailed in this table were designed to directly assess the long-term role of the hippocampus in contextual fear memory retrieval. Why do a few experiments show temporal gradients while the majority show flat gradients? This contradiction has been examined from several perspectives in attempt to solve the conflicting results. For instance, Lehmann, Lacanilao, and Sutherland (2007) and Sutherland et al. (2008) recognized that the extent of the hippocampal lesions performed in the studies showing a temporal gradient were often incomplete and restricted to the dorsal hippocampus (Kim & Fanselow, 1992; Maren et al., 1997; Anagnostaras et al., 1999). To examine this factor, hippocampal lesions were performed systematically to varying extent, some groups receiving partial hippocampal damage (40%) and other more complete damage (85%). Regardless of the extent of damage in these studies, Lehmann, Lacanilao, and Sutherland (2007) and Sutherland et al. (2008) always observed flat gradients.

Another factor of note in the studies showing temporal gradients is related to the details of the conditioning procedures. In some studies that reported temporal gradients, rats were conditioned by pairing a tone with foot-shock in the shock context. The effect of this procedural difference was examined by Sutherland et al. (2008), who trained groups of rats either with or without tone-signalled foot-shock, and then damaged the hippocampus at a recent (1-3 days) or remote (12-wks) training-to-surgery interval. Rats that received hippocampal damage at either time point exhibited robust retrograde amnesia, and this memory impairment was equivalent for the signalled and unsignalled groups. Sutherland et al. (2008) suggest that, regardless of whether the conditioning procedure includes tones signalling the foot-shocks, recent and remote contextual fear memories are equally affected by hippocampal damage, and it is unlikely that this parameter is the significant factor in the discrepant results in Table 4.2.

Using neurotoxins to create damage provides a means to target discrete regions with high spatial precision, but it is important to note that glutamate-analogue neurotoxins can

produce seizure activity that can propagate to other networks. The effects of lesion-induced seizure activity are relatively unexamined in the field of learning and memory, and may provide an explanation for the discrepant results listed in Table 4.2. Most of the studies showing flat gradients used the neurotoxin NMDA to create damage, and it might be possible that seizure activity produced during the lesion can disrupt contextual fear memories stored in non-hippocampal networks. This disruption may explain why remote memories were not expressed following hippocampal damage. To test for this possibility, we (Sparks et al., 2011b) designed an experiment to control the level of neurotoxin-induced seizure activity while at the same time using NMDA to damage the hippocampus at multiple time points following learning. Even though seizure activity was markedly reduced, rats still could not retrieve contextual fear memory following hippocampal damage. The severity of retrograde amnesia was similar at the recent (1-wk) and remote (5-wk) training-to-surgery intervals. This result parallels previous studies examining parameters described above, suggesting that the hippocampus does indeed play a long-term, if not permanent, role in contextual fear memory retrieval.

The present study examines the remaining hypothesis, that the strength of the initial memory used in the flat gradient studies was not strong enough to establish hippocampus independent representations through a time-based process of systems consolidation. As detailed in Table 4.2, most of the studies showing a temporal gradient used a high number of context-shock pairings (ranging from 10 to 15) during a single conditioning session (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Winocur et al., 2009). Given that this *strong* conditioning procedure does not produce hippocampus independent representations for recently acquired memories (also see Lehmann, Sparks, et al. (2009)), the passage of a sufficient period of time could still be required for systems consolidation to occur. In the present experiments rats were trained with either a *weak* or *strong* conditioning session (3 vs. 12 foot shocks) to test this hypothesis. If it is true that strong conditioning permits

a non-hippocampal memory to be created through a systems consolidation process, then we should see spared memory at the remote time points. Contrary to the predictions of the standard model, we did not find spared memory, for either the weak or the strong conditioned groups. At each training-to-surgery interval, the level of amnesia was the same, and this held true regardless of the strength of conditioning.

### *Conclusion*

Multiple experiments on the role of the hippocampus in recent vs. remote contextual fear memory designed to evaluate potentially relevant factors have not resolved the discrepancies found within the literature. Notably, the majority of recents studies have consistently produced flat gradients, supporting the idea of a permanent role for the hippocampus in contextual fear memory retrieval. That being said, these results do not stand alone in the field of learning and memory, but rather add to a growing body of work suggesting the same conclusion. Flat gradients have been found for tasks such as learning to fear a discrete stimulus (tone or light) (Sutherland et al., 2008; Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Lehmann et al., 2010), spatial navigation (Sutherland et al., 2001; Bolhuis et al., 1994; Mumby et al., 1999; Clark et al., 2005a, 2005b; Martin et al., 2005), object discrimination (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2001; Mumby et al., 1999; Lehmann, Glenn, & Mumby, 2007), object exploration (Gaskin et al., 2003), shock-probe memory (Lehmann, Sparks, et al., 2006), and picture memory (Epp et al., 2008). Here we have shown that weak **and** strong contextual fear conditioning produce memories that are equally dependent on the hippocampus for retrieval. This dependency is shown to exist at 1-wk, 2-mths, and 4-mths following training, and is confirmed by other work (Lehmann, Rourke, & Bernard, 2009). Damage to the hippocampus at each of these time points produced equivalent, severe retrograde amnesia, leading to a flat amnesia gradient. These results do not support the traditional idea of a process of systems consolidation

leading to a memory that is independent of the hippocampus, but rather the hippocampus remains necessary for the retrieval of contextual fear memory, regardless of how long ago the memory was acquired.

Table 4.2: Studies directly assessing temporally graded retrograde amnesia using contextual fear conditioning.

| **Memory task** | **Shock number** | **Damage** | **Lesion technique** | **RA duration (days)** | **Reference** |
|---|---|---|---|---|---|
| Context fear | 15 | 25% dorsal | Electrolytic | 1 (27) | Kim and Fanselow (1992) |
| Context fear | 10 | 25% dorsal | Electrolytic | 1 (49) | Anagnostaras et al. (1999) |
| Context fear | 3 | 40% dorsal | Neurotoxic (NMDA) | 28 (71) | Maren et al. (1997) |
| Context fear | 2 | 40% dorsal | Neurotoxic (NMDA) | Flat (180) | Lehmann, Lacanilao et al. (2007) |
| Context fear | 2 | 85% d+v | Neurotoxic (NMDA) | Flat (180) | Lehmann, Lacanilao et al. (2007) |
| Context fear | 3 | 40% dorsal | Neurotoxic (NMDA) | Flat (84) | Sutherland et al. (2008) |
| Context fear | 3 | 40% ventral | Neurotoxic (NMDA) | Flat (84) | Sutherland et al. (2008) |
| Context fear | 3 | 85% d+v | Neurotoxic (NMDA) | Flat (84) | Sutherland et al. (2008) |
| Context fear | 10 | 70% d+v | Neurotoxic (NMDA) | 1 (28) | Winocur et al. (2009) |
| Context fear | 5 | 85% d+v | Neurotoxic (NMDA) | Flat (35) | Sparks et al. (2011) |
| Context fear | 3/12 | 65% d+v | Neurotoxic (NMDA) | Flat (120) | Sparks et al. (current study) |

# Chapter 5

## Hippocampal Lesion-Induced Synchronous Activity and Memory Disruption


### Abstract

Damage to the hippocampus using the excitotoxin N-methyl-D-aspartate (NMDA) can cause retrograde amnesia for contextual fear memory. This amnesia is typically attributed to loss of cells in the hippocampus. However, NMDA is also known to cause intense neuronal discharge (seizure activity) during the hours that follow its injection. These seizures may have detrimental effects on retrieval of memories. Here we evaluate the possibility that retrograde amnesia is due to NMDA-induced seizure activity or cell damage *per se*. To assess the effects of NMDA induced activity on contextual memory, we developed a lesion technique that utilizes the neurotoxic effects of NMDA while at the same time suppressing possible associated seizure activity. NMDA and tetrodotoxin (TTX), a sodium channel blocker, are simultaneously infused into the rat hippocampus, resulting in extensive bilateral damage to the hippocampus. TTX, co-infused with NMDA, suppresses propagation of seizure activity. Rats received pairings of a novel context with foot shock, after which they received NMDA-induced, TTX+NMDA-induced, or no damage to the hippocampus at a recent (24 hours) or remote (5 weeks) time point. After recovery, the rats were placed into the shock context and freezing was scored as an index of fear memory. Rats with an intact hippocampus exhibited robust memory for the aversive context at both time points, whereas rats that received NMDA or NMDA+TTX lesions showed a significant reduction in learned fear of equal magnitude at both the recent and remote time points. Therefore, it is unlikely that observed retrograde amnesia in contextual fear conditioning is due to disruption of non-hippocampal networks by propagated seizure activity. Moreover, the memory deficit observed at both time points offers additional evidence supporting the proposition

that the hippocampus has a continuing role in maintaining contextual memories.[1]

## Introduction

In experiments designed to assess the role of the hippocampus in memory in rats, hippocampal damage produced by selective infusions of the neurotoxin N-methyl-D-aspartate (NMDA) have produced robust amnesia (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008, 2006, 2010). NMDA mediated hippocampal damage is based upon the excitotoxic effects of glutamate receptor agonism, and has proven effective in ablating the principal subfields, hilar cells, and dentate gyrus (Jarrard & Meldrum, 1993). Though the damage produced by neurotoxic lesions has a high degree of selectivity, lesions of the hippocampus can cause less obvious disruption to distal network circuitry (Jarrard & Meldrum, 1993; Albasser, Poirier, Warburton, & Aggleton, 2007). This consequence has been a concern using neurotoxic lesions, as well as other permanent lesion techniques (McClelland et al., 1995; Liang et al., 2000; Anagnostaras & Gale, 2002). Infusions of NMDA can produce hippocampus focused seizure activity that propagates to distal networks (Zaczek & Coyle, 1982). Because the seizure activity can be severe, it is important to determine the extent to which retrograde amnesia found after hippocampal lesions (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2001) is influenced by disruptive seizure activity propagating to non-hippocampal regions. This seizure activity may not be as great a concern when using non-neurotoxic lesion techniques, therefore leaving open the possibility that seizure activity could mediate differences in experimental results.

Retrograde amnesia for contextual fear memory is observed following damage to the hippocampus (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008, 2006, 2010). To as-

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sess the possibility that lesion-induced seizure activity contributes to retrograde amnesia for contextual fear memory, we employed a novel lesion technique that enabled control of seizure severity. This technique utilized the neurotoxic effects of NMDA and concurrently reduced hippocampus focused synchronous activity by blocking sodium channel conduction using tetrodotoxin (TTX). We damaged the hippocampus using the standard NMDA or the reduced seizure lesion technique at multiple time points following learning. Rats learned to fear a context during a single conditioning session, and extensive hippocampal lesions were produced following training.

Studies using lesions to investigate if contextual fear memory dependency on the hippocampus changes with the passage of time, have produced mixed results (see Sutherland et al. (2010) and Sutherland and Lehmann (2011) for in-depth review). The effects of hippocampal damage in contextual fear memory tasks typically do not differentially affect memories of different ages, meaning that recently acquired memories are just as susceptible to hippocampal damage as remote memories acquired long before the damage (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008, 2006, 2010). Though equivalent retrograde amnesia for recent and remote context fear memory is demonstrated in these studies, there are some accounts suggesting that remote context fear memory survives damage to the hippocampus, a pattern of results termed *temporally graded retrograde amnesia* (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Restivo, Vetere, Bontemi, & Ammassari-Teule, 2009; Winocur et al., 2009). Sparing of remote memories after hippocampal damage suggests that the hippocampus is no longer necessary for recall of these memories, and is taken as support for the idea of temporally based systems consolidation processes (McClelland et al., 1995; Squire & Alvarez, 1995; Anagnostaras et al., 2001; Meeter & Murre, 2004; Squire et al., 2004; Wiltgen et al., 2004; Frankland & Bontempi, 2005). Strong evidence for a systems consolidation process is not found within recent rodent experiments (Sutherland et al., 2010; Sutherland & Lehmann, 2011). There-

fore it is necessary to determine why a few studies do show temporally graded retrograde amnesia (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997). To test the possibility that neurotoxic lesion induced seizure activity is responsible for the discrepant results, we compared high and low seizure inducing lesions at multiple training-to-surgery intervals (1 week and 5 weeks). Thus our results include retention performance for recent and remote context fear memories. The dependence of context fear memory on the hippocampus is shown following NMDA mediated damage while modulating the amount of seizure activity.

## Materials and Methods

### *Subjects*

The University of Lethbridge Animal Care Committee approved all procedures under Protocol #0609, in accord with the guidelines set by the Canadian Council on Animal Care. Participants were 52 female Long-Evans rats (250-300 g) obtained from the Canadian Centre for Behavioural Neuroscience vivarium (University of Lethbridge, Alberta). Rats were housed in standard laboratory cages in a room with an ambient temperature of 21°C, 35% relative humidity, 12/12 hr light/dark cycle (lights on at 07:00), and were provided with food and water *ad libitum*. Behavioural testing was conducted during the light phase of the daily cycle.

### *Surgery*

The rats were first anaesthetized with isoflurane (Janssen, Toronto, Ontario) in 1.0 L/min oxygen at 14.7 PSIA at 21$^o$C (Benson Medical Industries, Markham, Ontario) and administered an analgesic (buprenorphine, 0.017 mg/kg, s.c.; Reckitt & Colman, Richmond, VA). They were then placed in a stereotaxic frame (Kopf instruments, Tujunga, CA) and a mid-

line scalp incision was made and periosteum excised to expose the top of the skull. Small burr holes were drilled through the skull using the anterior/posterior and medial/lateral coordinates in Table 5.1. The HPC lesions were made by intra-HPC infusions of either *N*-methyl-D-aspartic acid (NMDA; 7.5 $\mu$g/$\mu$l in 0.9% saline; Sigma Chemical Co., St. Louis, MO) or NMDA co-infused with Tetrodotoxin citrate (TTX; 4 $ng/\mu$l in 0.9% saline; Cedarlane Laboratories Ltd., Burlington, ON). The infusions were done sequentially through a 30-ga injection cannula attached to a 10 $\mu$l Hamilton syringe via polyethylene tubing (PE-50). At the most ventral sites, a total volume of 0.5 $\mu$l was infused at a flow rate of 0.15 $\mu$l per minute. At the remaining 5 sites, a volume of 0.4 $\mu$l was infused using the same flow rate. The injection needle was left in place for 3.5 min following the injection to facilitate diffusion. Following the lesions, the scalp incision was closed using sutures. As the rats recovered from the anaesthetic, a prophylaxis against seizures was administered (diazepam; 0.2cc; 10mg/ml, i.p.; Sabex, Boucherville, Quebec). The same surgical procedures were used for the Sham rats except that no damage was done to the skull or brain. The rats were allowed to recover for a minimum of 10 days before subsequent conditioning or testing.

### *Histology*

After completion of the experiments, all animals were sacrificed by administering an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with phosphate buffered saline (0.9% PBS) followed by 4% paraformaldehyde (PFA) in PBS. The brains were removed and post-fixed for 24 hr in PFA, then transferred and stored in 30% sucrose and PBS with sodium azide (0.02%) for at least 48 hr before sectioning. The brains were sectioned in the coronal plane 40 $\mu$m thick using a cryostat microtome (-19°C); every fourth section taken throughout hippocampus in all groups. Sections were wet-mounted on glass microscope slides and later stained with cresyl violet for visualization of hippocampal lesion induced damage and remaining tissue.

### *Lesion Quantification*

Volume of spared hippocampus tissue was calculated using the Cavalieri method. Images of cresyl violet stained sections from a single series (approximately 5 sections throughout the extent of the hippocampus) were taken using a Zeiss Axioskop 2 MotPlus epifluorescent scope attached to a QImaging Retiga CCD camera (Burnaby, British Columbia, Canada). Images were then analyzed using ImageJ software (http://rsb.info.nih.gob/ij/) in which a sampling grid with an area per point of $0.002mm^2$ was created and randomly thrown over each image. The total number of points in contact with the hippocampus tissue in each section was counted. The number of points per section was multiplied by the area associated with each point, the section thickness and then the section sampling fraction. These numbers were then summed to provide the total estimated volume of the spared hippocampus tissue. Percent damage in each of the the lesioned rats was calculated by dividing the quantified spared tissue volume by the average hippocampus volume of the control group, then multiplying by 100.

## ***Apparatus and Procedures***

### *Contextual Fear Conditioning*

Conditioning and testing were carried out in four identical observation chambers (30 x 24 x 21 cm; MED-Associates, Burlington, VT). The chambers were constructed from aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door) and were situated in sound-attenuating cabinets located in a brightly lit and isolated room. The floor of each chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center to center). Rods were wired to a shock source and solid-state grid scrambler (MED-Associates) for the delivery of footshock unconditioned stimuli. The chambers were cleaned with dilute Quatsyl and stainless steel trays cleaned with the same solution were

placed underneath the grid floors. Ventilation fans in each cabinet supplied background noise (65 dB, A scale).

For contextual fear conditioning procedures, rats were transported to the conditioning room four at a time in separate plastic transport tubs, placed in the conditioning chambers, and allowed to explore for 3 min before 5 foot shocks (2s duration, 1mA amplitude) were administered with an inter-shock interval of 58-s. The duration of the conditioning session for each rat was 8 min. Following the conditioning session, rats were immediately transported back to their home cage where they remained until retention testing. The retention session was conducted 11 days after surgery. Rats were transported to the conditioning room in the same manner as on the conditioning day; each animal was placed into the conditioning chamber for a 5 min extinction session. Behaviour while in the conditioning context was digitally recorded using FreezeFrame Video-Based Conditioned Fear System and analyzed by Actimetrics Software (Coulbourn Instruments, Wilmette, IL) for average freezing times. Freezing was defined as the absence of movement except for that due to respiration.

### *Evaluation of Post-Operative Behavioural Changes*

The behaviour of each rat was continuously evaluated for 3 h following surgery, and observations were made according to a rating scale previously described by Sperk et al., 1985(Sperk, Lassmann, Baran, Seitelberger, & Hornykiewicz, 1985) and Baran et al., 1985(Baran, Sperk, Hortnagl, Sapetschnig, & Hornykiewicz, 1985) with slight modifications, developed by direct comparison with seizure scores used in the amygdala kindling model. The following scores were used for rating severity of NMDA induced seizures: 0, no seizures; 1, eye closure, twitching of vibrissae, sniffling, facial clonus, staring; 2, head nodding associated with more severe facial clonus; 3, unilateral or bilateral forelimb clonus; 4, rearing, often accompanied by bilateral forelimb clonus; 5, rearing with loss of

balance and falling accompanied by generalized clonic seizures; 6, sustained generalized clonic convulsions (convulsive status epilepticus); 7, jumping/tonic seizure; and 8, respiratory arrest. The duration of each seizure score was recorded independently.

Table 5.1: Coordinates used for 7-site hippocampal lesion in adult female rate (measurements in millimetres relative to bregma).

| Site | Anterior | Lateral | Ventral |
|---|---|---|---|
| 1 | –3.1 | ±1.5 | –3.6 |
| 2 | –4.1 | ±3.0 | –4.0 |
| 3 | –5.0 | ±3.0 | –4.0 |
| 4 | –5.0 | ±5.2 | –7.3 |
| 5 | –5.8 | ±4.4 | –4.4 |
| 6 | –5.8 | ±5.1 | –7.5 |
| 7 | –5.8 | ±5.1 | –6.2 |

Table 5.2: Studies directly assessing temporally graded retrograde amnesia using contextual fear conditioning.

| Memory task | Damage | Lesion technique | RA duration (days) | Reference |
|---|---|---|---|---|
| Context fear | 25% dorsal | Electrolytic | 1 (27) | Kim and Fanselow (1992) |
| Context fear | 25% dorsal | Electrolytic | 1 (49) | Anagnostaras et al. (1999) |
| Context fear | 40% dorsal | Neurotoxic (NMDA) | 28 (71) | Maren et al. (1997) |
| Context fear | 40% dorsal | Neurotoxic (NMDA) | Flat (180) | Lehmann, Lacanilao et al. (2007) |
| Context fear | 85% d+v | Neurotoxic (NMDA) | Flat (180) | Lehmann, Lacanilao et al. (2007) |
| Context fear | 40% dorsal | Neurotoxic (NMDA) | Flat (84) | Sutherland et al. (2008) |
| Context fear | 40% ventral | Neurotoxic (NMDA) | Flat (84) | Sutherland et al. (2008) |
| Context fear | 85% d+v | Neurotoxic (NMDA) | Flat (84) | Sutherland et al. (2008) |
| Context fear | 85% d+v | Neurotoxic (NMDA) | Flat (35) | Sparks et al. (current study) |

## Results

### *NMDA Produces Pronounced Tissue Damage*

The NMDA injections produced extensive cell loss in all of the principal subfields (CA1-CA3) of the hippocampus, the dentate gyrus, as well as the anterior portion of the subiculum for each rat in both the NMDA alone and NMDA+TTX groups at the recent and remote

time points. Figure 5.1 shows a schematic reconstruction of the extent of brain damage caused by the NMDA infusions. Using unbiased stereology (Schmitz & Hof, 2005), it is estimated that 86.0% of the hippocampus was damaged by infusions of NMDA alone (SD = 6.7; Min = 74.5; Max = 93.7), and 84.8% of the hippocampus was damaged by the combined infusion of NMDA and TTX (SD = 5.5; Min = 77.7; Max = 93.8). Rats estimated to have less than 70% total damage were removed from analysis, yielding NMDA group n = 13 and NMDA+TTX n = 14 (see Figure 5.2B). The amount of hippocampal damage did not differ between these two lesion conditions ($F_{2,25} = .225, p = 0.640$) at either interval ($F_{2,25} = .016, p = 0.901$). Damage to the hippocampus extended throughout the dorsal and ventral hippocampus. The lowering and withdrawal of the injection cannulae also caused minor damage in the parietal cortex. No noticeable damage was found in the thalamus or basolateral and central region of the amygdala in any of the rats. Note that the lesion size estimates did not include one rat from the NMDA and three from the NMDA+TTX groups because of difficulties with the histology method, but the behavioural scores of these rats were retained and used in the behavioural analysis.

### *TTX Controls Seizure Severity*

NMDA induced seizures were assessed for the first 3 hours following surgery. The rating scale that was used ranks bouts of seizure activity by severity and was modified from that previously used in kindling studies (Sperk et al., 1985; Baran et al., 1985). The following scores were used for rating severity of NMDA induced seizures: 0, no seizures; 1, eye closure, twitching of vibrissae, sniffling, facial clonus, staring; 2, head nodding associated with more severe facial clonus; 3, unilateral or bilateral forelimb clonus; 4, rearing, often accompanied by bilateral forelimb clonus; 5, rearing with loss of balance and falling accompanied by generalized clonic seizures; 6, sustained generalized clonic convulsions (convulsive status epilepticus); 7, jumping/tonic seizure; and 8, respiratory arrest. A total

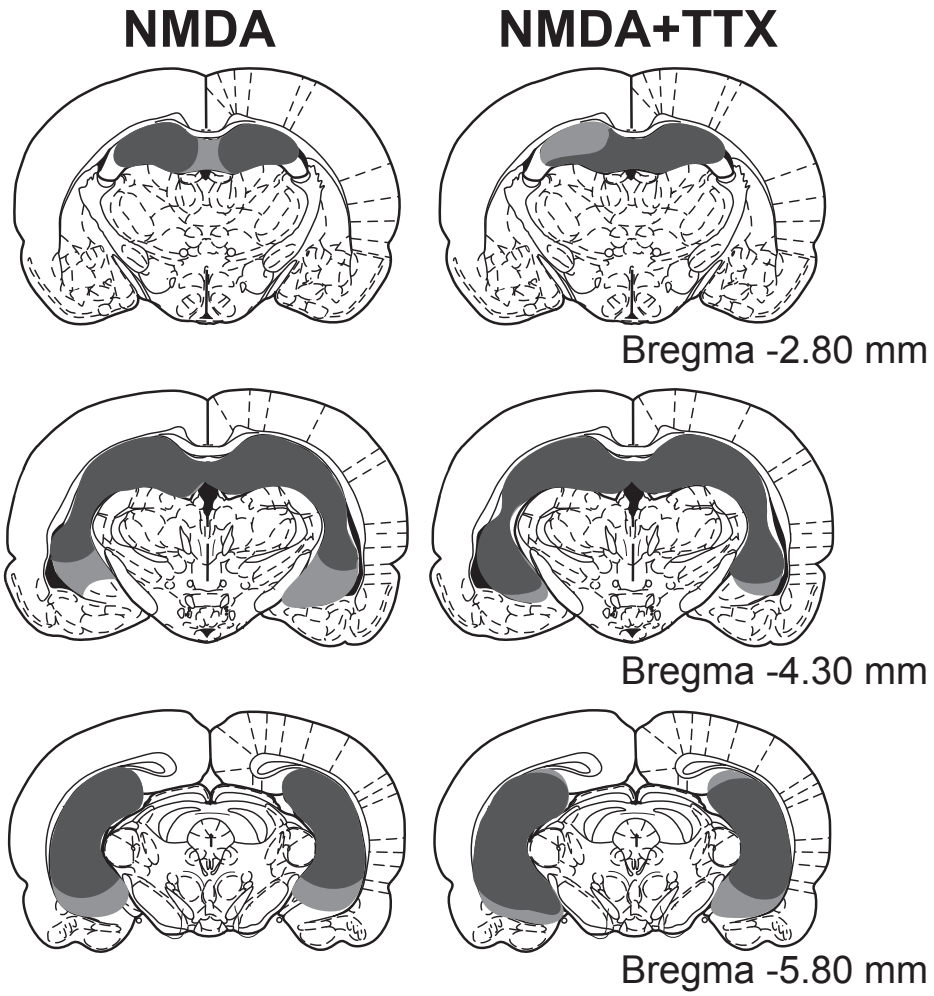


Figure 5.1: **Histology.** Illustration of the smallest (dark grey) and largest (light grey) lesion observed bilaterally through the rostral and caudal extent of the hippocampus for each lesion group.

seizure score was calculated for each rat by first taking the sum of the seizure durations for each rating, multiplying this sum by the rating number, and finally taking the sum of the rating x duration products. This seizure score allows for comparison of the duration and severity of seizures across groups (see Figure 5.1A). Details of the seizure score equation (1) are as follows: $S$ is the total seizure score for an individual rat, $r$ is the rating number, $d$ is the individual seizure durations for each rating $r$, and $n$ is the number of seizures for each rating number.

$$S = \sum_{r=1}^{8} \left( r \left( \sum_{j=1}^{n} d_j \right) \right) \tag{5.1}$$

The rats of the sham group underwent the same scoring procedure, and none showed signs of seizure activity, therefore they were not included in the analysis. In addition, two rats from the NMDA+TTX group had incomplete video data, so were excluded from the

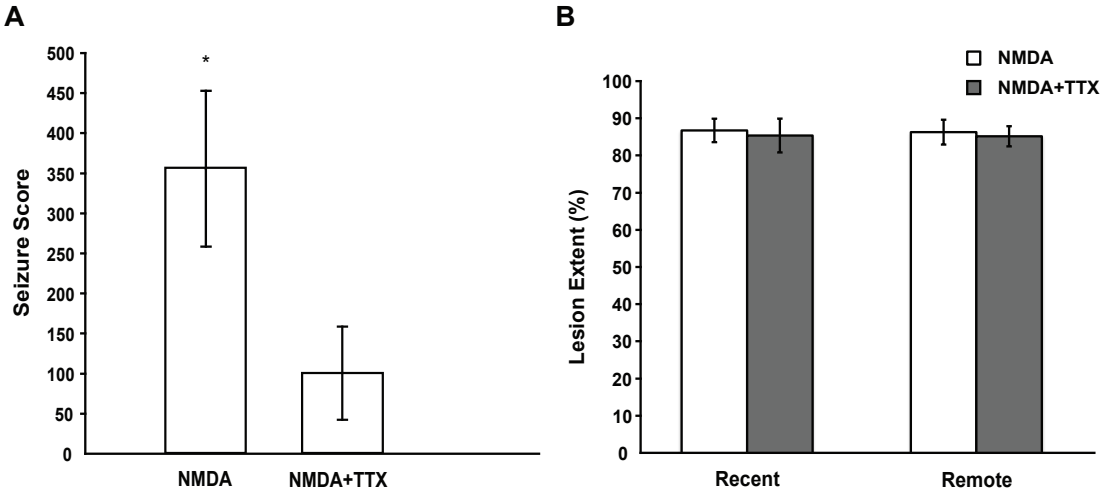


Figure 5.2: **Post-Operative Seizure Scores and Hippocampus Damage.** (A) Mean ± SEM seizure rating scores calculated for the 3 hr post-operative period, using equation (5.1). TTX significantly decreased NMDA mediated seizure activity ($p = .046$). (B) Mean ± SEM lesion extent calculated for each lesion group at the Recent and Remote intervals. All lesion groups sustained equivalent hippocampus damage.

analysis. An independent-samples *t*-test was conducted to compare seizure scores for the NMDA and NMDA+TTX lesion groups, revealing a significant difference ($t_{25} = 2.096, p = 0.046$) between the two groups. Moreover, the seizure scores for the NMDA+TTX lesion group were not significantly greater than the non-lesioned control group ($t_{11} = 1.340, p = 0.207$). Taken together, these results indicate that infusion of NMDA produced significant levels of seizure activity, and that TTX was effective at suppressing this NMDA mediated seizure activity to a level indistinguishable from controls. Furthermore this degree of seizure reduction did not affect the extent of NMDA-induced hippocampus damage.

### *Hippocampus Damage Results in Retrograde Amnesia for both Recent and Remote Memories*

Figure 5.3 shows the percentage of time spent freezing during the retention test for the recent (A) and remote (B) time points. A two-way ANOVA with lesion condition as between-group factor (Sham, NMDA, and NMDA+TTX) and learning-surgery interval as between-groups factor (1 week and 5 weeks) revealed a significant main effect of lesion ($F_{2,45} = 28.236, p < 0.001$) and no main effect of learning-surgery interval ($F_{2,45} = .032, p = 0.860$). The lesion-interval interaction was not statistically significant ($F_{2,45} = .280, p = 0.758$) suggesting that treatment equally impaired both recent and remote memories. Analysis of the main effect of lesion revealed that hippocampal lesions caused retrograde amnesia. Specifically, pairwise comparisons of data collapsed across learning-surgery intervals indicated that the level of freezing by the Sham group was significantly greater than both NMDA and NMDA+TTX groups ($p < 0.001$). A significant difference was also found between freezing levels of the NMDA and NMDA+TTX groups ($p = 0.032$).

NMDA mediated hippocampus damage significantly impaired both recent and remote contextual fear memory. When NMDA lesion-induced seizure activity was reduced, the

severity of the memory impairment was also reduced, which suggests that the seizure activity contributed to the levels of retrograde amnesia observed in the NMDA alone groups. It is important to note that although the memory impairment was reduced, the NMDA+TTX groups still showed severe retrograde amnesia, and that this impairment was equivalent at both the recent and remote time points. Recent and remote contextual fear memories are equally susceptible to disruption by seizure activity, but this disruption in and of itself does not account for the full extent of retrograde amnesia observed following NMDA lesions.

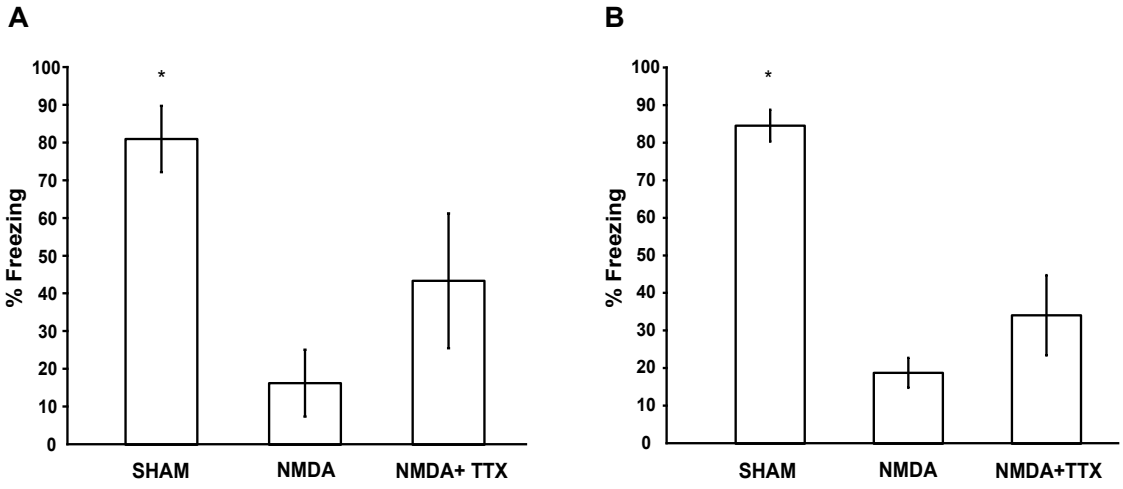


Figure 5.3: **Fear Conditioning Retention Sessions.** Mean ± SEM (A) Recent time point. Percentage freezing during the context memory retention session for rats that underwent contextual fear conditioning 1 week prior to hippocampus surgery. Sham rats froze significantly more than either lesion group. (B) Remote time point. Percentage freezing during the context memory retention session for rats that underwent contextual fear conditioning 5 weeks prior to hippocampus surgery. Sham rats froze significantly more than either lesion group.

## Discussion

The current experiments were designed to assess the effects of neurotoxic lesion induced seizure activity on the retrieval of recent and remote contextual fear memory. Seizure severity associated with NMDA lesions of the hippocampus was effectively limited by co-

infusion with the sodium channel blocker TTX. We determined that NMDA induced hippocampus damage caused severe retrograde amnesia for contextual fear memory, regardless of the age of the memory. Importantly, this memory impairment was also severe for recent and remote memory when lesion-induced seizure severity was suppressed. Taken together, these experiments demonstrate that seizure activity following NMDA mediated hippocampal lesions is not completely responsible for the impairment of contextual fear memories, regardless of the age of the memory. We show that the hippocampus is necessary for the recall of recent as well as remote contextual fear memory, and confirm that the loss of hippocampal cells per se is driving the amnesia reported following hippocampal lesions in contextual fear tasks.

### *Seizure Activity can Cause Memory Impairments*

Seizure activity with a HPC focus is thought to have the potential to disrupt memories (Jarrard & Meldrum, 1993; McClelland et al., 1995; Liang et al., 2000; Anagnostaras & Gale, 2002). The connection between seizure activity and amnesia has been well documented by research on humans (Butler et al., 2007; Butler & Zeman, 2008; Hornberger et al., 2010; Milton et al., 2010), cats (Kesner & Doty, 1968), and rodents (Sideroff, 1975). In all of these cases, studies are presented showing either retrograde amnesia, anterograde amnesia, or both following periods of seizure activity. Studies in rats have assessed the relation between seizure activity and memory processes by manipulating the induction of seizure activity to affect the ability of a region such as the hippocampus or amygdala to consolidate or retrieve information, and this can be done pharmacologically (Genkova-Papazova & Lazarova-Bakarova, 1995; Genkova-Papazova, Shishkova, & Lazarova-Bakarova, 2001) or using electrical stimulation (Schmitz & Hof, 2005).

Given the known effects of seizure activity on learning and memory, it is reasonable to ask whether certain lesion techniques produce misleading results when assessing effects of

loss of a specific neural structure on memory system function. NMDA, a potent excitatory amino acid, has severe effects on neurons by increasing the frequency of neuronal activity (Olney, 1978), creating sustained depolarization (Olney, 1978), and permitting excessive calcium influx from the extracellular space (Berdichevsky, Riveros, Sanchez-Armass, & Orrego, 1983; Griffiths, Evans, & Meldrum, 1983; Retz & Coyle, 1984). The neurotoxic effects of NMDA prompt an increase in osmolarity of the cell resulting in lysis (Rothman, 1985). Through these processes, many excitatory amino acids can have convulsant effects to varying degrees, of which NMDA has been found to produce the most pronounced seizure activity, that lasts for the longest duration (Zaczek & Coyle, 1982). We have shown that selective neurotoxic NMDA lesions of the hippocampus can lead to seizure activity (see Figure 5.1). Given that seizure activity can disrupt memory processes, and NMDA lesions cause seizure activity, these results leave open the possibility that NMDA induced seizure activity is responsible for retrograde amnesia found in certain studies of memory.

Consistent with other studies showing memory impairments following seizures, our current study suggests that seizure activity makes a modest contribution to retrograde amnesia following NMDA lesions. Indeed, when the groups at the recent and remote time points are collapsed, pairwise comparisons show that the NMDA alone group froze significantly less than the NMDA+TTX group. Although this effect is small when compared to the overall effect of hippocampus damage on freezing performance, decreasing seizure activity resulted in slightly less severe retrograde amnesia. Though a decrease in seizure activity resulted in better memory performance, lesioned rats were equally impaired at both the recent and remote time points. Because the contribution of seizure activity to the overall memory impairment is small for contextual fear conditioning, that is not to say that memory in other tasks will be affected to a greater degree. Careful consideration should be given to this factor in all experimental designs that use neurotoxic lesions of the hippocampus.

### *Equivalent Retrograde Amnesia for Recent and Remote Contextual Fear Memories Following Complete NMDA Lesions*

The current findings are consistent with similar studies showing that lesions of the hippocampus made at a recent or remote time point after learning, produce robust retrograde amnesia for contextual fear memories (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008; Lehmann, Rourke, & Bernard, 2009; Sparks, Spanswick, Lehmann, & Sutherland, 2009). By manipulating the level of seizure activity produced by NMDA lesions, we have shown that the retrograde amnesia of rats in the high seizure versus low/no seizure groups is significant compared to the Sham control groups. This comparison is consistent across both recent and remote intervals. These results confirm that retrograde amnesia was due to removal of a memory system that contributes to the maintenance and expression of recent and remote contextual fear memories, and not due to disruptive seizure activity.

Equally robust retrograde amnesia for recent and remote memories is labeled a *flat gradient*, where the retrieval of the target memory is dependent on the integrity of the hippocampus. Our finding of a flat gradient following hippocampus damage adds support to the idea that the hippocampus plays a long-term, likely permanent, role in the maintenance of contextual fear memory. This permanent role of the hippocampus in memory maintenance/retrieval is supported by other studies designed to directly assess the time-dependent role of the rat hippocampus in tasks such as learning to fear a discrete stimulus (tone or light) (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Sutherland et al., 2008; Lehmann et al., 2010), spatial navigation (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001; Clark et al., 2005a, 2005b; Martin et al., 2005), object discrimination (Sutherland et al., 2001; Mumby et al., 1999; Lehmann, Glenn, & Mumby, 2007; Lehmann, Lacanilao, & Sutherland, 2007), object exploration (Gaskin et al., 2003), shock-probe memory (Lehmann, Lecluse, et al., 2006), and picture memory (Epp et al.,

2008). Though the pattern of flat gradients is predominant, a number of studies show a change in the dependence of retrieval on the hippocampus as the learning-to-lesion interval increases, where recent memories are more dependent on normal hippocampus functioning than remote memories—a term called *temporal gradient*. This change in hippocampal dependence has been reported in context fear conditioning (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Winocur et al., 2009), trace eyeblink conditioning (Takehara et al., 2003), trace fear (Quinn et al., 2008), and flavour/odour memory (Winocur, 1990; Winocur et al., 2001; Clark et al., 2002; Ross & Eichenbaum, 2006; Tse et al., 2007). The Standard Model of Systems Consolidation (SMSC) (Squire et al., 2004) accounts for temporal gradients by stating that certain memories that are dependent on the HPC, through a temporally based consolidation process become independent of the hippocampus. Though evidence supporting this theory in the rat is limited (see Sutherland et al. 2011, 2010 for comprehensive reviews), it stands as an essential part of the dominant view of hippocampal function in the rat literature.

One might predict that because of associated seizure activity, NMDA lesions would have greater disruptive effects on distal memory networks than other lesion techniques. If this were true, then memories contained in distal networks might be disrupted to a degree that, after hippocampal lesion, the original memories cannot be expressed. This level of disruption would make interpretation of retrograde amnesia following hippocampus damage difficult to attribute to the function of the hippocampus *per se*. Remote memories that are weakly established in non-hippocampal networks due to a systems consolidation process might be especially susceptible to disruptive seizure activity. If this is the case, and if context memories are consolidated outside the hippocampus with the passage of time, then the seizure activity produced during/after NMDA lesions could be responsible for the flat gradients observed in Table 5.2. Our present results count against this possibility by clearly showing that if levels of seizure activity are reduced during NMDA lesions,

retrieval of both recent and remote contextual fear memory is equally impaired. Hence, NMDA induced seizure activity does not disrupt contextual fear memories that might be stored in non-hippocampal networks. The equivalent retrograde amnesia seen at both recent and remote time points adds direct support to the view that if contextual fear memories are initially dependent on the hippocampus, they will always require the hippocampus for retrieval—flat gradients should always be observed.

Clearly, flat gradients have not always been observed using contextual fear conditioning in the rat. Maren et al. (1997) used small NMDA lesions of the dorsal hippocampus, and reported that contextual fear memories 100 days old are less susceptible to hippocampus damage than recent memories (either 1 or 28 days old). At first glance, this result shows that with NMDA lesions a temporal gradient can be observed in this task. Though, after closer examination of the methods and data, the findings are not compelling and do not add strong support to the conclusions. The analysis in this paper requires more explanation in order to make explicit why the data may show temporally graded retrograde amnesia. For instance, the means for each group were calculated by selecting a single 64-s time bin (out of the 8 bins in the retention session) in which each independent rat showed the highest level of freezing. Using this method, the authors present a figure that shows a strong temporal gradient as the training-to-lesion interval increases. An approach as selective as this is not in line with the typical methodology of contextual fear analysis in the field. The typical method of quantifying freezing behaviour is to record freezing levels across a pre-defined extinction session and then average the levels of freezing found in each time bin (bin size being irrelevant) (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008). Between-bin variability is not accounted for by selecting and reporting one bin from each rat.

Other evidence for temporally graded retrograde amnesia following hippocampus damage in context fear paradigms comes from studies using electrolytic lesions (Kim & Fanselow,

1992; Anagnostaras et al., 1999). As with the Maren et al. (1997) (Maren et al., 1997) study, these experiments used relatively small lesions of the hippocampus. As seen in Table 5.2, the two studies reporting a temporal gradient report damaged approximately 25% of the dorsal hippocampus (Kim & Fanselow, 1992; Anagnostaras et al., 1999). Studies in which hippocampal volume was quantitatively assessed following lesions show that spared tissue can support memory retrieval in tasks that involved picture discrimination memory and contextual fear memory (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008; Epp et al., 2008). The spared tissue in the studies by Kim and Fanselow (1992) and Anagnostaras et al. (1999) could have supported memory retrieval at the remote time points in their studies using electrolytic lesions. Because of the small amount of hippocampal tissue damaged in these studies, it is difficult to interpret the results as being strong support for the SMSC.

Of the experiments using large hippocampal lesions, a flat gradient is the norm (Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008). The one exception to this pattern is a study conducted by Winocur et al. (2009), in which temporally graded retrograde amnesia was observed when hippocampus damage was performed either 1 or 28 days following training. One difference that sets this study apart from others using large lesions is that the rats were conditioned to a tone-shock pairing within the context. It is not clear how this procedural difference would lead to spared memories at a remote training-to-surgery interval. Using a range of small to large NMDA lesions, we have attempted to replicate studies showing temporally graded retrograde amnesia following tone-shock conditioning, and have consistently found flat gradients (Sutherland et al., 2008). In further support of flat gradients, we have also investigated weak vs strong contextual fear conditioning (Sparks et al., 2009; Lehmann, Sparks, et al., 2009), small vs large lesions in dorsal, ventral, or dorsal+ventral hippocampus (Sutherland et al., 2008), and found significant retrograde amnesia at both recent and remote surgery-to-lesion time points. In all cases, no evidence of

*temporally graded retrograde amnesia* have been found. On the contrary, *flat gradients* are the norm.

Reduction of seizure activity produced during neurotoxic (NMDA) lesions of the hippocampus at a recent or remote time point did not spare contextual fear memories. Memories that were established 1 week or 5 weeks prior to surgery were equally susceptible to hippocampus damage. Together with the results from studies in rats using tasks such as fear to a discrete stimulus (tone or light) (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Sutherland et al., 2008; Lehmann et al., 2010), spatial navigation (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001; Clark et al., 2005a, 2005b; Martin et al., 2005), object discrimination (Sutherland et al., 2001; Mumby et al., 1999; Lehmann, Glenn, & Mumby, 2007; Lehmann, Lacanilao, & Sutherland, 2007), object exploration (Gaskin et al., 2003), shock-probe memory (Lehmann, Lecluse, et al., 2006), and picture memory (Epp et al., 2008), the current study supports the supposition that if the hippocampus is involved in establishing a memory, it will always be involved in retrieval of that memory, either at a recent or remote time point. These results score against the view that the hippocampus plays a time limited role in the retrieval of certain types of memory, a view purported by the Standard Model of Systems Consolidation. Seizure activity produced by NMDA lesions of the hippocampus is unlikely to be responsible for the degree of retrograde amnesia for remote memories found in studies of the hippocampus and fear conditioning. A simpler view is supported here, that retrograde amnesia following hippocampal lesions is due specifically to the loss of cells within the hippocampal network.

# Chapter 6

## Putting the Interactive Role of the Hippocampus in Context

The results of the present experiments suggest a surprisingly simple view on hippocampus and memory. We review experiments that provide evidence of multiple memory systems that can support contextual fear behaviour. These hippocampal and non-hippocampal systems interact in such as way as to provide appropriate behavioural output. This interaction can be one of interference, whereby the hippocampus inhibits learning in other systems. Hippocampal interference can occur at the time of learning **and** at the time of remembering. We also review recent experimental evidence that supports the view that if the hippocampus is involved in the initial storage of a memory, then it will always be involved in the accurate retrieval of that same memory. When the hippocampus is damaged following learning contextual fear, the result is profound amnesia. This result is consistent with leading theories of hippocampal function, though these theories predict that only recently formed memories will be affected, and those more remote from the time of learning will be spared disruption. Our results do not support this prediction. We evaluate the status of the Standard Model of Systems Consolidation in light of the experiments presented here and those within the recent rat literature. Alternative theories to the standard model are then introduced and critically assessed.

## Hippocampal Interactions During Learning

As discussed in Chapter 1, there is a number of studies that suggests that the hippocampus interacts with non-hippocampal systems in significant ways (Frank, Rudy, & O'Reilly, 2003; McClelland et al., 1995; Sherry & Schacter, 1987; White & McDonald, 2002; Fanselow, 2010). Memory in several tasks can be supported by the hippocampus as well as non-hippocampal systems. Support for this assertion is evident in the dissociation of retro-

grade and anterograde amnesia following hippocampal damage—characterized by amnesia for task-specific memory following damage, and no impairment in learning the same task in the absence of a hippocampus. So far, this finding has been demonstrated for contextual fear memory (Lehmann, Sparks, et al., 2009; Wang et al., 2009; Wiltgen et al., 2006; Maren et al., 1997; Sparks et al., 2011a), object recognition (Broadbent, Squire, & Clark, 2007; Gaskin et al., 2003), shock-probe conditioning (Lehmann, Carfagnini, Yamin, & Mumby, 2005; Lehmann, Sparks, et al., 2006), visual discrimination (Driscoll et al., 2005; Sutherland et al., 2001), fear-potentiated startle (Lehmann et al., 2010), pattern discrimination (Broadbent et al., 2007), and home base memory (Lehmann, Clark, & Whishaw, 2007; Travis et al., 2010). These results have been interpreted to mean that: *a*) When the hippocampus is intact during learning it interferes with other systems and prevents them from acquiring an independent contextual fear conditioning memory; and *b*) when the hippocampus is absent, these other systems are released from this interference and are able to rapidly acquire an independent memory (Maren et al., 1997; Frankland et al., 1998; Fanselow & Poulos, 2004; Driscoll et al., 2005; Lehmann, Sparks, et al., 2006; Sutherland et al., 2006). Even though both the hippocampal and non-hippocampal systems can acquire memory to support task performance, because of the differences in connectivity and architecture of the systems, it is likely that the memories are qualitatively different.

Evidence for hippocampal interference during contextual fear learning was presented in Chapters 2 & 3. In these experiments, one group of rats sustained extensive hippocampal damage prior to training, that subsequently removed hippocampal interference associated with learning. The non-hippocampal systems gained the appropriate associations that would later manifest in learned freezing behaviour (see Figure 3.2B). Conversely, another group received hippocampal damage following learning, and when later returned to the training context displayed a substantial deficit in freezing (Figure 3.2A). Because the hippocampus was present in this second group during learning, a contextual fear repre-

sentation was not formed in non-hippocampal systems that would allow for hippocampal-independent retrieval. Both of these groups received the same conditioning and testing parameters, so we can infer that within one conditioning session the hippocampus and non-hippocampal systems have the capacity to establish a contextual fear representation that produces equivalent learned freezing.

There are a number of ways to interpret the dissociation between retrograde and anterograde amnesia following hippocampal damage: there could be a direct inhibition of other systems by the output from the hippocampus, there could be a competitive interaction at the time of action or response selection, or there could be a competition for associative strength mediated by a third structure. The view taken here is that during learning the hippocampus interferes with, or prevents, other systems from acquiring a memory representation that can be expressed in the absence of the hippocampus. It is unknown where the interference takes place, and it is reasonable to hypothesize that the interference occurs in multiple systems as part of a complex process.

One possible candidate for locus of hippocampal interference comes from the work of Biedenkapp and Rudy (2009) on the ventral subiculum and basolateral amygdala. Extending from work performed by Guarraci et al. (1999) in which infusion of the dopamine D1 receptor agonist SKF82958 into the basolateral amygdala increased the amount of conditioned fear, Biedenkapp and Rudy (2009) reasoned that a similar procedure may attenuate interference if this region is in fact where the competition occurs. Biedenkapp and Rudy (2009) data supported this hypothesis by allowing the non-hippocampal systems to establish a representation of contextual fear that could be later expressed in the absence of the hippocampus.

Given the evidence supporting hippocampal interference of other systems during learning certain tasks, it is reasonable to ask whether some set of learning parameters would make it possible for this interference to be overcome. Can non-hippocampal systems estab-

lish a hippocampal-independent representation in the presence of an active hippocampus?

One learning parameter that we evaluated (Chapter 2) involved varying the temporal distribution of learning episodes. Distributed learning may establish stronger memories than the same amount of conditioning performed in a single session (Fanselow, DeCola, & Young, 1993; Fanselow & Tighe, 1988). Using this premise, we reasoned that by distributing a contextual fear conditioning session over multiple days, a stronger representation could be formed in non-hippocampal systems such that after removing the hippocampus the contextual fear memory would be expressed (Lehmann, Sparks, et al., 2009). Distributed conditioning sessions are theorized to be different in that each session incrementally adds information to non-hippocampal memory representations. Therefore, non-hippocampal systems can acquire a strong memory representation over many sessions. Rats received either context fear conditioning (total of 12 context-foot shock pairings) within one session, or the same number of context-shock pairings distributed across six days (total 11 sessions). Importantly, both the total time spent in the conditioning context and the number of shocks were consistent for each group. Extensive hippocampal lesions were made in the rats of each group to assess memory recall in the absence of the hippocampus. Consistent with the contextual fear literature, rats that underwent a single conditioning session exhibited severe retrograde amnesia when returned to the conditioning context. Conversely, the rats that experienced distributed learning displayed good memory retention, and did not differ from their respective control group. Distributed learning created a memory resistant to very extensive hippocampal damage by overcoming hippocampal interference of non-hippocampal memory systems during learning.

The data presented here are the first to demonstrate that a recent hippocampal-dependent contextual fear memory can survive hippocampal damage. There is consensus within the field that hippocampal damage soon after learning produces robust retrograde amnesia (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Lehmann, Lacanilao, & Sutherland,

2007; Maren et al., 1997; Sutherland et al., 2008; Lehmann, Sparks, et al., 2009; Sparks et al., 2011b). Importantly, all of these studies used massed conditioning. Is it reasonable to suggest that making context memories independent of the hippocampus through distributed learning is a model for some patterns of temporally graded retrograde amnesia? This question is addressed below, in *Hippocampal Interactions Over Time*.

## Hippocampal Interactions During Remembering

If the hippocampus can interfere at the time of learning with the acquisition of an independent contextual fear memory in non-hippocampal systems, then it is reasonable to ask if it can also interfere, with memories that are already well established in other systems. In Chapter 3 we present experiments that were designed to address this hypothesis. As described above, single-session contextual fear conditioning can establish independent memory in non-hippocampal networks when conditioning takes place in the absence of a functional hippocampus (Lehmann, Sparks, et al., 2009; Wang et al., 2009; Wiltgen et al., 2006; Maren et al., 1997; Sparks et al., 2011a). Once this memory is established, restoring hippocampal function at the time of remembering should produce an opportunity for the hippocampus to interfere with retrieval of that memory. To achieve this design, we employed temporary inactivation techniques that allowed for "turning off" the hippocampus for a prescribed period of time during learning, remembering, or both time points (refer to Chapter **??** for detailed description of this technique).

Consistent with the permanent lesion data showing no anterograde amnesia for context fear following hippocampal damage, inactivating the hippocampus prior to learning as well as prior to remembering did not affect memory performance (Sparks et al., 2011a). In this case, non-hippocampal systems acquired a context fear memory that was expressed during the retention session (all in the absence of the hippocampus). Another group of rats underwent the same conditioning procedure with an inactive hippocampus, but dur-

ing the retention session had reinstated hippocampal function prior to remembering. In this situation, the non-hippocampal system does have a context fear representation that can be expressed, and the hippocampus has no record of the experience of receiving foot shock within the conditioning context, or of the context itself. There is an incongruence of memory representation between the two systems, and the hippocampal representation dominated for expression of the memory. For these rats, the hippocampus expressed a memory that did not reflect prior experience congruent with the context-shock interaction, and they displayed no fear of the context. To further confirm that the non-hippocampal fear memory could indeed be expressed, we performed a second retention test the following day, though this time with an inactive hippocampus. Because the hippocampal interference was removed during this retention session, the rats showed significant levels of contextual fear memory no different than the control group.

We propose that the hippocampal interference found during remembering is not unlike that found during learning. The interference could result from the hippocampal output being a significant modulator of non-hippocampal system representation at the time of memory expression. Again, this interaction could be happening at the level of the amygdala as is likely the case during learning (Biedenkapp & Rudy, 2009). When a rat enters a conditioning context for the first time, the hippocampus is naïve to this experience and quickly established a contextual representation that can be associated with a foot shock (Fanselow, 1990). At this time, the hippocampus acts to inhibit other systems from establishing an independent representation (or association with the amygdala). Likewise, when the naïve hippocampus enters the conditioning context during the retention session, it is possible that this naïve hippocampal representation interferes with the "appropriate" context fear memory stored in non-hippocampal regions.

## Hippocampal Interference and Multiple Memory Systems

Data presented so far suggests that hippocampal interference during learning and remembering involves at least two memory systems, one being the hippocampus and the other non-hippocampal regions. Each of these systems exhibit a learning rate dependent parameters set by their intrinsic network circuitry as well as modulatory interactions from other systems. For contextual fear memory, the hippocampal system is fast learning when compared to the non-hippocampal system, though when hippocampal interference is removed from the non-hippocampal learning rate parameters, the non-hippocampal system can learn just as quickly (Lehmann, Sparks, et al., 2009; Wang et al., 2009; Wiltgen et al., 2006; Maren et al., 1997; Sparks et al., 2011a). Each of these systems has the capacity to learn and express contextual fear memory. Contextual fear is not the only memory that can be similarly supported by multiple memory systems (i.e., hippocampal and non-hippocampal systems), as evidence has been also shown for object recognition (Broadbent et al., 2007; Gaskin et al., 2003), shock-probe conditioning (Lehmann et al., 2005; Lehmann, Sparks, et al., 2006), visual discrimination (Driscoll et al., 2005; Sutherland et al., 2001), fear-potentiated startle (Lehmann et al., 2010), pattern discrimination (Broadbent et al., 2007), and home base memory (Lehmann, Clark, & Whishaw, 2007; Travis et al., 2010).

Traditional views of how multiple memory systems operate cannot accommodate the described dissociation between retrograde and anterograde amnesia. Traditional theories are founded upon the premise that each memory system contains circuitry that performs a hard-wired style of processing essential in carrying out specific forms of learning (Squire, 1992; White & McDonald, 2002). Therefore, removing a system before or after learning is predicted to result in equivalent retrograde and anterograde amnesia—removing a system disables learning a certain memory, or it does not. The situation of multiple memory systems capable of learning and supporting similar task behaviour requires a more dynamic view of multiple-systems interaction, and how such a model might operate has been sug-

gested by Fanselow (2010). According to this framework, memory systems can be viewed as primary and alternate pathways able to mediate similar behaviours. These pathways are distinct in their efficiency, primary pathways being more efficient than alternates. During learning, primary pathways may compete and interfere with (or inhibit) learning within alternate pathways.

Fanselow (2010) summarizes the principles of a dynamic model of memory systems as: " *a*) there are primary and alternate pathways able to mediate fear behaviour; *b*) the alternate pathways are less efficient compared with the primary pathway; *c*) the more-efficient primary pathway dominates the learning and simultaneously prevents significant learning in the alternate pathway(s); *d*) the alternate pathways compensate when the dominant pathway is compromised; *e*) plasticity in these circuits might be regulated by the same mechanisms responsible for competitive learning between stimuli and described by Pavlovian principles (Fanselow, 1980); and *f*) this model provides a set of rules for how the brain can select specific and efficient circuits for production of specific adaptive behaviours". An examination of the data within this thesis showing systems-interaction between the hippocampus and non-hippocampal networks shows strong support for the idea of a dynamic model of memory systems.

## Hippocampal Interactions Over Time

### *Hippocampal Involvement in Long-Term Contextual Memory: Implications for the Idea of Systems Consolidation*

Reading through a stack of introductory psychology and neuroscience textbooks, you will notice that the leading conception of hippocampal function states that the role of the hippocampus in memory is limited to an initial period of time after learning. This idea is part of the Declarative Memory Theory proposed by Cohen and Squire (1980) that takes into ac-

count observations in human amnesics stemming from the initial observations reported by Milner and Colleagues (Scoville & Milner, 1957; Penfield & Milner, 1958; Milner, 1959). According to this view, the hippocampus is involved in a process of systems consolidation whereby long-term memories become established in non-hippocampal systems, and after a prescribed period of time no longer require hippocampal activity for recall. The principle of temporally graded retrograde amnesia is fundamental to the Declarative Memory Theory and subsequently the Standard Model of Systems Consolidation (SMSC) (Squire & Spanis, 1984; Squire, 1992; Squire et al., 2004).

As discussed throughout this thesis, the SMSC is an attempt to account for the extremely compelling observations initially made by Scoville and Milner (1957) with patient HM, and has been the focus of many research programs on human, as well as non-human animal, memory processes. Unfortunately, evidence from animal models supporting the SMSC is surprisingly limited. Looking primarily at the data from work with rat models, we postulate that a time-limited period of hippocampal involvement in long-term memory recall might not exist at all (Sutherland et al., 2010; Sutherland & Lehmann, 2011). When the hippocampus is involved in memory storage, it appears to always be involved in memory retrieval, and this has been shown for tasks such as learning to fear a discrete stimulus (tone or light) (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Maren et al., 1997; Sutherland et al., 2008; Lehmann et al., 2010), spatial navigation (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001; Clark et al., 2005a, 2005b; Martin et al., 2005), object discrimination (Sutherland et al., 2001; Mumby et al., 1999; Lehmann, Glenn, & Mumby, 2007; Lehmann, Lacanilao, & Sutherland, 2007), object exploration (Gaskin et al., 2003), shock-probe memory (Lehmann, Lecluse, et al., 2006), and picture memory (Epp et al., 2008). The significance of the existence of equivalent retrograde amnesia for recent and remote memories (flat gradients) in all of these tasks has not been fully appreciated, though as the field of hippocampal research broadens its view of the how the

role of the hippocampus might not qualitatively change with the passage of time, fundamental perceptions of long-term memory processes are slowly changing.

The traditional view of HM's amnesia has been changing as more stringent assessment methods of his condition and neural damage developed. For instance, the duration of retrograde amnesia initially reported was on the scale of a few years preceding his surgery (Scoville & Milner, 1957). Upon further investigation and testing, this retrograde interval extended out to include two decades (Corkin, 2002). Finally, in the latest evaluation of HM's autobiographical memories, Steinvorth, Levine, and Corkin (2005) found no indication of spared personal memory, regardless of how far back in HM's life they tested. If this trend does not raise the ire of memory researchers, then one fact should, that "H.M. was unable to supply an episodic memory of his mother or his father–he could not narrate even one event that occurred at a specific time and place" (Corkin, 2002). This final assessment of HM cannot undo the morass created by the attempts to replicate the observations by Scoville and Milner (1957), but can shed light on reasons for the discrepancies within the literature found today.

### *Flat vs. Temporal Gradients: Examining the Rat Literature*

We have identified a few possibilities for how a number of temporal gradients have been found within the rat literature on contextual fear (see Table 1.1 for a comprehensive list of studies), and designed experiments to directly assess them. First, it was conceivable that the strength of initial training may have an effect on future hippocampal dependent systems consolidation process—in enabling a hippocampal-independent memory retrieval. In our experiments designed to investigate this possibility (detailed in Chapter 4), we trained rats with either *weak* or *strong* conditioning parameters, and then performed hippocampal damage after three different lengths of training-to-surgery intervals (Sparks et al., 2009). Our results replicated other studies using weak conditioning parameters showing flat gradients

(Lehmann, Lacanilao, & Sutherland, 2007; Sutherland et al., 2008; Sparks et al., 2011b), but failed to replicate the temporal gradients reported in studies using stronger conditioning parameters (Kim & Fanselow, 1992; Anagnostaras et al., 1999; Winocur et al., 2009). We conclude that it unlikely that strong memories have an increased likelihood of undergoing systems consolidation, and that there must be some other reason for the differences in results.

The various experiments examining retrograde amnesia after hippocampal damage have used several different lesion methods. It is possible that contextual fear studies showing temporal gradients have used lesion techniques that are less generally disruptive to non-hippocampal networks (Kim & Fanselow, 1992; Anagnostaras et al., 1999). The most widely used method of selectively damaging the hippocampus is via infusion of glutamatergic neurotoxins such as NMDA or ibotenic acid. Neurotoxic damage of the hippocampus produces seizure activity through synchronous discharge of hyper-excited neurons, and this activity could disrupt synapses serving memory processes in networks outside the hippocampus (Jarrard & Meldrum, 1993; McClelland et al., 1995; Liang et al., 2000; Anagnostaras & Gale, 2002). Therefore, it stands to reason that hippocampal neurotoxic lesion-induced seizure activity could disrupt older, weaker memories that have transferred to non-hippocampal regions. By developing a novel lesion technique that utilized the neurotoxic effects of NMDA, but at the same time suppressing lesion-induced seizure activity by co-infusing the sodium channel blocker TTX, we were able to directly test this hypothesis. Chapter 5 details how neurotoxic lesion-induced seizure activity does indeed contribute moderately to the extent of memory loss following hippocampal damage (Sparks et al., 2011b). Though what is important to focus on here, is that suppression of seizure activity did not specifically alleviate remote memory disruption. Comparing the degree of retrograde amnesia for recent and remote contextual fear memories revealed equivalent memory loss. Regardless of the severity of the seizures, remote memories were just as

susceptible to hippocampal damage as recent memories.

To completely disrupt a contextual fear memory, extensive damage of the hippocampus is required; partial damage can result in sparing of memory (Sutherland et al., 2008). Therefore, the remaining hippocampal tissue following an incomplete lesion can support contextual fear memory retrieval. Because of the ability of remaining tissue to support memory performance, deciding which regions are supporting contextual fear memory following small hippocampal lesions is ambiguous. Is it possible that sparing 75% of the hippocampus as achieved by Kim and Fanselow (1992) and Anagnostaras et al. (1999), or the 60% by Maren et al. (1997), allowed for remaining hippocampal tissue to support memory retrieval at the remote time point? In a direct test of this possibility, Sutherland et al. (2008) and Lehmann, Lacanilao, and Sutherland (2007) performed partial or extensive hippocampal damage on rats that had been trained in a contextual fear task. What they discovered was that the extent of damage affected the severity of retrograde amnesia. Partial and extensive lesions both produce flat retrograde amnesia gradients. Though spared hippocampal tissue can support a level of memory recall, it is not responsible for the temporal gradients or selective sparing of remote memories found in studies using small lesions.

Up to this point, we have discussed a discrepancy between flat and temporal gradients found between contextual fear experiments, though temporal gradients have also been shown using trace eyeblink (Takehara et al., 2003), trace fear (Quinn et al., 2008), and flavour/odour tasks (Winocur, 1990; Winocur et al., 2001; Clark et al., 2002; Ross & Eichenbaum, 2006; Tse et al., 2007). What sets most of these studies apart from the contextual fear task is the relatively short retrograde amnesia durations. For instance, experiments that involve social transmission of food preference or flavour map memory (flavour and odour tasks), appear to have a rapid period of systems consolidation that enable hippocampal independent recall after a few days (Winocur, 1990; Winocur et al., 2001; Clark et al., 2002; Tse et al., 2007). Another interpretation of these results has been suggested

that appeals to the possibility of the retrograde amnesia reported for recent flavour/odour memories was due to disruption of cellular consolidation processes—a product of initial learning (Rudy & Sutherland, 2008). Cellular consolidation processes act to strengthen the synapses, and this cellular/molecular process is thought to occur over a period of less than $10^2$ hours (Rudy & Sutherland, 2008; Sutherland et al., 2010). Damage to the hippocampus within the period of cellular consolidation, could in effect disrupt memories that are actively becoming established through the same processes in non-hippocampal regions. It is difficult to appreciate the suggestion that a rapid systems consolidation process occurs in flavour/odour tasks, when the duration of the systems consolidation falls within, or overlaps, with the timeframe of cellular consolidation processes.

Given the evidence presented in the rat literature including that offered within this thesis, of the role of the hippocampus in long-term memory processes, there is only weak support for the idea of systems consolidation. A process whereby memories become independent of hippocampal activity, via a protracted post-learning interaction between the hippocampus and non-hippocampal systems, is not supported by the consistent findings of flat gradients. The evidence evaluated in this thesis provide a great challenge to the Standard Model of Systems Consolidation, and requires proponents of the standard model, textbook publishers, and current/future theorists to revaluate their views surrounding the long-term role of the hippocampus in memory.

## Alternatives to the Standard Model

Contextual fear memories can be established by means of a single conditioning session in non-hippocampal systems, in the absence of a functional hippocampus (Lehmann, Sparks, et al., 2009; Wang et al., 2009; Wiltgen et al., 2006; Maren et al., 1997; Sparks et al., 2011a). In the presence of a functional hippocampus, these other systems are inhibited from forming a representation that can be retrieved after hippocampal damage. By dis-

tributing fear conditioning across multiple days—for instance across 11 sessions instead of a single session—a non-hippocampal context fear memory can be established even in the presence of hippocampal interference. This memory can even survive complete hippocampal damage shortly after learning. This evidence of spared context fear memory following hippocampal damage suggests that there is a mechanism other than a time based process of systems consolidation that acts to make memories independent of the hippocampus. There is limited evidence supporting the existence of a systems consolidation process that relies on "off-line" hippocampal mediated rehearsal to establish memories in non-hippocampal systems over a period of time (McClelland et al., 1995; Squire, 1992). In a review of the most recent (past 5 years) rodent research on the long-term role of the hippocampus in memory retrieval, it is concluded that a process of systems consolidation is not necessary to explain the data. Therefore, to provide a framework to move beyond the Standard Model of Systems Consolidation, we propose two alternatives that can account for the data (Sutherland et al., 2010):

1. **Distributed Reinstatement Theory**. A learning episode rapidly creates a stored memory representation that is quite strong in hippocampus and very weak in non-hippocampal networks, bouts of hippocampus-dependent replay incrementally strengthen the non-hippocampal memory representation, but the bouts of replay become extremely infrequent or non-existent on the time-scale of hours (Sutherland et al., 2010).

2. **Dual-Store Model**. Different memory systems acquire information independently, based upon learning rate parameters set by their intrinsic network properties and by modulatory interactions from other systems.

### *Post-Event Hippocampal Activity and Consolidation: The Distributed Reinstatement Theory*

The Distributed Reinstatement Theory (DRT) was conceived after our observations that distributed training promotes sparing of memories after hippocampal damage (Chapter 2. During conditioning, the hippocampus quickly forms a representation that can be associated with foot shock, while the representation in non-hippocampal regions is relatively weak. A period of post-event activity strengthens the non-hippocampal representation, though this activity is brief and insufficient to create a hippocampal-independent memory after one episode. Distributing the reinstatement of post-event activity by repetitious conditioning sessions, incrementally strengthens the non-hippocampal memory. Learning contextual fear in one session is insufficient to enable hippocampal-independent recall. When contextual fear conditioning is distributed across multiple sessions, the contextual fear memory survives complete hippocampal damage (Lehmann, Sparks, et al., 2009).

Both the SMSC and the DRT hold that post-event activity mediated by the hippocampus influences the strength of relevant memory representations in non-hippocampal networks (McClelland et al., 1995; Squire, 1992; Sutherland et al., 2010). One neural candidate for post-event hippocampal activity has been recorded during periods of restfulness (sleep) and has been hypothesized to play a crucial role in the process of memory consolidation (Buzsaki, 1989, 1996; Pennartz, Uylings, Barnes, & McNaughton, 2002; Born, Rasch, & Gais, 2006). This activity called sharp-wave ripple events (SPW-R) were first described by Buzsaki, Leung, and Vanderwolf (1983) and are associated with a synchronous discharge of large number of hippocampal neurons. SPW-Rs are mainly present during slow-wave sleep (SWS), though also occur during behaviours such as eating, drinking, grooming, and drowsiness (Buzsaki et al., 1983; Buzsaki, 1986). Because SPW-Rs are not associated with behavioural states associated with the processing of new information, they are considered a candidate for providing a substrate for "off-line" memory consolidation. In

addition, SWS has been documented to be associated with learning-associated changes in cortical spindle activity in rats (Eschenko, olle, Born, & Sara, 2006), and details coordinated hippocampal-neocortical communication during slow oscillations (Siapas & Wilson, 1998; Battaglia, Sutherland, & McNaughton, 2004). SPW-Rs provide highly selective coordinated neural activity between hippocampal and neocortical ensembles that provides optimal conditions for alterations of synaptic plasticity in afferent neurons (Buzsaki, 1989). Hippocampal neurons that fire together during behavioural activity also fire together during SWS (Pavlides & Winson, 1989; Wilson & McNaughton, 1994). This activity was taken as evidence of the hippocampus "replaying" recent experiences as part of a consolidation process (McClelland et al., 1995). Patterned replay is considered to occur over the period of hours following behavioural activity (Pavlides & Winson, 1989; Ribeiro et al., 2004; Wilson & McNaughton, 1994). Limited evidence suggests that replay can be sustained for 24 hours (Kudrimoti, Barnes, & McNaughton, 1999) and even 48 hours (Ribeiro et al., 2004), though the evidence for extended replay is not well founded (Tatsuno, Lipa, & McNaughton, 2006). The duration of systems memory consolidation processes described by the SMSC take place on the timescale of weeks to months. Although replay is considered a strong candidate for the process of memory consolidation, it is unclear how replay (lasting on the timescale of hours) can be a mechanism for systems consolidation lasting months.

### *Examining the Distributed Reinstatement Theory*

Although the association between replay and memory consolidation is largely correlational, it does provide a framework from within which multiple hypotheses can be tested. We aimed to examine the DRT directly by disrupting post-event replay during a distributed fear conditioning regime and test the hypothesis: is hippocampal mediated post-event replay necessary for the consolidation of context fear memory in non-hippocampal networks (Gulbrandsen et al., 2011). Rats were first implanted with chronic guide cannula aimed at

the dorsal and ventral hippocampus bilaterally (cannula and surgery methods according to those developed and detailed in Chapter **??**) to enable the infusion of the sodium channel blocker ropivacaine or artificial cerebral spinal fluid (aCSF) for the purpose of temporarily inactivating the hippocampus. A pilot study revealed that infusion of ropivacaine into the dorsal and ventral hippocampus produced more than an 90% decrease in the amount of the immediate early gene *c-fos* expression following electroconvulsive shock, confirming that ropivacaine infusion effectively inactivates activity throughout the hippocampus.

Distributing fear conditioning over 11 sessions establishes a memory resistant to hippocampal damage (Lehmann, Sparks, et al., 2009), though the minimum number of sessions to produce the same result is unknown. To examine this parameter, we exposed rats to either three or six conditioning sessions (1 foot shock per session; 0.9mA, 2sec) distributed across three days. Twenty-four hours following the final conditioning session, rats were infused with either ropivacaine or aCSF and returned to the conditioning context. All of the rats with an active hippocampus displayed robust freezing behaviour, suggesting that three and six conditioning sessions produced similar levels of contextual fear memory. Rats that underwent three conditioning sessions, and then had an inactive hippocampus during the retention session displayed a significant decrease in the amount of expressed fear, while those that had six sessions had freezing levels comparable to the respective control group. This result indicates that three conditioning sessions are not enough to establish a contextual fear memory resistant to hippocampal inactivation, whereas six sessions fulfil the minimum threshold.

If hippocampal mediated post-event replay is necessary to consolidate memory in non-hippocampal systems, then blocking this activity following repeated conditioning sessions should prevent memory performance when tested in the absence of the hippocampus. We assessed this possibility by training in the same six conditioning sessions as described above, though inactivated the hippocampus of one group of rats following the second ses-

sion each day (total three out of six post-event replay periods inactivated), preventing replay for the fear conditioning event. Prior to memory retention testing, all of the rats received infusions of ropivacaine, thereby inactivating the hippocampus and any context fear memory associated with that structure. When returned to the conditioning context, all of the rats expressed robust freezing behaviour, indicating that all groups had good retention of the fear memory. Had the reduced-replay group shown reduced freezing behaviour we would have concluded that replay was necessary to consolidate the non-hippocampal memory, though this was not the case. Because all of the groups showed equivalent memory retention, our assessment is that distributed post-event replay is not necessary for establishing a hippocampal-independent contextual fear memory.

Central to the DRT is the premise that post-event hippocampus-dependent replay incrementally strengthens non-hippocampal memory representations. Our test of this premise does not lend support for the DRT, as rats with reduced replay activities remembered just as well as the respective control group. There must be another mechanism by which memories initially dependent on the hippocampus become independent following distributed training.

### *Dual-Store Model*

Considering the discussion thus far on the DRT as an alternative to the SMSC, a simpler framework might be that of a dual-store model. In this model, multiple memory systems acquire long-term memories in parallel, though the learning rate is dependent upon parameters within each system as well as interactions with other systems that act to modulate learning. Evidence presented on the interfering role that the hippocampus plays during learning, and the absence of this interaction when the hippocampus is removed (see Chapter 2) suggests that multiple systems can acquire a context-shock association memory. That being said, significant interaction between systems modulates the rate at which this association is learned. The hippocampus is theorized to have a relatively fast rate of learning

and can express the memory after as few as one context-shock exposure, whereas the hippocampus normally acts to inhibit the learning rate of non-hippocampal systems which in comparison learn at a much slower rate.

According to this model, a process of between-systems consolidation over time, is not necessary to establish a memory resistant to hippocampal damage. Associative strength in non-hippocampal networks is increased by way of repeated learning sessions. With each successive session, the associative connections in non-hippocampal networks are incrementally strengthened according to the learning parameters of the system. It is not known at this time what these parameters are, or how they change with learning experience. Though through further experimentation, specific learning parameters could be unveiled. Additional support for this model comes from a patient that had sustained bilateral hippocampal damage (Maguire et al., 2006). Patient TT, for approximately 40 years drove the streets of London as a taxi driver. London taxis undergo rigorous route training lasting three to four years before granted license as a driver. Part of TT's training was to learn 25,000 streets within the city. Once a licensed driver, TT spent the following 35+ years driving some of those thousands of streets more than others, and among those streets establishing well known routes. After he sustained damage to the hippocampus, TT experienced robust retrograde amnesia for much of the layout of London, though the routes most often driven were remembered. Knowledge of all of the routes was initially learned during training, 40 years prior to hippocampal damage, and a time-based process of systems consolidation would suggest that all of these routes would be remembered after his injury. TT's pattern of retrograde amnesia did not support the SMSC, rather a process whereby multiple reiterations (or experiences) of the routes driven established hippocampal-independent memories.

## Conclusions

The hippocampus interacts with other memory systems during the learning and remembering of contextual fear memories. This interaction has been discussed within this thesis to be one of interference. The relationship between the hippocampus and non-hippocampal systems during the memory process, suggests that the two systems are regulated by different learning parameters. It is theorized that the hippocampus is a fast learner while, due to the interfering effect of hippocampus, the other system is relatively slow. The learning rate parameters of these memory systems are dynamic, where removal of the primary system (i.e., hippocampus) in turn results in adjustment to the parameters of the alternate system. These interactions are not fully supported by the traditional view of multiple memory systems, but rather a dynamic systems model.

Hippocampal interactions over time likely retain this effect, and in our data there is no suggestion of a process of between-systems memory consolidation. Memories can become independent of the hippocampus by way of multiple reinstatements over time, enabling the slower non-hippocampal system to acquire required information. Within the context of the role of the hippocampus in long-term memory processes, we believe that these results will further our understanding of hippocampal-mediated learning and remembering, and how these events fit into and modify the current conception of memory.

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## Appendix 1

There are various strategies that can be used to temporarily inactivate the hippocampus. The two most common approaches are to either a) inhibit sodium channel conduction using tetrodotoxin (TTX) or a local anaesthetic such as lidocaine, or b) potentiate neural hyper-polarization by using endogenous inhibitory mechanisms to increase intracellular chloride concentrations (i.e., using a GABA agonist such as muscimol; 3-hydroxy-5-aminomethylisoxazole), or infusing potassium chloride (KCl) into the extracellular space. Both of these strategies have proved effective at temporarily inhibiting hippocampal function. For example, Bast et al. (2001) used an auditory fear conditioning task to examine and compare the effects of TTX and muscimol when infused into the ventral hippocampus. In this case, TTX blocked conditioning to the tone and context, while the effects of muscimol were restricted to conditioning to the context. Conversely, some of the first studies to temporarily inactivate the hippocampus were performed using KCl (Avis & P.L.Carlton, 1968; Hughes, 1969). The use of muscimol remains the most common method of temporary neural inactivation within the hippocampal learning and memory field (rodent).

Muscimol is a structural analogue of γ-aminobutyric acid (GABA), and is a potent agonist of the GABA receptor (Beaumont, Chilton, Yamamura, & Enna, 1978; Johnston, Krogsgaard-Larsen, Curtis, Game, & McCulloch, 1975). Hippocampal pyramidal cells are highly connected with interneurons that provide GABAergic inhibition (Andersen, Gross, Lomo, & Sveen, 1969; Curtis, Felix, & McLennan, 1970). It stands to reason that because of the role of GABAergic neurons in modulating hippocampal pyramidal cell activity, that the GABA agonist muscimol should be effective in reducing hippocampal network activity. Indeed, the inhibitory effects of muscimol have been demonstrated electrophysiologically, and the range of effective diffusion quantified (Edeline, Hars, Hennevin, & Cotillon, 2002). Importantly, these effects are transient and reversible, after which the cells return to normal activity. In contrast with sodium channel blockers (e.g., TTX) or local anaesthetics (e.g.,

lidocaine), which prevents action potential within local neurons as well as inhibits sodium conduction along fibres of passage (Hilles, 1966, 1977; Ritchie, 1979), muscimol only directly inhibits the activity of local neurons. These properties make muscimol an attractive candidate for use in behavioural experiments, where multiple acute inactivations restricted to the hippocampus are required during a learning and memory paradigm.

Nonetheless, in previous experiments examining the efficacy of muscimol, electrophysiological recordings were performed over a short period of time, and also used small amounts of muscimol that likely targeted a discrete and restricted portion of the hippocampal network. For the purpose of learning and memory experiments, it is necessary to know the time course of muscimol inhibition in the awake animal. To examine the efficacy of muscimol in potentiating neural inhibition, electrophysiological recordings of evoked potentials were performed in the dentate gyrus of the dorsal hippocampus. Evoked potentials in the dentate gyrus provide a potent measure of neural inhibition, and were chosen as a means of demonstrating the situation of maximal inhibition. This technique has been successfully used to quantify other pharmacological inhibitors of neural activity *in vivo*, namely a selective AMPA/kainate receptor antagonist (Riedel et al., 1999). This chapter describes the time course of muscimol mediated inhibition before detailing the method and techniques used to build the chronic guide cannula, acute infusion cannula, chronic implantation surgical procedures, and electrophysiology testing.

## Results and Discussion

To examine the efficacy of muscimol in potentiating neural inhibition, electrophysiological recordings of evoked potentials were performed in the dentate gyrus of the dorsal hippocampus. Evoked potentials in the dentate gyrus provide a potent measure of neural inhibition, and were chosen as a means of demonstrating the situation of maximal inhibition. Figure 6.1 details the population spike amplitude over a 6 hour period. Following

muscimol infusion at the 30 min mark, fEPSPs were rapidly affected and the population spike amplitude diminished from baseline. The rapid decrease in population spike amplitude continued for 30 min post-infusion to a point approximately 20% baseline amplitude, after which continued to diminish relatively slowly to 0% baseline over the next hour. The inhibitory effects of muscimol began to wear off as indicated by the population spike amplitude increasing approximately 165 min post-infusion, and continued over the following 75 min until reaching pre-infusion baseline amplitude.

The rapid suppression of population spike amplitude following muscimol infusion indicates that the number of neurons responding to perforant path stimulation decreased. As the post-infusion time continued, the inhibitory effects of muscimol, as recorded in the dentate gyrus, continued to increase to a point of maximal inactivation. At this point, few if any neurons in the dentate gyrus were responding to stimulation. The profile of inhibition provided by these electrophysiological recordings suggests that a window of temporary inactivation of the hippocampus begins approximately 35 minutes after muscimol infusion and lasts for slightly over 2 hours. Therefore, experiments designed to utilize muscimol mediated hippocampal inactivation should consider training and/or testing within the window of inactivation. The full recovery of the population spike amplitude to pre-infusion baseline levels is indicative of recovery of the network to what is assumed to be a normal state.

## Cannula Construction Techniques

For the purpose of the currents studies, four cannulae were chronically implanted bilaterally into the dorsal and ventral hippocampus. Because of the physically close nature of this configuration, it was necessary to build a custom set of guide, injection, and dummy cannula that would allow enough space for proper placement. *Guide cannulae* were constructed using stock 23 gauge 304 stainless steel hypodermic tubing (inside diameter .0125/.014”,

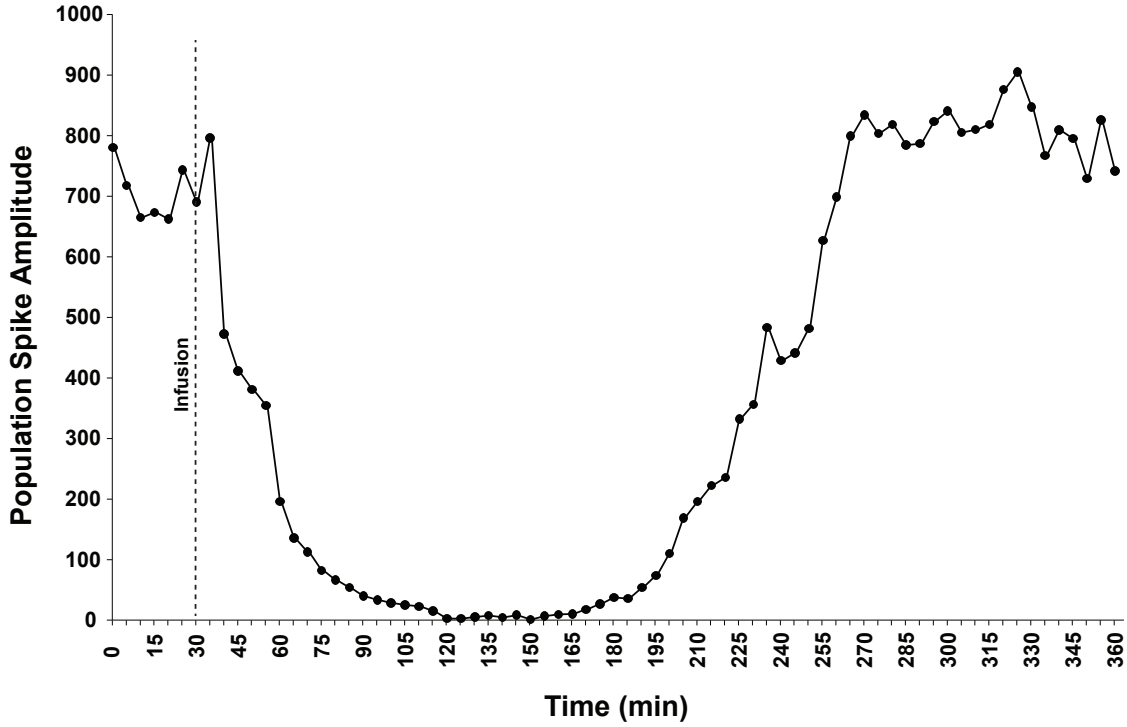


Figure 6.1: **Time-course of Muscimol Inactivation.** Plot of population spike amplitude following evoked potentials in the dentate gyrus. Electrophysiological recording of perforant path stimulation was performed over the course of 6 hours to determine the time course of muscimol mediated neural inhibition.

outside diameter .025/.0255") (Component Supply Co., Fort Meade, FL). Precise lengths of 10mm and 13mm were cut for implantation in the dorsal and ventral hippocampus respectively. A hand rotary tool (Dremel®) with a cutting wheel served as a means to cut, grind, and sand the cannulae. A 30 gauge hypodermic needle was used to free the inside of the cannula from debris. Two shallow notches were also ground into the side of the cannula to prevent slipping within the head cap. Prior to surgery, the guide cannulae were flushed and rinsed with 1mol hydrochloric acid to remove residual metal particles, and then stored in saline. *Injection cannulae* were constructed using the same technique by inserting a section of 30 gauge 304 stainless steel hypodermic tubing (inside diameter .0055/.007", outside diameter .012/.0125") (Component Supply Co., Fort Meade, FL) into a section of 23 gauge, and then the two soldered together. The inside of the 30 gauge cannula was cleaned using .005" music wire. The injection cannulae were designed to extend 1mm past the end of the guide cannula by placing a slight bend at the appropriate length. Following construction, the injection cannula were each connected to PE-50 tubing attached to a 10$\mu$l Hamilton® syringe (Reno, NV), and a rubber washer placed on the tubing to measure drug displacement. *Dummy cannulae* were constructed from .012" 304 stainless steel music wire (Component Supply Co., Fort Meade, FL), to fit flush with the bottom of the guide cannulae, and bent at the top to keep in place.

### Surgical Techniques for Chronic Quad Cannulae Implantation

Rats are anesthetized by isoflurane inhalation (Janssen, Toronto, ON, Canada) (3.5% with 1 litre/min oxygen, reduced to 1% after a surgical plane is established) and an analgesic (buprenorphine, 0.017 mg/kg, s.c.; Reckitt & Colman, Richmond, VA, USA) is administered. They are then placed in a stereotaxic frame (Kopf® instruments, Tujunga, CA, USA), a midline scalp incision made, and periosteum excised to expose the top of the skull. Small burr holes are drilled through the skull using anterior/posterior and medial/lateral co-

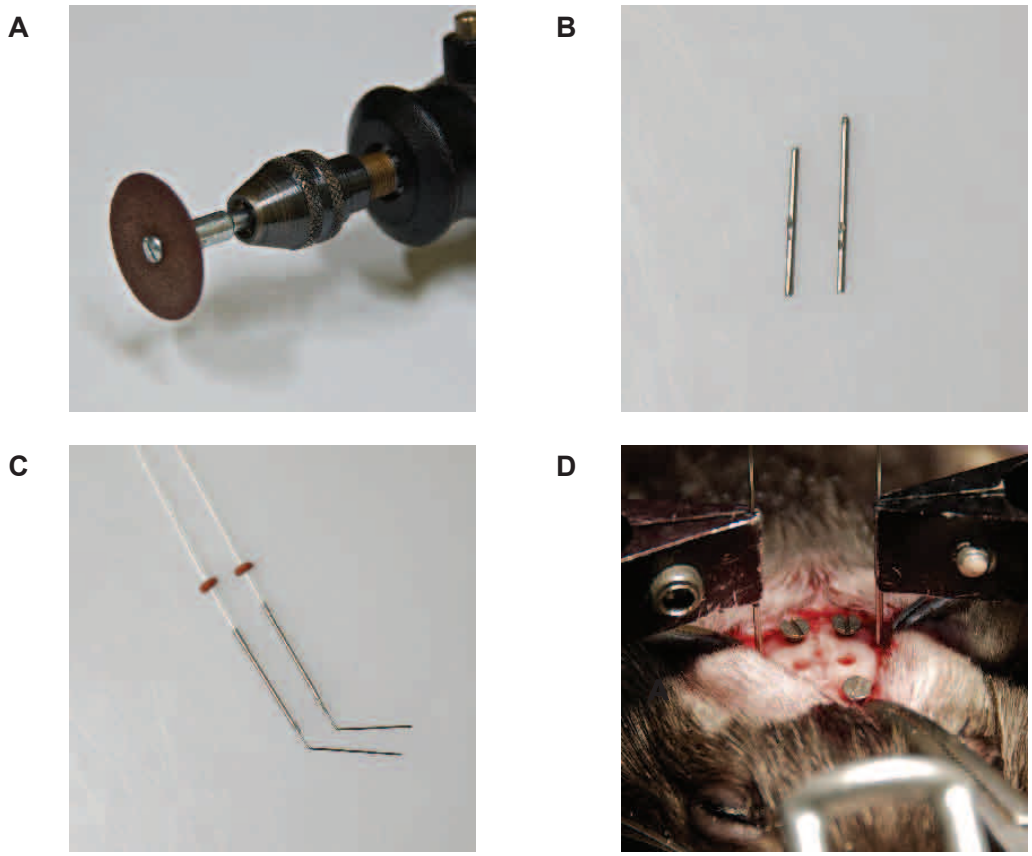


Figure 6.2: **Guide Cannula Construction.** (A) Cutting wheel used to cut cannula lengths and grind/smooth ends. (B) Examples of dorsal (left) and ventral (right) cannula after construction. (C) Constructed infusion cannula attached to PE-50 tubing. (D) Arrangement of quad infusion cannula during drug administration.

ordinates relative to Bregma as detailed in Table 6.1. An additional three holes are drilled, and stainless steel jewellers screws tapped and inserted flush with the bottom of the bone.

The main steps in the cannulae implant process are detailed in Figures 6.3 and 6.4. Ventral cannulae are lowered on bregma to take dorsoventral measurement and then lowered into the two most posterior sites. The skull is rinsed with sterile saline and allowed to dry before application of dental acrylic. Dental acrylic is applied to the base of the two most posterior screws and ventral cannulae only. Care is taken to avoid covering the dorsal cannula holes. Multiple layers of acrylic are applied, with time permitted for drying in between applications. Acrylic is built up to cover the notches in the cannulae. After the acrylic has hardened, the cannula holders are retracted and dorsal cannulae placed on the holders. The dorsal cannula are placed on bregma to take the dorsoventral measurement and then lowered into their respective holes. The skull is checked for dryness before applying an amount of acrylic that flows around the anterior screw and the dorsal cannulae. The acrylic is built up to cover the notches in the dorsal cannulae and allowed to dry before removing the cannula holders. After the cannula holders are removed, the head cap is finished by applying successive layers of acrylic to produce a smooth surface, free from sharp protrusions around the edge of the cap thereby preventing irritation of the skin. If the initial incision was too long, a single stitch is placed either in front or behind the head cap to snug the scalp to the acrylic. The scalp should not be stretched tight, and at the same time should not have open spaces. Figure 6.4 details proper head cap construction.

Table 6.1: Coordinates used for 4-site hippocampus cannula implantation in adult male rat (measurements in millimetres relative to bregma).

| **Site** | **Anteriorposterior** | **Mediolateral** | **Dorsoventral** |
|---|---|---|---|
| Dorsal | –3.5 | ±2.0 | –4.0 |
| Ventral | –5.8 | ±5.2 | –6.5 |

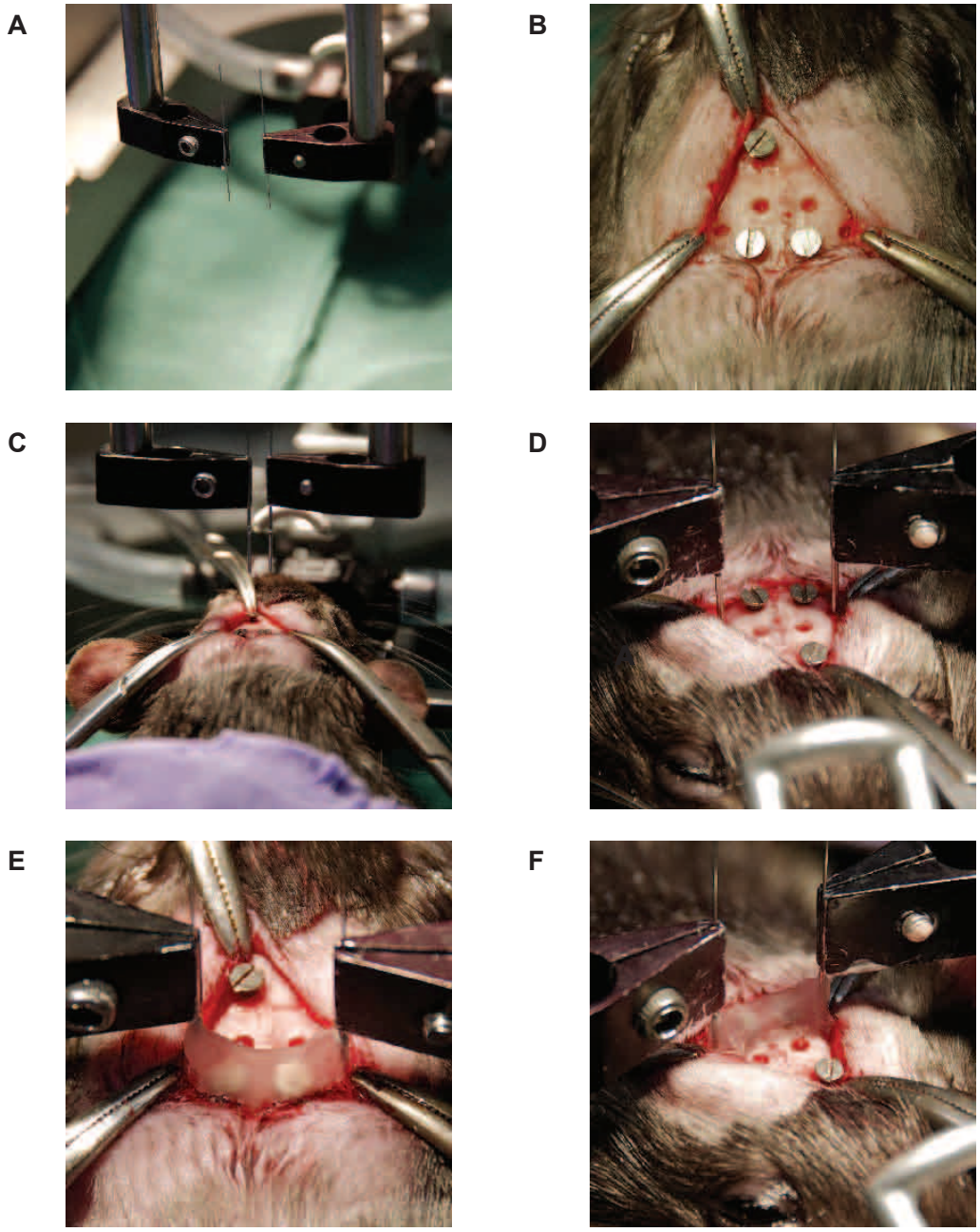


Figure 6.3: **Surgical Diagram for Guide Cannula Implantation.** Surgical procedure for implantation of guide cannulae. (A) Fasten 0.012" music wire into cannula holders to hold guide cannula during procedure. Place very slight bend in wire to provide friction against guide cannula. (B) Drill appropriate holes in skull and insert stainless steel jewellers screws as shown. (C) Slide ventral guide cannulae onto wires with the notches toward the top. (D) Insert ventral cannula to calculated depth. (E,F) Apply dental acrylic around base of cannulae and screws, avoiding covering dorsal cannulae holes. Build up acrylic to cover notches in cannulae.

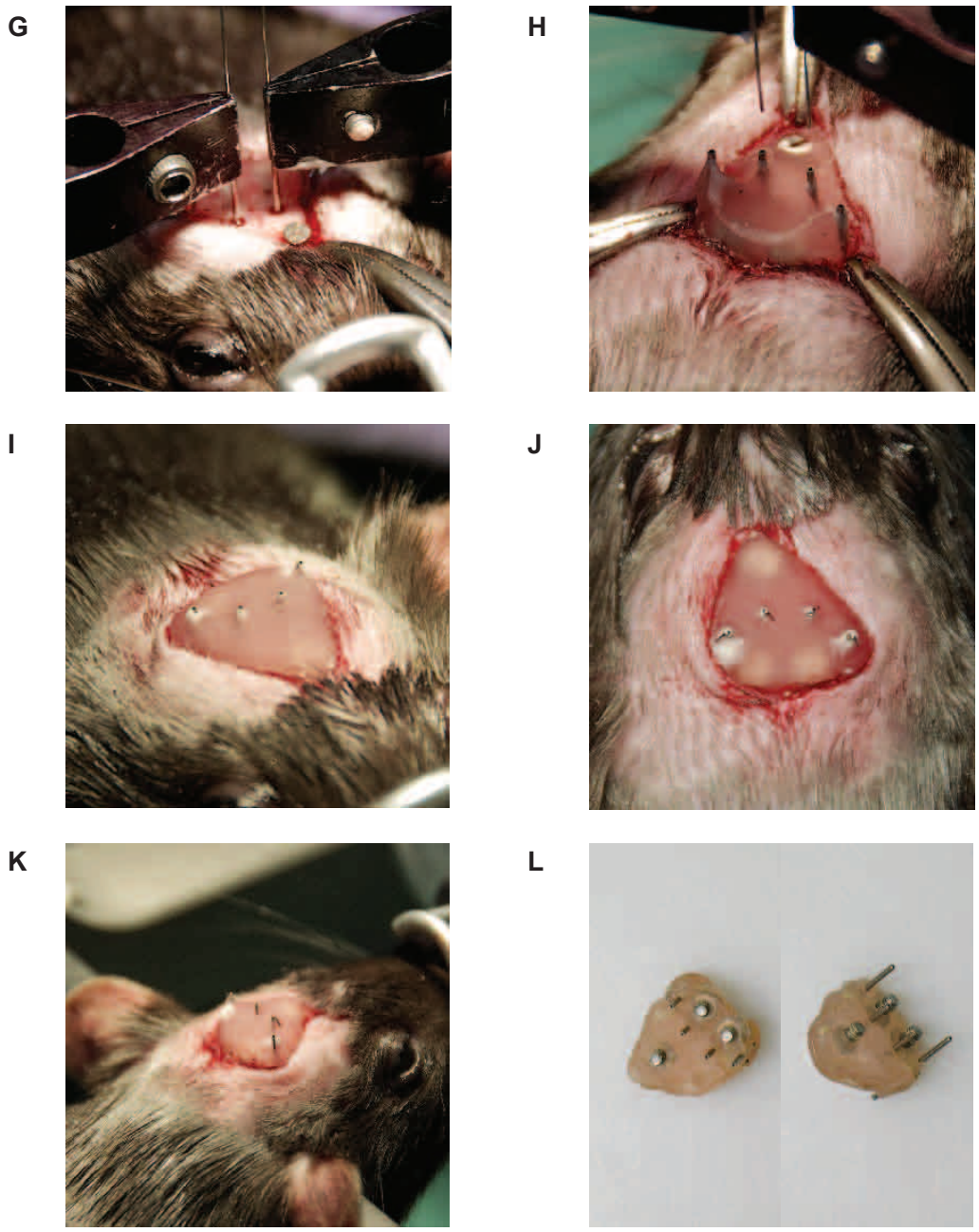


Figure 6.4: **Surgical Diagram for Guide Cannula Implantation.** Continuation of surgical procedure for implantation of guide cannulae. (G) Insert dorsal cannula to calculated depth. (H) Apply acrylic to cover base of cannulae and remaining screw, and remove cannula holders after the acrylic has dried. (I) Build up acrylic to form a smooth head cap to within 1mm of tops of cannulae. (J,K) Finished head cap with dummy cannulae inserted. (L) Complete head cap after removal from skull.

## Materials and Methods

### *Subjects*

The University of Lethbridge Animal Care Committee approved all procedures under Protocol #0609, in accord with the guidelines set by the Canadian Council on Animal Care. Participants were 1 male Long-Evans rat (400 g) obtained from the Canadian Centre for Behavioural Neuroscience vivarium (University of Lethbridge, Alberta). The rat was housed in a standard laboratory cage in a room with an ambient temperature of $21^oC$, 35% relative humidity, 12/12 hr light/dark cycle (lights on at 07:00), and was provided with food and water *ad libitum*. Electrophysiological testing was conducted during the light phase of the daily cycle.

### *Surgery: Cannula and Electrode Implantation*

Rats were anesthetized by isoflurane inhalation (Janssen, Toronto, ON, Canada) (3.5% with 1 litre/min oxygen, reduced to 1% after a surgical plane was established) and administered an analgesic (buprenorphine, 0.017 mg/kg, s.c.; Reckitt & Colman, Richmond, VA, USA). They were then placed in a stereotaxic frame (Kopf instruments, Tujunga, CA, USA), a midline scalp incision was made, and periosteum excised to expose the top of the skull. For placement of guide cannulae, jewellers screws and electrodes, small burr holes were drilled through the skull with care not to damage the underlying tissues. Stainless steel guide cannulae (23 gauge; 10 mm in length for dorsal hippocampus, 13 mm for ventral hippocampus) were bilaterally implanted in to the dorsal (Anterior/Posterior -3.5 mm, Medial/Lateral +/-2mm, Dorsal/Ventral -3 mm on the basis of the Paxinos and Watson (1998) rat brain atlas) and ventral (Anterior/Posterior -5.8 mm, Medial/Lateral +/-5 mm, Dorsal/Ventral -5 mm) hippocampus. Cannulae were fixed to the skull with three jewellers screws and dental acrylic. After surgery, stainless steel stylets (dorsal -10 mm and ventral

-13 mm) were placed in the guide cannula to prevent clogging. The rat was allowed to recover for 10 days before commencement of the behavioural procedures.

### *Intracranial Drug Infusions*

One hour before recording, the rat was transported from its home cage to the recording room where it remained in the transport cage. 30 min into the recording session, the rat received a bilateral infusion of muscimol (5-Aminomethyl-3-hydroxyisoxazole hydrobromide dissolved in 0.9% sterile saline, 1 $\mu$g/$\mu$l; Sigma-Aldrich Canada, Oakville, ON, Canada; M group) at a rate of 0.32 $\mu$l/min for 94-s. A 0.5 $\mu$l infusion was made concurrently in each of the dorsal and ventral sites, for a total hemispheric infusion of 1 $\mu$l (i.e., 1 $\mu$g muscimol per hemisphere). Injection cannulae (30 gauge; stainless-steel) attached to polyethylene tubing (PE-50; Small Parts Inc., Lexington, KY, USA) were placed in, and extended 1 mm beyond (11 mm dorsal, 14 mm ventral) the indwelling guide cannulae. The distal ends of the PE-50 tubing were attached to 10 $\mu$l Hamilton syringes (Hamilton Co., Reno, NV, USA), which were attached to a micro-infusion pump (Harvard Apparatus, South Natick, MA, USA). After the infusion pumps were turned off, the injection cannulae were left in place for 1 min to allow for diffusion of the drug. After infusions were completed, the rat was placed back in the recording chamber for the remainder of the recording session.

### *Apparatus*

Electrophysiology recording was carried out in a plexiglass box approximately 30 cm x 45 cm in size with standard housing bedding on the floor. The connecting plug on the head of the rat was fastened to a cable that connected to an amplifier (A-M Systems, Carslborg, WA) as well as an isolated stimulator (A.M.P.I. ISO-flex, Jerusalem, Israel). Signals from

the amplifier were passed through an analogue/digital board (DataWave®Technologies, Loveland, CO) before running to the computer to be recorded. The computer software (SciWorks; DataWave®Technologies) controlled the stimulation timing via a Master-8 controller (A.M.P.I., Jerusalem, Israel). An audio monitor (Grass Technologies, West Warwick, RI) received input from the amplifier.

### *Electrophysiological Procedures*

For drug infusion and electrophysiology recording, the rat was transported to the recording room in a plastic transport tub. After 30 min, the rat was connected to the recording apparatus and placed into the recording box. And I/O curve was performed using six stimulus intensities (50, 100, 200, 300, 400, and 500 $\mu$A). Each stimulus intensity was repeated 10 times at an inter-stimulus interval of 0.05 Hz. The fEPSP slope and population spike amplitude was averaged at each stimulus intensity to provide an I/O curve for the rat. The stimulus intensity producing 70% of the maximum population spike amplitude was calculated and used throughout the testing.

Testing consisted of 100$\mu$sec stimulations with a frequency of 0.05 Hz extending for a period of 6 hours. 30 min of stimulation was recorded and saved as a baseline prior to infusion of muscimol. At this point, the rat was removed from the recording chamber, stimulating/recording wires disconnected, and drug infusion protocol performed. At completion of drug infusion (approximately 5 minutes), the wires were reconnected to the rat and placed back into the recording chamber. Stimulating and recording recommenced and continued uninterrupted for the next 5.5 hours.