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Characterizing the Effect of a Novel CA1 Lesion on Memory in Rats

A dissertation submitted in partial satisfaction of the
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by

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2017

The dissertation of Amber Chantelle Ocampo is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

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DEDICATION

I dedicate this dissertation to my family, friends, advisors, and lab.

Thank you all for supporting me over the past five years and for giving me the chance to pursue my dreams.

EPIGRAPH

Remembering is not the re-excitation of innumerable fixed, lifeless and fragmentary traces. It is an imaginative reconstruction… built out of the relation of our attitude towards a whole active mass of organized past reactions or experience…

Frederic Bartlett (1932)

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ABSTRACT OF THE DISSERTATION

Characterizing the Effect of a Novel CA1 Lesion on Memory in Rats

by

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Doctor of Philosophy in Psychology

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The hippocampus plays an important role in memory. Hippocampal subfield CA1 serves as the primary output of the hippocampus to neocortex and thus is important for supporting hippocampal function. Based on this role, a lesion targeting the entire CA1 region should block hippocampal output to neocortex and disrupt hippocampus-dependent memory as severely as a large hippocampal lesion. However, some findings contradict this idea, demonstrating more severe impairments following DG or CA3 lesions than after CA1 lesions. This could be explained by (i) information leaving the hippocampus through descending CA3 projections, or (ii) the incompleteness of existing

CA1 lesions, which separately target only dorsal or ventral CA1. Overall, it remains unclear if CA1 output to neocortex is required to support memory.

We resolve this issue with a novel lesion that targets the entire dorsoventral CA1 axis. First, we tested the effect of the lesion on retrograde memory and found that complete CA1 lesions caused severe impairments in the watermaze, context fear conditioning, and trace fear conditioning tasks (similar to previous findings with large hippocampal lesions). We next tested the effect of the CA1 lesion and large hippocampal lesions on anterograde memory. Both lesions impaired performance in the watermaze task and delayed match-to-position (DMP). However, when rats were given prior DMP training, CA1 lesions no longer impaired watermaze performance, and when rats were given prior watermaze training, CA1 lesions no longer impaired DMP performance. In contrast, rats with hippocampal lesions were impaired in both tasks regardless of prior training.

These studies demonstrate that CA1 output to neocortex is normally needed to support memory, but prior experience can ameliorate anterograde memory impairments caused by the loss of CA1 output. Because prior experience only benefited rats with CA1 lesions, we suggest that descending CA3 efferents may be able to support some forms of memory. However, we also consider that CA1 sparing or a reduction in remote lesion effects might also account for the superior CA1 performance. Additionally, we suggest that the concept of the schema may be particularly important for understanding the beneficial effect of prior experience.

CHAPTER 1:
INTRODUCTION: A REVIEW OF THE CA1 LESION

Since the discovery of its involvement with memory, the hippocampus has become one of the most extensively investigated structures in the brain. Early patient findings showed that removal of the medial temporal lobe (MTL), including the hippocampus, results in anterograde and temporally graded retrograde amnesia (Manns et al., 2003; Scoville and Milner, 1957). These observations were later extended to patients with MTL damage limited to the hippocampus (Bayley et al., 2006; Rempel-Clower et al., 1996). Following the human findings, anterograde and retrograde impairments were also observed in animals with hippocampal lesions across a variety of memory tasks (Broadbent and Clark, 2013; Clark et al., 2001; Clark et al., 2002; Morris et al., 1982; Zola-Morgan et al., 1989). Together, decades of human and animal research have demonstrated that the hippocampus is an integral component in a system that supports episodic and semantic memory.

The hippocampus is composed of three distinct subfields, areas CA1, CA3, and the dentate gyrus (DG), and primarily receives input from the entorhinal cortex (EC). Figure 1 shows a diagram depicting the circuitry of these structures. EC layers I and II project to the DG and area CA3 through the perforant pathway (van Strien et al., 2009). Through the temporoammonic pathway, the EC also sends a small subset of projections from layer III directly to area CA1. Within the hippocampus, the DG projects to area CA3 through the mossy fiber pathway, and area CA3 projects to area CA1 via Schaffer collaterals. Area CA1 then projects out of the hippocampus to the subiculum and EC layers V and VI, serving as the primary output pathway of the hippocampus to the

neocortex. A fourth, smaller hippocampal subfield, area CA2, lies between areas CA1 and CA3. However, much less is known about its connectivity and function.

Significant interest has been focused on the CA1 subfield because of its unique place in hippocampal circuitry. The combined information it receives from the EC and the rest of the hippocampus as well as its efferent connections to the neocortex have inspired various predictions about its potential functions in memory formation and retrieval. To uncover CA1 function, numerous studies in both patients and animals have investigated the effects of CA1-specific damage on different types of memory.

CA1 Lesions in Humans

The CA1 field is particularly susceptible to neurological insult. Studies in rats have shown that forebrain ischemia induces comprehensive damage to area CA1 while leaving the other hippocampal subfields intact (Davis et al., 1986; Volpe et al., 1984; Volpe et al., 1989). Similar findings have been observed *in vitro* with studies of oxidative stress (Wang et al., 2009). A recent neuroimaging study in patients has shown that selective CA1 damage is linked to various neurological conditions, such as hippocampal ischemia, limbic encephalitis, status epilepticus, and transient global amnesia (Barstch et al., 2015). Because of this unique vulnerability, a number of CA1 lesions have been documented in humans and have provided some of the earliest insights into CA1 function.

Zola-Morgan et al. (1986) were the first to systematically characterize the cognitive impairment in a CA1 lesion patient. They investigated the case of Patient R.B., who developed memory deficits after experiencing an ischemic episode at the age of 52.

R.B. demonstrated a moderate, but clinically significant and lasting anterograde memory impairment, as measured by tests of recall and recognition. For example, 6 months after his ischemic episode, R.B. was given the Rey-Osterreith complex figure test. He was able to copy the figure, but could not reproduce it from memory after a short delay (Figure 2A). He was still impaired when retested 17 months later (Figure 2B). When tested for information that he had acquired before his ischemic episode (e.g., biographical information, famous faces, television programming, and public events), he showed little to no impairment. This contrasts with findings from patients with more complete hippocampal lesions, who typically lose memory from the few years prior to the onset of amnesia (Bayley et al., 2006; Manns et al., 2003). Performance on these tests, however, can be variable, and the tests that were used may not have been sensitive enough to detect more subtle deficits. Thus, some uncertainty remains about R.B.'s retrograde memory impairment. Nevertheless, it is important to note that these same tests were able to detect retrograde impairments that spanned at least ten years in patients with complete hippocampal lesions (Rempel-Clower et al., 1996).

Similar findings were reported a decade later by Rempel-Clower et al. (1996) with another CA1 lesion patient, G.D, who suffered from an ischemic episode during surgery at the age of 43. After his surgery, he displayed moderate but significant anterograde memory deficits that lasted until his death, 9.5 years later. His performance on the Rey-Osterreith complex figure test was comparable to Patient R.B. (Figure 2C). With retrograde memory tests, on the other hand, his performance was mixed. While he showed virtually no impairment in autobiographical memory, he was impaired in tests of famous faces and public events. The authors note that his poor performance may have

been caused by low motivation during testing and his lack of education. Given these factors, his retrograde performance was difficult to interpret.

Patients R.B. and G.D. performed normally on tests of other types of cognitive ability, indicating that their cognitive impairments were limited to memory loss (Rempel-Clower et al., 1996; Zola-Morgan et al., 1986). After their deaths, the authors were able to carry out extensive neurohistological analyses to confirm that the lesions were restricted to the hippocampus. Within the hippocampus, both patients exhibited thorough and highly localized cell loss in the CA1 field (although G.D. displayed some sparing in proxmial CA1). R.B also displayed small, focal lesions in the subiculum and CA3 (observed in only two histological sections), as well as other minor damage in the left globus pallidus, right primary somatosensory cortex, anterior amygdaloid area, left internal capsule, and cerebellar cortex. G.D. exhibited some cell loss in the subiculum, particularly at the border of CA1, but CA2, CA3 and DG were intact. Other affected regions include EC layers III and V, left ventromedial amygdala, left mammillary nucleus, left mediodorsal thalamic nucleus, right globus pallidus, and the vermis of the cerebellum. However, the damage in these areas was minor. Together, these early findings demonstrated that damage limited primarily to area CA1 is sufficient to produce clinically significant and lasting anterograde memory impairments in humans.

Studies have also examined transient global amnesia (TGA), an example of acute memory impairment, to uncover the role of the CA1 field in specific types of memory. TGA involves both retrograde and anterograde memory deficits (without other cognitive impairments) that last up to 24 hours. The exact cause of TGA is unknown. However, Barstch et al. (2006) observed that TGA is frequently associated with transient focal

abnormalities of area CA1 that are detectable using diffusion-weighted imaging (DWI) and T2-weighted MRI. These abnormalities are observable 24-72 hours after the onset of amnesia and are reversed within 14 days. Barstch et al. (2010) suggest that they reflect the temporary disruption of the CA1 field and its circuitry in the hippocampus. While their neuroimaging techniques have been useful in detecting abnormalities in area CA1 in TGA patients, is important to keep in mind that the severity of the memory impairment observed with TGA indicates that the disruption may be more widespread than what is observed with their methods (Barstch et al., 2011; Kritchevsky et al., 1997).

Barstch et al. (2010) investigated the effects of TGA on place memory using a virtual watermaze task. Patients presenting with TGA symptoms underwent virtual training several hours after the onset of amnesia. Then, 48-72 hours later, DWI and T2-weighted MRI was undertaken to determine if the patients displayed CA1 abnormalities. Only those with focal CA1 changes were included in the study. Patients with CA1 abnormalities were impaired during the acquisition phase of virtual watermaze training (when the subjects were required to form a place memory for the platform location), but not during a cued exploration task and virtual training (in which participants practiced using a joystick to travel in a virtual environment). They were also impaired during the follow-up probe tests, conducted approximately two weeks later (after TGA recovery) to assess watermaze recall performance. Based on these findings, the authors propose that human CA1 neurons play a critical role in place memory. It should be noted, however, that TGA patients are also impaired at recall and recognition of word lists and other tests that have no obvious spatial component (Kritchevsky et al., 1988; Kritchevsky et al., 1997).

In a separate study with a similar experimental paradigm, Barstch et al. (2011) assessed the role of the CA1 field in autobiographical memory. TGA patients were given autobiographical memory interviews several hours after the onset of their amnesic episode and, afterward, those with CA1 abnormalities were selected using the same MRI-detection methods. Interestingly, the patient group displayed a memory impairment that was severe across all time periods (beyond 30 years into the past). The authors interpreted these findings to show that area CA1 is necessary for autobiographical memory retrieval in humans.

Although TGA studies have been able to link specific functions to the CA1 subfield in humans, they conflict with the original findings from patients with permanent CA1 damage and more chronic forms of amnesia. In contrast to the severe remote impairment observed by Barstch et al. (2011), Patients R.B. and G.D. displayed minimal, if any, autobiographical retrograde memory loss (Rempel-Clower et al, 1996; Zola-Morgan et al., 1986). Even patients with more complete hippocampal damage display retrograde impairments that typically cover a maximum of only 10 years (Bayley et al., 2006). The patients in the Barstch et al. (2011) study are more reminiscent of patients with lesions that extend beyond the hippocampus, such as E.P., who lost over 30-40 years of autobiographical memory (Insausti et al., 2013). Patient E.P. exhibited extensive MTL damage, as well as cortical thinning and atrophy in the surrounding temporal lobe (Inausti et al., 2013). These considerations raise the concern that TGA could involve additional dysfunction or aberrant activity in cells outside of area CA1 (perhaps throughout the MTL and surrounding cortex). This extended dysfunction may have been too subtle to be

detected by their neuroimaging methods, but could still have had a significant impact on memory performance.

Barstch et al. (2010) have argued that it is difficult to compare chronic and acute forms of amnesia because of potential compensatory mechanisms that take place when the hippocampus is permanently damaged and MTL circuits are given time to reorganize. This may be possible considering that patients with permanent damage are typically tested weeks or months after the onset of amnesia, which would allow time for circuit reorganization. However, there is little to no evidence that memory improves over time in patients with permanent CA1 damage. Patients R.B. and G.D. demonstrated stable memory impairments that remained clinically significant even years after their injuries (Rempel-Clower et al, 1996; Zola-Morgan et al., 1986). Considering the rapid time course of TGA, it can be argued that sudden changes in area CA1 could induce widespread MTL circuit abnormalities that prevent learning and memory. With enough time, these circuit abnormalities resolve and learning can occur. This scenario might explain the brief manifestation of the memory impairment in TGA, and again raises the possibility that TGA affects areas outside of the CA1 subfield.

Overall, the human work has shown that CA1 damage impairs memory. It has also shown that TGA is often associated with disruption in area CA1, although concerns remain regarding the extent of the disruption. While these findings suggest that area CA1 is an important component in the memory system, more experimentation is needed to determine the exact nature of its involvement.

CA1 Lesions in Animals

Following the human findings, hippocampal lesions were extensively studied in animals, primarily in rodents and monkeys. Some of the earliest studies involved models of ischemia. Because of the vulnerability of the CA1 field, these models often featured CA1-selective damage.

Models of ischemia. In animals, it was initially thought that damage to both the hippocampus and the amygdala were required to create a significant memory impairment (Mishkin, 1978). This idea, however, could not be reconciled with findings from ischemic patients that demonstrated that hippocampal lesions alone could impair memory (Zola-Morgan et al., 1986). Bachevalier and Mishkin (1989) questioned whether the impairment observed in ischemic patients was caused solely by hippocampal damage or by possible undetected ischemic damage outside of the hippocampus. They tested their inquiry experimentally, using an ischemic procedure in the monkey. The procedure produced three bilateral hippocampal lesions (20-55% damage) with little to no detectable extra-hippocampal damage. Using the delayed nonmatching to sample task (DNMS), they demonstrated that monkeys with these ischemic lesions were impaired in recognition memory, thus corroborating the human findings. It is important to note that while the ischemic lesions in this study were not solely limited to the CA1 field, areas CA1 and CA2 were the only damaged structures that the three subjects had in common. Interestingly, they also found that monkeys with ischemia were more severely impaired than monkeys with more extensive neurosurgical lesions of the MTL. The authors interpreted these findings to show that ischemia may involve more extensive damage than

what is observed in the histology. However, upon closer inspection of the data, Squire and Zola (1996) proposed that this unusual finding could have been caused by differences in the testing histories of their subjects.

In another study conducted in the monkey (Zola-Morgan et al., 1992), ischemia produced more selective damage to areas CA1 and CA2, except for some loss of somatostatin-immunopositive cells in the dentate hilus. Outside of the hippocampus, they observed patchy cell loss in the Purkinje cell layer of the cerebellum, but no damage in areas implicated in memory function. Monkeys with ischemic lesions were impaired on DNMS, object retention, and eight-pair concurrent discrimination tasks. Interestingly, these monkeys were less impaired on the latter two tasks than monkeys with more extensive aspiration lesions of the hippocampus (Zola-Morgan et al., 1994), indicating that the ischemic lesions likely involved less tissue damage than aspiration lesions. These findings suggest that the memory impairment observed in the ischemic group was caused by the observed damage, mainly within areas CA1 and CA2, and not by undetected widespread damage.

The ischemic lesion was also developed in rodents, and has been shown to cause impairments in a wide variety of memory tasks. Rats with ischemic lesions were impaired in spatial memory with the radial arm maze task (Volpe et al., 1984; Volpe et al., 1989) and the watermaze (Auer et al., 1989). They were also impaired on DNMS, which is similar to the testing conducted with both monkeys and patients (Wood et al., 1993). In each of these studies, the majority of the cell loss was observed in area CA1. However, various other structures, most commonly the dentate hilus and striatum, also exhibited varying levels of damage.

The behavioral effects of ischemic lesions in rats have also been compared against targeted neurotoxic lesions of area CA1. Volpe et al. (1992) tested the effects of the two lesion methods (separately) on spatial memory using a spatial delayed discrimination task. They found that in both groups, the severity of CA1 damage was predictive of performance regardless of overall hippocampal damage, suggesting that area CA1 is critical for performance in this task.

Overall, the ischemic models in the monkey and rodent support the human findings. Similar to Patients R.B. and G.D., ischemia in animals leads to cell loss primarily in the CA1 field and impairments in memory performance across a variety of tasks. It is important to consider, however, that ischemia is widespread, occurring across various brain regions. While the human and animal work have repeatedly shown that ischemic damage predominantly occurs within area CA1, they have also shown that other regions, particularly the dentate hilus, cerebellum, and striatum, can be affected. Additionally, it is possible that ischemia leads to other types of cellular damage or dysfunction that is not observed in the histology.

A study by Mumby et al. (1996) provided some evidence that ischemia may involve undetected damage outside of the hippocampus. Similar to previous findings (Wood et al., 1993), they showed that rats with ischemic lesions were severely impaired on the DNMS task. Interestingly, when they removed the hippocampus 1 hour after producing ischemia in another group of rats, they observed a milder DNMS impairment. Based on these findings, the authors suggest that ischemia induces a pathogenic process that involves the hippocampus and leads to extra-hippocampal damage. Upon closer

inspection of the data, however, Squire and Zola (1996) found that the two experimental groups performed similarly ($P>0.10$), leaving the outcome of the study unclear.

Another study, by Nunn et al. (1998), demonstrated how extra-CA1 damage can go undetected with conventional histological techniques. They compared the behavioral effects of ischemia and neurotoxic CA1 lesions and found that ischemic lesions impaired performance on the watermaze and match-to-position tasks more severely than neurotoxic lesions. Using a standard histological stain (cresyl violet), they found that the lesions were specific to area CA1 and comparable across both groups. However, using the Fink and Heimer (1967) method of silver impregnation, they observed additional cell loss in the DG, CA2, and CA3 in all rats with ischemic lesions. This extra-CA1 damage may explain why the ischemic group exhibited greater memory impairments.

While the two previous studies provide some indication that ischemic damage extends beyond area CA1, a study by Zola-Morgan et al. (1994) suggests that ischemic damage is still limited to the hippocampus. They compared the memory deficits of monkeys with ischemia and neurosurgical MTL lesions of various extents and loci. They found that ischemic monkeys performed most closely to those with lesions limited to the hippocampus. Additionally, monkeys with ischemia and hippocampal lesions were significantly less impaired than those with more extensive MTL lesions that also include the entorhinal and parahippocampal cortices. Their findings indicate that potential ischemic damage outside of the CA1 likely does not extend beyond the hippocampus.

Neurotoxic CA1 lesions. Current studies typically use neurotoxic methods to generate CA1 lesions, mainly in rodents. Neurotoxic lesions are generally more reliable

and have highly targeted effects (Jerman et al., 2005). Thus, they have been useful in more precisely identifying different types of memory that specifically require area CA1.

Spatial memory. The hippocampus has long been associated with spatial memory. Over the years, there have been numerous reports of spatial impairments following hippocampal damage (Broadbent et al., 2004; Clark et al. 2005; Jarrard, 1983; Morris et al., 1982). Given these findings and the discovery of place cells in area CA1 (O'Keefe and Dostrovsky, 1971), the spatial function of the CA1 field became a natural target of memory research.

Studies first determined whether area CA1 plays an important role in spatial learning using the watermaze task, a benchmark test of spatial memory. Stubley-Weatherly et al. (1996) tested rats with dorsal (d) CA1 and ventral (v) CA3 lesions in the watermaze. Following histological analysis, they found that the lesions were fairly restricted to their respective targets, although the vCA3 lesion group sustained significantly more cellular damage. Despite this discrepancy, they observed equal impairments in watermaze acquisition in the two groups, suggesting that both structures are needed in spatial learning, although area CA1 may play a more significant role.

Similar observations have been reported in mice using different spatial tasks. Dillon et al. (2008) used mice to test the effects of CA1 lesions on spatial memory in the Y-maze spontaneous alternation task and the 8-arm radial maze. Histological analysis revealed that the lesions varied in severity, but were largely specific to the CA1 field. They found that mice with CA1 lesions (even those with small lesions restricted only to

the dorsal portion of area CA1) were significantly impaired on both spatial tasks. Based on these findings, the CA1 field appears to be necessary for spatial learning in mice.

It has been suggested that spatial memory requires both topological and metric spatial information processing (Goodrich-Hunsaker et al., 2005). Topological information involves crude representations of space, such as basic relationships between objects (e.g., object A is above object B). Metric information involves more precise spatial information, such as the distance or angle between objects (e.g., object A is closer to object B than it is to object C). To determine if the individual hippocampal subfields are preferentially involved with processing either type of spatial information, Goodrich-Hunsaker et al. (2008) tested rats with dorsal CA1, CA3, and DG lesions using separate metric and topological memory tasks. Their topological task tested the ability of the rats to recognize differences in the configuration of four objects, whereas their metric task tested their ability to identify differences in the distance between two objects. They found that metric memory was impaired following lesions of dorsal DG, CA3, and CA1, while topological memory was only impaired following dCA1 lesions. These findings demonstrate that of the three hippocampal subfields, area CA1 is uniquely required for both metric and topological information processing.

Together, these findings show that area CA1 is required in a variety of spatial tasks. They are consistent with reports on spatial memory following hippocampal lesions. Additionally, two of the presented studies directly compared the effects of CA1 lesions against lesions of the DG and CA3, and both provided some indication that spatial memory is particularly dependent on area CA1.

Fear memory. Several lesion studies have also investigated the role of area CA1 in fear memory. In the Stubley-Weatherly et al. (1996) study, rats with dCA1 and vCA3 lesions underwent passive avoidance conditioning. They habituated the rats to a dark compartment, then later presented them with a footshock in the compartment. They tested the rats by placing them in a light compartment with access to the familiar dark compartment, and measured the time it took for the rats to enter the dark compartment. They found that both lesion groups entered the dark compartment faster than the controls, and were therefore impaired in remembering the association with the dark compartment and the shock. These findings indicate that areas CA1 and CA3 are necessary in a fear-motivated memory. Interestingly, they observed that the impairment was greater in the vCA3 lesion group than in the dCA1 group. In the reports discussed thus far, CA1 lesions have caused equal or greater memory impairment than CA3 or DG lesions. The current finding is the first to count against this trend. The authors suggest that this could be attributed to the severity of the cell loss reported in their vCA3 lesions. It might also be explained by the particular involvement of the ventral hippocampus with fear memory.

It has been shown that the ventral hippocampus is closely connected to structures necessary for regulating fear, anxiety, and stress behaviors, such as the amygdala and the prefrontal cortex (Goldman-Rakic et al., 1984; Henke, 1990), whereas the dorsal hippocampus is closely connected with regions that support spatial processing, such as the retrosplenial and dorsolateral medial entorhinal cortices (Cenquizca and Swanson, 2007; Fyhn et al., 2004; Harker and Whishaw, 2004). Given the differences in connectivity along the dorsoventral axis of the hippocampus, it has been suggested that the ventral region is particularly involved with fear memory while the dorsal region is

more important for spatial memory (Moser & Moser, 1998). It might be expected, then, that the ventral region of the CA1 subfield would play a greater role in fear conditioning than the dorsal region.

Hunsaker and Kesner (2008a) used a delay fear conditioning paradigm to assess context and auditory-cued fear learning along the dorsoventral axis of area CA1. They found that dCA1 lesions impaired contextual encoding (freezing during the intertrial intervals of the conditioning phase) and retention (freezing during the context test), while vCA1 lesions impaired only context retention. In the auditory-cued fear tests, neither group showed any impairment in encoding or retrieval, although this is not unexpected considering that the same finding is often observed with full hippocampal lesions (Kim and Fanselow, 1992; Phillips and LeDoux, 1992).

The previous study shows that dCA1 is recruited during context fear encoding and retrieval. Similar findings have been reported by Lee and Kesner (2004). The ventral portion of area CA1, on the other hand, is shown to be recruited only during retrieval. Interestingly, this does not align with the expectation that vCA1 would play a greater role in fear conditioning. This might be explained by the association between contextual and spatial learning (Nadel and Willner, 1980; Sutherland and Rudy, 1989). If the dorsal hippocampus is important for spatial memory and contextual learning involves information about space, it is not surprising that the dCA1 is heavily recruited for context fear conditioning.

A similar study was conducted by Rogers et al. (2006) to assess the effects of dCA1 and vCA1 lesions on trace fear conditioning. Trace fear conditioning is identical to delay fear conditioning, except that it incorporates a trace interval that separates the

presentation of the tone and shock. In this study, they did not observe encoding impairments in either lesion group during the context or trace acquisition periods. They did, however, find impairments during the retention tests. The dCA1 lesion group displayed a moderate impairment in context fear retention, while the impairment in the vCA1 lesion group was more severe. They also found that the vCA1 lesion group was impaired during the trace retention test. These observations suggest that both regions are needed for context fear retention in the trace conditioning paradigm, but the ventral portion of the CA1 may play a more important role. This conflicts with the report from Hunsaker and Kesner (2008a), but supports the idea that vCA1 is more closely involved with fear conditioning than dCA1. Additionally, this study shows that area CA1 is necessary in auditory-cued fear conditioning when the tone and shock are separated by a trace interval.

Temporal processing. Why is area CA1 involved only in cued fear conditioning when a trace interval is introduced? It might be because the trace interval incorporates the element of time. More specifically, area CA1 may play a role in associating events that are separated in time.

Early evidence for the relationship between the hippocampus and time comes from a rodent study that used the Y-maze spontaneous alternation task (Mikulka and Freeman, 1975). They found that hippocampal lesions impaired performance when reinforcement was given after a 10 s delay, but did not impair performance when the reinforcement was given immediately. Additional evidence comes from observations in rabbits that show that the hippocampus is only necessary in eyeblink conditioning when it

involves a trace interval (Moyer et al., 1990; Solomon et al., 1986). The same finding was observed in rats with delay and trace fear conditioning (Kim and Fanselow, 1992; McEchron et al., 1998; Phillips and LeDoux, 1992). Together, these studies led to the idea that the hippocampus is responsible for processing temporal information.

Based on the findings from Rogers et al. in 2006 (unpublished at the time), Kesner et al. (2005) suspected that within the hippocampus, the CA1 subfield is particularly important for temporal processing. They modified an existing object-odor paired associate task (known to be independent of the hippocampus) to include a trace interval between the presentations of the object and the odor. They then tested to see if the addition of the trace interval recruited either dCA1 or dCA3. They found that rats with dCA1 lesions were impaired on the task, while rats with dCA3 lesions performed similarly to controls. Their findings show that area CA1 is recruited in another hippocampus-independent task following the addition of a trace interval, while area CA3 is not.

While the previous studies have demonstrated that the CA1 field is needed when bridging information across time gaps, temporal information can manifest in other forms - for example, through sequences. Lee et al. (2005) investigated the roles of areas CA1 and CA3 in spatiotemporal processing using a spatial sequence memory task. The newly designed task, called the Tulum maze, required rats to remember the sequence of four identical sections of the maze presented one at a time, as well as a specific location marked within each section. After exposure to each section and a short delay, the rat was returned to one of the four sections and was required to find the location in that section that was marked during the rat's previous visit (using only background cues as spatial

guides). This task requires rats to hold four locations in memory that are separated and organized in temporal order.

With the Tulum maze, Lee et al. (2005) found that dCA1 lesions impaired performance in all serial positions in the sequence, while dCA3 lesions impaired performance only on the first three serial positions. Additionally, both lesions led to more severe impairments when the locations were presented in the middle of the sequence (the two positions most subjected to interference from neighboring sequential positions). These findings suggest that dCA1 and dCA3 may need to work together to perform temporal pattern separation for spatial locations. They also indicate that both the CA1 and CA3 fields play an important role in spatial sequential learning.

Hunsaker and Kesner (2008b) aimed to more finely characterize the involvement of the hippocampal subfields in spatial and temporal processing using a different sequence memory task. Their task specifically tested the ability of rats to process spatiotemporal information with either high or low spatial interference. They found that rats with dCA1 lesions were not affected by the level of spatial interference (in contrast to rats with dDG lesions that were impaired only when interference was high), which indicates that the CA1 is not involved with discriminating spatial locations. Similar observations were reported by Gilbert et al. (2001), in which dDG lesions led to spatial pattern separation impairments, while dCA1 lesions did not. Instead, Hunsaker and Kesner (2008b) observed that rats with dCA1 lesions displayed a preference for the most recent locations in the sequence. Based on these findings, the authors propose that area CA1 contributes to temporal processing by regulating recency judgements. They suggest that area CA1 biases the rat towards primacy (favoring items presented at the beginning

of the sequence) to counteract biases supported in other brain regions toward recency (favoring the items presented last). These findings, however, are not supported by the previous report from Lee et al. (2005), which showed that dCA1 lesions impair performance even when the tested location was the most recently presented.

The two previous reports suggest that the CA1 field plays an important role in sequence memory, which supports the idea that area CA1 is needed to process information about time. However, both studies involved memory for spatial information and area CA1 has been shown to be an important structure in spatial memory (Dillon et al., 2008; Goodrich-Hunsaker et al., 2005; Stubley-Weatherly et al., 1996). It could be the case that these findings do not apply to sequence memory for non-spatial information.

To determine if CA1 involvement with sequence memory applies more broadly, other studies focused their investigation on the effects of CA1 lesions on non-spatial sequence memory tasks. It was reported that dCA1 and vCA1 lesions impair performance on a sequence memory task for visual objects (Hoge and Kesner, 2007; Hunsaker et al., 2008). It was also shown that vCA1 lesions impair sequence memory for olfactory information (Hunsaker et al., 2008; Kesner et al., 2010). Together, these findings show that area CA1 is involved in sequence memory across various modalities. Interestingly, Hoge and Kesner (2007) reported that rats with dCA1 lesions showed a strong preference for the most recently encountered visual objects, similar to what was found in the Hunsaker and Kesner (2008b) study with spatial locations.

The question remains about how the CA1 subfield supports sequence memory. Does it directly code for order? To test this idea, Farovik et al. (2009) designed an order processing task that required rats to remember the order of two sequentially presented

odors. They used 10 different odor pairs that were unique to each trial. They found that rats with dCA1 lesions performed normally on the task when the delay between the two paired odors was limited to 3 s, suggesting that area CA1 is not critical for processing order. In contrast, rats with dCA1 lesions were impaired when they increased the delay between the paired odors to 10 s. It is unclear why performance was impaired with a longer inter-item interval, but the finding may reflect the involvement of area CA1 in linking associated events that are separated in time. Area CA1 may be needed to link paired events when they are separated by long delays that tax attention and working memory.

Interestingly, in contrast to their observations with dCA1 lesions, the authors found that rats with dCA3 lesions were severely impaired regardless of the inter-item interval condition (Farovik et al., 2009). Thus, when the delay between the paired odors was limited to 3 s, rats with dCA3 lesions were impaired while rats with dCA1 lesions were not, providing an additional instance in which CA3 lesions led to more severe memory impairments than CA1 lesions.

Summary

Overall, these studies have demonstrated that area CA1 plays a critical role in memory. In humans, CA1 lesions led to performance deficits in various types of recall and recognition tests. Similar findings were observed in monkeys. In rodents, CA1 lesions impaired performance in a variety of memory tasks, including those involving space, fear, and time. Together, the human and animal work demonstrate that area CA1 supports memory across different types of tasks and modalities.

However, it is notable that the memory impairments caused by CA1 lesions were quite broad, which indicates that the findings might not simply reflect impairments in functions that are specific to area CA1. Instead, these impairments might reflect the fact that CA1 lesions disrupt hippocampal output to neocortex. Consistent with this idea, many of the impairments observed with CA1 lesions mirrored the findings from complete hippocampal lesions. For example, both lesions impaired performance in the watermaze task, context fear conditioning, and trace, but not delay fear conditioning (Hunsaker and Kesner, 2008a; Kim and Fanselow, 1992; McEchron et al., 1998; Morris et al., 1982; Phillips and LeDoux, 1992; Rogers et al., 2006; Stubley-Weatherly et al., 1996). Additionally, there were many instances in which CA1 lesions impaired memory equally or more severely than either DG or CA3 lesions, which would be expected if CA1 lesions prevented information processed in the DG and CA3 from reaching neocortex.

There were a few findings, however, that did not fit these patterns. For example, rats with dDG lesions were impaired on a spatial pattern separation task with high spatial interference, while rats with dCA1 lesions were unimpaired regardless of interference level (Gilbert et al., 2001). Also, rats with dCA3 lesions were impaired on an order processing task regardless of inter-item interval, while rats with dCA1 lesions performed normally when the inter-item interval was limited to 3 s (Farovik et al., 2009).

These conflicting findings raise an interesting question. If area CA1 provides the primary hippocampal output pathway to neocortex, why would a CA1 lesion, which should disrupt hippocampal output to neocortex, lead to better performance than a lesion in the DG or CA3? One potential explanation involves the descending projections from area CA3 to subcortical structures (Swanson and Cowan, 1979). While many regard CA1

output to the neocortex to be the main pathway by which memory-related information leaves the hippocampus, it is possible that information sent to subcortical structures could support some forms of memory as well.

Another potential explanation involves CA1 sparing. Area CA3 is known to project broadly along the dorsoventral axis of area CA1 (Ishizuka et al, 1990) and the vast majority of CA1 lesions in the literature have only targeted either the dorsal or ventral halves of CA1 (separately). These lesions typically encompass between 70-90% of their respective targets (Goodrich-Hunsaker et al., 2008; Hoge and Kesner, 2007; Hunsaker et al., 2008; Lee et al., 2005; Rodgers et al., 2006), which results in damage to less than 50% of the total CA1 subfield. Therefore, with these partial lesions, area CA3 may have been able to send information to the neocortex through its projections to the spared half of area CA1.

Overall, it remains unclear if CA1 output to neocortex is needed to support memory. This question could be more clearly answered with a complete CA1 lesion, which would block all hippocampal output to neocortex. However, a neurotoxic lesion encompassing the entire CA1 subfield has not yet been reported in the literature. To resolve these issues, the main goals of the work described in this dissertation were to develop a complete CA1 lesion, to characterize its effects on memory in rats, and to compare these to the effects of large hippocampal lesions.

In the next chapter (Chapter 2), we examine the effects of the CA1 lesion on retrograde memory. Large hippocampal lesions often produce temporally graded retrograde amnesia (TGRA), whereby recent memory is impaired more than remote memory. However, TGRA has not been observed with the watermaze task, and the

findings have been inconsistent with context fear conditioning. In these cases, both recent and remote memory are severely impaired after large hippocampal lesions. To examine if complete CA1 lesions produce the same ungraded impairments as large hippocampal lesions, we tested rats with complete CA1 lesions on the watermaze, context fear conditioning, and trace fear conditioning tasks at recent and remote time points. We considered the possibility that the CA1 lesion might spare remote memory because it minimizes damage to the hippocampus and could reduce indirect disruption to structures that project to the hippocampus. Yet, as described in Chapter 2, we found no evidence of remote sparing after complete CA1 lesions with any of the three tasks. These findings support the idea that CA1 output to neocortex is needed to support memory. However, the impairments with CA1 lesions (and those reported with large hippocampal lesions) were always severe and demonstrated floor effects. Therefore, these retrograde experiments might not have been able to detect all differences between the two lesions.

In Chapter 3, we more finely compare the two lesions by testing their effects on anterograde memory. Specifically, rats with complete CA1 lesions, large hippocampal lesions, or sham surgeries were tested in two different tasks in the watermaze: the delayed match-to-position task, followed by the standard watermaze task. Additionally, different rats with the same lesions were tested on the watermaze task, followed by delayed match-to-position. As described in Chapter 3, we found striking differences between the effects of the two lesions on these tasks, particularly when rats had prior experience in the opposing task. Our findings indicate that memory may not always require CA1 output to neocortex depending on the experience that an animal brings to a new task.

CHAPTER 2:

RECENT AND REMOTE RETROGRADE MEMORY ARE IMPAIRED AFTER COMPLETE CA1 LESIONS

Systems consolidation refers to the process by which new memories become independent of the hippocampus as they are gradually reorganized into a stable, long-lasting form in neocortex. Support for this idea comes from the phenomenon of temporally graded retrograde amnesia (TGRA), whereby recently acquired memories are more vulnerable to hippocampal damage than older, remotely acquired memories (Frankland and Bontempi, 2005; Squire and Bayley, 2007; Squire et al., 2015). TGRA after hippocampal damage has been well documented in humans (Kapur and Brooks, 1999; Manns et al., 2003) and experimental animals (Clark et al., 2002; Kim and Fanselow, 1992; Kim et al., 1995; Takehara et al., 2003; Zola-Morgan and Squire, 1990).

An exception to these findings is found in studies with the watermaze task, which tests memory for locations in space. Rats with hippocampal lesions have consistently exhibited severe, ungraded retrograde memory impairment in this task, with remote memory as severely impaired as recent memory (Bolhuis et al., 1994; Clark et al., 2005a; Mumby et al., 1999; Sutherland et al., 2001). The same ungraded impairment was also observed using variations of the watermaze task: cued platform locations (Clark et al., 2007; Martin et al., 2005), annular tracks (Clark et al., 2005a; Hollup et al., 2001), the dry-land Oasis maze (Clark et al., 2005a), and when prolonged training was given early in life (Clark et al., 2005b).

Why is remote memory impaired in rodents with hippocampal lesions when testing occurs in the watermaze? Typically, rodent studies have involved large, excitotoxic hippocampal lesions that encompass areas CA1, CA3, and the dentate gyrus (DG). These large lesions might indirectly disrupt the function of neighboring regions, similar to the disruption observed in area CA1 after excitotoxic lesions of the entorhinal cortex (Miettinen et al., 1998). Indeed, large excitotoxic hippocampal lesions have been reported to cause volume loss in the cortex (Anagnostaras et al., 2001; Anagnostaras et al., 2002; Jarrard and Meldrum, 1993). The affected regions could include areas important for task performance or cortical areas thought to be important for storing consolidated memories (Frankland et al., 2004; Maviel et al., 2004). These possibilities might be explored by preparing a discrete lesion that targets only area CA1. Because area CA1 provides the sole output pathway from the hippocampus to neocortex (van Strien et al., 2009), a selective CA1 lesion should disrupt hippocampal output to neocortex but preserve the majority of the hippocampus and reduce potential indirect disruption in neighboring regions by sparing the majority of the projections to the hippocampus.

We tested the effects of a CA1 lesion in rats on recent (1-3 days old) and remote (31-33 days old) memory in the watermaze. We also tested the effects of this lesion on context and trace fear conditioning. TGRA has been reported previously for trace fear conditioning after dorsal hippocampal lesions (Quinn et al., 2008). TGRA has also been reported for context fear conditioning after hippocampal lesions (Anagnostaras et al., 1999; Kim and Fanselow, 1992; Maren et al., 1997; Winocur et al., 2009), but the literature is mixed and ungraded retrograde memory impairment has also been reported

(Broadbent and Clark, 2013; Lehmann et al., 2007; Sparks et al., 2011; Sutherland et al., 2008).

METHODS

Subjects

Subjects were 64 experimentally naive, male Long-Evans rats that were first trained in the watermaze and fear conditioning tasks. Following training, rats were assigned to receive either CA1 or sham lesions (based on watermaze performance at the end of training). Rats in the recent condition received surgery 1-3 days post-training (CA1 n=16, sham n=16), while rats in the remote condition received surgery 31-33 days post-training (CA1 n=16, sham n=16). Following recovery from surgery, all rats were tested in both behavioral tasks. In the recent condition, rats weighed between 320-350 g at the beginning of training, and in the remote condition they weighed 290-320 g. Rats were maintained on a 12:12 h light:dark cycle and were initially housed in pairs, then housed individually post-surgery. Food and water were freely available. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego.

Apparatus

Watermaze. Testing was conducted in a pool of water (1.8-m diameter at the water level) that was rendered opaque by the addition of powdered milk. The testing room contained a number of constant, salient visual cues (posters, objects, equipment). A

video camera mounted on the ceiling directly above the pool was used in conjunction with a video tracking system (San Diego Instruments) to record the swim path of each rat. An Atlantis platform (12.7 cm diameter) was used that could be raised or lowered remotely (Spooner et al., 1994). In the lowered position, the platform was undetectable and unavailable. In the raised position (1.5 cm below the surface of the water), the platform remained invisible, but provided a means to escape the water.

Context and trace fear conditioning. Conditioning and testing were conducted in 8 identical fear-conditioning chambers housed within polyvinyl chloride (PVC) sound-attenuating cubicles (Med Associates Inc, Georgia, VT). The conditioning chambers were constructed from aluminum and Plexiglas. The floor of each chamber consisted of 19 stainless steel rods (0.5 cm diameter) spaced 1.6 cm apart (center-to-center). The rods were connected to a shock generator and scrambler. Each chamber was fitted with a ventilation fan that also provided background noise (75 dB). A video camera connected to a computer was positioned at the front of each chamber, which digitally recorded behavior for off-line analysis using Video Freeze V2.1.0 software (Med Associates Inc.).

Behavioral Training

Watermaze acquisition. Rats were given eight trials/day for 10 days. The first and fifth trials of each day were reinforced probe trials. During these trials, rats were placed in the water facing the pool wall at one of four start points (counterbalanced across animals). The platform remained lowered for the first 60 s of the probe trial. The platform was then raised, and the rat had an additional 60 s to reach the platform. If the rat did not reach the platform within the additional 60 s, it was guided to the platform by the

experimenter. After escaping the water, the rat remained on the platform for 30 s and was then returned to its home cage. During the remaining six standard training trials, the platform remained in the raised position, allowing the rats to escape from the water. Rats were given up to 2 min to escape the water before being guided to the platform by the experimenter. As with the probe trials, the rats remained on the platform for 30 s before being returned to their home cage. Following training, rats were assigned to receive either CA1 or sham lesions based on their performance during the first probe trial on the last day of training. Specifically, the assignment was based on the percentage of time that the rat spent in the quadrant of the pool that had contained the platform (chance = 25%).

Context and trace fear conditioning. Following completion of watermaze training, rats underwent context and trace fear conditioning. Each rat was placed in a fear-conditioning chamber for approximately 25 min. The session began with a 240-s baseline period, followed by five tone-shock trials. Each trial consisted of a 20-s pure tone (5 kHz, 90 dB), a 30-s stimulus-free trace interval, and a 2-s foot-shock (1.0 mA). The inter-trial interval was 240 s and the conditioning session ended 60 s after the last trial.

Surgery

At the prescribed time after training, rats received either excitotoxic CA1 lesions or sham surgeries. Anesthesia was maintained throughout surgery with isoflurane gas (0.8-2.0% isoflurane delivered in O_2 at 1 L/min). The rat was placed in a Kopf stereotaxic instrument, and the incisor bar was adjusted until Bregma was level with Lambda. For CA1 lesions, ibotenic acid (IBO; Biosearch Technologies) dissolved in 0.1 M PBS (concentration: 10 mg/ml, pH 7.4) was injected along the dorsoventral CA1 axis

(bilaterally) using a 10 ml, 30-g Hamilton syringe, which was held in a Kopf microinjector (model 5000) and mounted on a stereotaxic frame. The syringe was first lowered to the target coordinate and left in place for 1 min. After injection (at a rate of 0.1 ml/min), the syringe stayed at the target coordinate for 2 min to prevent IBO from spreading up the syringe tract upon its retraction. For certain injection sites in ventral CA1 (noted below), the syringe was left in place for 5 min to ensure that IBO would not spread up the syringe track (where it might cause unintended damage to CA3 or the DG). IBO (0.025 µl) was injected at each site (unless otherwise noted). Injections were made at multiple locations. All coordinates are in millimeters, anteroposterior (AP) relative to Bregma, mediolateral (ML) relative to Lambda, and dorsoventral (DV) relative to the brain surface at -4.8 mm from Bregma and ±4.2 mm from Lambda: AP -2, ML ±1, DV -2.9; AP -3.6, ML ±1, DV -2.7; AP -3.6, ML ±2, DV -1.9; AP -4.5, ML ±1.4, DV -3.3; AP -4.5, ML ±2.7, DV -1.8; AP -4.5, ML ±4.5, DV -7.9 (waited 5 min before retracting syringe); AP -5.3, ML ±3, DV -1.7; AP -5.3, ML ±4.8, DV -8 (waited 5 min before retracting syringe); AP -5.3, ML ±4.8, DV -2.4; AP -5.3, ML ±5.8, DV -7.5; AP -5.3, ML ±5.8, DV -5.7; AP -5.3, ML ±5.8, DV -3.9; AP -6.3, ML ±5.4, DV -3 (injected 0.05 µl IBO); AP -6.3, ML ±6.3, DV -5.7 (injected 0.05 µl IBO). Rats given sham surgeries underwent the same surgical procedures up to the point of the craniotomy. Once awake and responsive, each rat was returned to its home cage for a 12-14 day recovery period.

Behavioral Retention Tests

Watermaze retention test. Following recovery from surgery, rats were given a reinforced probe trial (as described in Behavioral Training) to test their memory for the

trained platform location. As in training, the start location for the trial was counterbalanced across animals. Spatial memory retention was calculated by measuring the percentage of time each rat spent in the quadrant of the pool where the platform had been located during training (chance = 25%), as well as the percentage of time that each rat spent in the circular zone directly above the platform location (chance = 4%).

Context fear retention test. Following the watermaze test probe, rats were tested for their retention of context fear memory. Rats were placed in the fear-conditioning chambers that they were originally conditioned in for 8 min, while freezing behavior was measured. Context fear retention was calculated as the percentage of time that each rat spent freezing during the 8-min test.

Trace fear test. The next day, rats were habituated to a new context for 8 min. This new context involved a different fear-conditioning chamber with triangular walls, flat plastic flooring, altered lighting, new olfactory cues, a modified transportation experience, and a new experimenter (who handled each animal for two 5-min sessions prior to this phase of testing). One day later, rats were returned to the context in which they were habituated the previous day and given an 8-min tone test to assess their retention of trace fear memory. The test began with a 240-s baseline period, followed by a 20-s tone, and then 220 s without tone. Trace fear retention was calculated as the percentage of time that each rat spent freezing during the 240 s after the onset of the tone.

Histology

At completion of testing, the rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by

10% formaldehyde solution (in 0.1 M phosphate buffer). The brains were removed and cryoprotected in 20% glycerol/10% formaldehyde. Coronal sections (50 um) were cut with a freezing microtome beginning at the level of the anterior commissure and continuing caudally through the length of the hippocampus. Every fifth section was mounted and stained with thionin to assess the extent of the lesions.

An additional series of sections (with the same section intervals) was prepared for immunolocalization of neuron-specific nuclear protein (NeuN) by using an anti-NeuN (1:15,000, Chemicon) monoclonal mouse antibody. A biotinylated anti-mouse IgG (H+L) (1:1,000, Vector BA-2000) was used as the secondary antibody. Images of the NeuN-stained tissue sections were acquired using a DM6000 microscope (Leica Microsystems, Inc.). The images from every other mounted section were then analyzed using Stereo Investigator software (mbf Bioscience; MicroBrightField, CA, USA). The volumes of spared tissue were calculated using the Cavalieri method, which involved overlaying a sampling grid (one grid point/150 μm^2) on the tissue image and counting the total number of grid points in contact with each of the following anatomical regions: dorsal(d)CA1, ventral(v)CA1, dCA3, vCA3, dDG, and vDG. The total estimated volume of the spared tissue in each region was calculated by summing the section thickness, the section sampling fraction, and the number of selected grid points per section multiplied by the area associated with each grid point. We then determined the percent damage in each region, calculated by dividing the volume of damaged tissue by the average volume of tissue in the sham rats and multiplying by 100. This analysis was conducted for all lesion rats and 8 sham rats in the recent and the remote conditions. Calculations were conducted

separately for rats in the two conditions. The experimenter was not blind to the retention intervals during the analysis.

RESULTS

Neurohistological Findings

All lesion animals sustained significant damage to area CA1, including both its dorsal and ventral portions. Figure 3A shows a series of sections from a sham animal and Figure 3B shows the extent of a representative CA1 lesion. The average percent damage was 80.2%. Sparing occurred most frequently in the posterior-most and ventral-most extent of CA1. Still, overall damage to vCA1 was substantial. There was also typically some extra-CA1 damage in the DG and area CA3. On average, area CA3 sustained 38.0% damage and the DG sustained 11.5% damage. Figure 4 reports the percent damage to the separate hippocampal subregions in the lesion groups from the recent and remote conditions. Additionally, it is worth noting that there was no damage to structures immediately adjacent to area CA1 (other than the normal and unavoidable cortical damage observed above the dorsal hippocampus).

Interestingly, there was a significant discrepancy in the amount of damage sustained by the lesion groups in the two conditions. Specifically, lesion rats in the remote condition had less CA1 damage than those in the recent condition (mean ± SEM: recent: 88.7 ± 2.1%; remote: 71.7 ± 3.0%; $t(30)=4.7$, $p<0.0001$). This finding may have resulted from differences in rat size at the time of surgery. In any case, despite this potential advantage for the remote group, TGRA was not observed.

Behavioral findings.

Watermaze. A two-way ANOVA was conducted to test the effects of Group (CA1 vs. sham) and Retention Interval (recent vs. remote) on the percent time that rats spent in the target quadrant and also in the platform location during the probe test. There was a main effect for Group with both measures (quadrant: $F_{(1, 60)}$=52.6, p<0.0001; platform: $F_{(1, 60)}$=33.4, p<0.0001), indicating that the two CA1 groups spent less time than the two sham groups in the target quadrant (recent CA1: 22.6 ± 2.0%, recent sham: 47.2 ± 3.5%, remote CA1: 27.7 ± 3.9%, remote sham: 53.7 ± 4.1%; Figure 5A) and in the platform location during the probe test (recent CA1: 3.9 ± 0.7%, recent sham: 14.5 ± 1.8%, remote CA1: 6.9 ± 2.1%, remote sham: 16.4 ± 2.0%; Figure 5B). There was no main effect for Retention Interval with either the quadrant ($F_{(1, 60)}$=2.7, p>0.1) or the platform measures ($F_{(1, 60)}$=1.9, p>0.1). Also, there was no Group x Retention Interval interaction for either measure (quadrant: $F_{(1, 60)}$=0.04, p>0.1; platform: $F_{(1, 60)}$=0.1, p>0.1). Additionally, in both the recent and remote conditions, the CA1 group performed no better than chance (all ts<1.4; all ps>0.1), while the sham group performed above chance in both conditions and for both measures (all ts>5.8; all ps<0.05).

Context and trace fear conditioning. A two-way ANOVA was conducted to test the effects of Group and Retention Interval on the percent time that rats spent freezing during the context fear test and trace fear test. There was a main effect for Group with context fear ($F_{(1, 60)}$=89.8, p<0.0001; Figure 6A), showing that the two CA1 groups froze significantly less than the two sham groups during the context fear test (recent CA1: 4.8 ± 1.2%, recent sham: 50.5 ± 5.4%; remote CA1: 12.5 ± 5.6%, remote sham: 65.2 ± 6.8%). The same effect was found with trace fear conditioning ($F_{(1, 60)}$=57.1, p<0.0001; Figure

6B). During the trace fear test, the two CA1 groups were impaired in comparison to controls (recent CA1: 6.5 ± 1.0%, recent sham: 45.5 ± 8.3%; remote CA1: 9.2 ± 2.0%, remote sham: 57.7 ± 7.8%). For the context fear test, there was an additional main effect for Retention Interval ($F_{(1, 60)}$=4.6, p<0.05), indicating that remote performance was better than recent performance for the combined CA1 and sham groups. However, there was no interaction between Group and Retention Interval ($F_{(1, 60)}$=0.5, p>0.1). For the trace fear test, there was no main effect for Retention Interval ($F_{(1, 60)}$=1.7, p>0.1) and no Group x Retention Interval interaction ($F_{(1, 60)}$=0.7, p>0.1).

Behavioral findings excluding rats with extra-CA1 damage. To examine if the observed behavioral impairments were caused by unintended extra-CA1 damage, we analyzed the data after excluding rats that sustained more than 30% damage to the combined areas of the DG and CA3. This approach excluded six rats from the recent CA1 group and two rats from the remote CA1 group. Overall, the results were the same. Two-way ANOVAs demonstrated main Group effects across the three behavioral tests, indicating that the CA1 groups were impaired in comparison to controls during the watermaze probe, context fear, and trace fear tests (all Fs>24.4, all ps<0.0001). Also, there were no main effects for Retention Interval (all Fs<3.6, all ps>0.06) and no Group x Retention Interval interactions (all Fs<0.5, all ps>0.1). Lastly, we analyzed the data again after excluding rats with a more strict cutoff for extra-CA1 damage (more than 20%, which excluded nine rats from the recent condition and five rats from the remote condition), and obtained the same results.

DISCUSSION

Rats were trained in the watermaze, in context fear conditioning, and in trace fear conditioning before receiving either bilateral CA1 lesions or sham surgeries. Surgery was scheduled either 1-3 days or 31-33 days after training. The CA1 lesion was intended to reduce disruption in neighboring areas that project to the hippocampus with the idea that the restricted CA1 lesion might spare remote memory. Yet, rats that received CA1 lesions long after training were impaired in all three tasks and performed similarly to rats that received CA1 lesions shortly after training. Thus, our findings appear to exclude the possibility that impaired remote memory can be attributed to retrograde disruption in structures projecting to the hippocampus (for example, to DG and CA3).

The findings from all three behavioral tasks provide no support for the standard model of systems consolidation—the idea that the hippocampus plays a gradually diminishing role in the storage of long-term memory. Instead these data are consistent with a number of studies in the rodent literature finding that hippocampus-dependent memories remain hippocampus-dependent (Sutherland et al., 2010). One proposal is that during acquisition the hippocampus interferes with, or overshadows, the contribution of other brain areas that would otherwise encode information (Sutherland et al., 2010). A more extended account suggests that, because "the hippocampus receives a broad range of input through convergent cortical afferents, and influences activity dynamics in cortical and subcortical regions… the hippocampal representation [remains] essential for memory retrieval" (Lee et al., 2016). For discussion of other perspectives on the role of the hippocampus, particularly in remote spatial memory, see Martin et al. (2005).

Nonetheless, there are examples where TGRA has been observed in rodents following hippocampal damage, even in spatial tasks (for reviews, see Clark, 2011; Frankland and Bontempi, 2005). Although the reasons for this discrepancy (TGRA vs. no TGRA) are unclear, we consider here possible factors that could mitigate against finding TGRA in tasks such as ours. We first discuss the results from the watermaze. As in earlier studies with larger lesions (Clark et al., 2005a; Clark et al., 2005b; Clark et al., 2007; Martin et al., 2005), our findings suggest that even limited hippocampal damage impairs performance on this spatial task, regardless of how long after training the damage occurs. Note, though, that spared remote spatial memory has been observed in memory-impaired patients (for review see Squire and Bayley, 2007). For example, patient E.P., who developed profound amnesia at age 72 following bilateral medial temporal lobe damage, could mentally navigate the streets in the region where he had grown up (Teng and Squire, 1999). Similarly, patient K.C., who sustained bilateral damage to the hippocampus and parahippocampal gyrus, as well as regions of neocortex, was able to draw maps of his childhood neighborhood that included an accurate layout of the streets (Rosenbaum et al., 2000). Neither patient could learn or remember new routes (Rosenbaum et al., 2000; Teng and Squire, 1999).

Why is remote memory in the watermaze dependent on the hippocampus in rodents when patients with hippocampal damage can remember and navigate environments learned long ago? One possibility is that there are important differences between rodents and humans that affect performance in this task. Support for this idea comes from recent studies of path integration, where subjects search for a target in the dark and then try to return to the start location. Patients with hippocampal lesions

performed well at path integration, but rats with hippocampal lesions could not perform the task no matter how simple the outward path (Kim et al., 2013; Sapiurka et al., 2016). It was suggested that path integration in humans can be supported by working memory (in the neocortex), but that rodents cannot construct an effective working memory of spatial environments. Accordingly, for rodents, spatial working memory may require coordination between the hippocampus and neocortex (Sapiurka et al., 2016).

One perspective along these lines suggests that the rodent hippocampus organizes egocentric spatial information from the posterior parietal cortex in order to construct allocentric representations (Byrne et al., 2007). These representations might then support performance in spatial tasks. If so, hippocampal lesions should affect performance whenever there is a need to engage spatial working memory. In the watermaze, successful performance requires rodents to construct in working memory a coherent representation of the spatial environment and to navigate in the environment. By this account, a hippocampal lesion would impair performance regardless of whether the lesion was made shortly after training or long after training.

We next discuss the results from context fear conditioning. We did not find TGRA, despite the fact that TGRA has frequently been reported after hippocampal lesions with this task (Anagnostaras et al., 1999; Kim and Fanselow, 1992; Maren et al., 1997; Winocur et al., 2009). It is notable that, with one exception (Winocur et al., 2009), the lesions in the earlier studies were limited to dorsal hippocampus, whereas our lesion targeted both the dorsal and ventral regions of area CA1. vCA1 originates projections to the amygdala (van Groen and Wyss, 1990), which is critical for both recent and remote context fear memory (Maren et al., 1996). One possibility is that the CA1 lesion

disrupted activity in the amygdala because of the loss of input from vCA1 (also see Anagnostaras et al., 2001). Note, however, that ungraded retrograde amnesia has sometimes been reported with this task even after limited dorsal hippocampal lesions (Broadbent and Clark, 2013; Lehmann et al., 2007; Sutherland et al., 2008). Additionally, context fear acquisition is sometimes spared after large hippocampal lesions (Cho et al., 1999), which would not be expected if large lesions caused significant disruption in the amygdala.

Lastly, we discuss the results from trace fear conditioning, where again both recent and remote memory were impaired. One earlier study found temporally graded memory impairment in rats after dorsal hippocampal lesions (Quinn et al., 2008). A second study found the same trend, but without clear evidence of spared remote memory (Beeman et al., 2013). There are two important differences between our study and the earlier one that found TGRA (Quinn et al., 2008). First, in the earlier study TGRA was evident across a training-lesion interval of 200 days. We tested remote memory after a training-lesion interval of only 31-33 days. Second, in the earlier study the lesion targeted only dorsal hippocampus, whereas our CA1 lesion targeted both dorsal and ventral hippocampus. As discussed above, it is possible that the ventral portion of our CA1 lesion might have disrupted amygdala function. It is also relevant that the medial prefrontal cortex (mPFC) receives the majority of its hippocampal input from vCA1 (Cenquizca and Swanson, 2007), and mPFC lesions impair remote memory for trace fear conditioning (Beeman et al., 2013; Quinn et al., 2008). Accordingly, indirect anterograde disruption of mPFC might be particularly important for understanding the remote memory impairment that we observed in trace fear conditioning.

It is perhaps worth emphasizing that the key issue is not how hippocampal lesions affect recent and remote memory. The key issue is the status of systems consolidation, the idea that the hippocampus becomes less important for memory as time passes after learning, and an idea that hippocampal lesions could potentially illuminate. The principles of systems consolidation are well supported (Kitamura et al., 2017), especially by studies of hippocampal function that use tools and methods more temporally and spatially discrete than ibotenic lesions of hippocampus (Bontempi et al., 1999; Goshen et al., 2011; Hales et al., 2016; Maviel et al., 2004; Wiltgen et al., 2010). Nevertheless, TGRA has been inconsistently found, especially in rodents, and interpretations of retrograde memory impairment have been suggested that do not incorporate a long process of systems consolidation (Sutherland et al., 2010). Still, it is worth considering the possibility that the failure to find TGRA in the current study might reflect specific limitations of the conventional lesion technique and features specific to certain tasks. For example, the spatial demands of the watermaze task may place a burden on working memory, a problem related to the organization of rodent neocortex, not hippocampal function itself. And after large hippocampal lesions, or even CA1 lesions that include ventral hippocampus, disruptive effects may occur in other structures important for fear conditioning.

In summary, rats were impaired in the watermaze, in context fear conditioning, and in trace fear conditioning both when CA1 lesions were made shortly after training and when these lesions were made long after training. Our CA1 lesions were intended to reduce the volume of hippocampal damage and minimize indirect disruption of areas that project to the hippocampus. Our findings could reflect in part the fact that our discrete

lesion nevertheless included ventral tissue and that damage to this tissue may have disrupted function in other areas important for fear conditioning. In addition, limitations in spatial working memory in the rodent might be important in understanding watermaze performance. Moving forward, modern methods and tools that improve upon traditional lesion techniques will be useful for expanding the understanding of hippocampal function and memory consolidation.

Acknowledgments

Chapter 2, in full, is a reprint of the material as it appears in Learning & Memory, 2017. Ocampo, Amber C.; Squire, Larry R.; Clark, Robert E. The dissertation author was the primary investigator and author of this paper.

CHAPTER 3:

ANTEROGRADE MEMORY IS IMPAIRED AFTER COMPLETE CA1 LESIONS, BUT IMPROVES WITH PRIOR EXPERIENCE

INTRODUCTION

Schemas refer to preexisting knowledge structures into which newly acquired information can be incorporated (Bartlett, 1932; Tse et al., 2007; Dragoi and Tonegawa, 2013; McKenzie et al., 2014). A variety of studies have demonstrated that preexisting knowledge (or schemas) is advantageous for human learning (Bransford & Johnson, 1972; Maguire et al., 1999; van Kesteren et al., 2013; Race et al., 2015). Although the schema concept is fundamental to the psychological science of human memory, the concept has only recently become relevant in work with experimental animals.

A number of studies have now documented striking effects of prior experience on learning in rodents. For example, in one notable study, rats learned to associate six specific flavors with six places in a familiar arena (Tse et al., 2007). Initial learning was slow, but after rats had accumulated experience in the task, they learned new flavor-place pairings in a single trial. Prior experience can also benefit learning in the watermaze task (Bannerman et al., 1995). Specifically, D-AP5 normally impairs watermaze acquisition in rats, presumably by blocking the induction of LTP, but this treatment had no effect on acquisition when rats received prior watermaze training with a different platform location in a different environment. The same benefit of prior experience has been reported using other methods to block LTP (Inglis et al., 2013; Otnaess et al., 1999) and with other tasks (Dragoi and Tonegawa, 2013; Wiltgen et al., 2011). Interestingly, the benefit of prior

watermaze training was not obtained in rats with conventional hippocampal lesions (Bannerman et al., 1995).

It is unclear to what extent the beneficial effects of prior experience depend on the kind of experience that an animal brings to a new task. Can beneficial effects occur when the task to be learned is different than the task that has provided prior experience? In the current study, rats with two kinds of hippocampal lesions and control rats were given experience with the delayed match-to-position task (DMP; Steele and Morris, 1999) before training on the watermaze. Separate groups of rats were given experience with the watermaze task before training on the DMP task. The DMP task is similar to the watermaze task but requires rats to learn a new platform location each day. Thus, the DMP task does not provide any specific spatial information that would be useful in the watermaze task (and vice versa). Training in each task does, however, provide substantial experience in a circular pool of water and experience with the features common to the two tasks (e.g., a platform is to be found, the platform is not located near the walls, and distal spatial cues are important).

We tested rats with conventional hippocampal lesions and control rats. Because beneficial effects of prior experience have not been found after conventional lesions, we also tested animals with a recently developed, novel lesion restricted to field CA1 that encompasses the entire dorsoventral extent of the hippocampus (Ocampo et al., 2017). Area CA1 serves as the primary output pathway from the hippocampus to neocortex (van Strien et al., 2009). Accordingly, a complete CA1 lesion should block hippocampal output to neocortex but leave the majority of the hippocampus intact. In this way we evaluated the effects of a more limited disruption of hippocampal function. The CA1

lesion might be advantageous because it spares efferent projections from CA3 and/or because it reduces remote effects to structures that project to the hippocampus. Hippocampal lesions have been reported to cause volume loss in the cortex (Anagnostaras et al., 2001; Jarrard and Meldrum, 1993).

METHODS

Subjects

Subjects were 63 experimentally naive, male Long-Evans rats that received either hippocampal lesions, CA1 lesions, or sham surgeries. After recovery, rats were trained in two spatial tasks: the watermaze task and the DMP task, a variation of the watermaze task that involves one-trial learning. In one condition, training occurred first in the DMP task, followed 2 d later by the watermaze task (DMP First; H n=8, CA1 n=8, sham n=7). In a second condition, training occurred first in the watermaze task, followed 2 d later by the DMP task (WM First; H n=8, CA1 n=8, sham n=8). Timelines are shown in Figure 7. Additionally, in a third condition, training in the watermaze task occurred at the same interval after surgery as the DMP First group (43 d), but without prior DMP training (WM After Delay; H n=8, CA1 n=8). The timeline for this condition is shown in Figure 11. All rats weighed between 320-350 g at the time of surgery. They were maintained on a 12:12 h light:dark cycle and were housed individually. Food and water were freely available. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego.

Surgery

Anesthesia was maintained throughout surgery with isoflurane gas (0.8-2.0% isoflurane delivered in O_2 at 1 L/min). The rat was placed in a Kopf stereotaxic instrument, and the incisor bar was adjusted until Bregma was level with Lambda. For hippocampal and CA1 lesions, ibotenic acid (IBO; Biosearch Technologies) dissolved in 0.1 M PBS (concentration: 10 mg/ml, pH 7.4) was injected into dorsal and ventral regions of the hippocampus using a 10 ml, 30-g Hamilton syringe. The syringe was held in a Kopf microinjector (model 5000) and mounted on a stereotaxic frame. The syringe was first lowered to the target coordinate and left in place for 1 min. After injection (at a rate of 0.1 ml/min), the syringe stayed at the target coordinate for 2 min to prevent IBO from spreading up the syringe tract upon its retraction. For CA1 lesions, IBO was injected into 14 sites per hemisphere (0.025 µl/site, unless otherwise noted). For certain injection sites in ventral CA1 (noted below), the syringe was left in place for 5 min to ensure that IBO would not spread up the syringe track (where it might cause unintended damage to CA3 or the dentate gyrus). All coordinates are in millimeters, anteroposterior (AP) relative to Bregma, mediolateral (ML) relative to Lambda, and dorsoventral (DV) relative to the brain surface at -4.8 mm from Bregma and ±4.2 mm from Lambda: AP -2, ML ±1, DV -2.9; AP -3.6, ML ±1, DV -2.7; AP -3.6, ML ±2, DV -1.9; AP -4.5, ML ±1.4, DV -3.3; AP -4.5, ML ±2.7, DV -1.8; AP -4.5, ML ±4.5, DV -7.9 (waited 5 min before retracting syringe); AP -5.3, ML ±3, DV -1.7; AP -5.3, ML ±4.8, DV -8 (waited 5 min before retracting syringe); AP -5.3, ML ±4.8, DV -2.4; AP -5.3, ML ±5.8, DV -7.5; AP -5.3, ML ±5.8, DV -5.7; AP -5.3, ML ±5.8, DV -3.9; AP -6.3, ML ±5.4, DV -3 (injected 0.05 µl IBO); AP -6.3, ML ±6.3, DV -5.7 (injected 0.05 µl IBO). For hippocampal

lesions, IBO was injected into 18 sites per hemisphere as in Clark et al. (2000). For sham surgeries, rats underwent the same surgical procedures up to the point of the craniotomy. Once awake and responsive, each rat was returned to its home cage for 13-21 d of recovery.

Apparatus

DMP and watermaze training were conducted in a pool of water (1.8-m diameter at the water level) that was rendered opaque by the addition of powdered milk. The testing room contained a number of constant, salient visual cues (posters, objects, equipment). A video camera mounted on the ceiling directly above the pool was used in conjunction with a video tracking system (San Diego Instruments) to record the swim path of each rat. An Atlantis platform (12.7 cm diameter) was used that could be raised or lowered remotely (Spooner et al., 1994). In the lowered position, the platform was undetectable and unavailable. In the raised position (1.5 cm below the surface of the water), the platform remained invisible, but provided a means to escape the water.

Behavioral Training

DMP. Rats learned a new platform location each day during three phases of training. Briefly, the platform was moved to a new location at the beginning of each training day, and in the first trial rats had to find the platform without any knowledge of its location. In subsequent trials on the same day, rats could find the platform by recalling where it was located in trial 1.

The first phase consisted of five training days. Each day, a trial began when a rat was placed in the water facing the pool wall at one of four start points (which were changed for each trial and counterbalanced across animals during training). The platform was kept in the raised position throughout the trial, allowing the rats to escape from the water. Rats were given up to 2 min to escape the water before being guided to the platform by the experimenter. After escaping the water, rats remained on the platform for 30 s before being returned to their home cage. Three more training trials were given on the same day using the same platform location (15-s delay between trials). Performance was measured as the distance traveled to reach the platform, averaged across trials 2-4 each day.

The second phase consisted of 12 training days. Each day, rats received two training trials with either a 1-min, a 20-min, or a 6-hr delay between the two trials. Rats were tested with each of the three delays on four separate days. The order in which the delays were given was mixed and counterbalanced across animals. Performance with each delay was measured as the distance traveled to reach the platform during trial 2, averaged across the four times the rats experienced that delay.

The third phase of the DMP task was conducted in one day. Rats received one training trial with a new platform location and one reinforced probe trial separated by a 1-min delay. The probe trials were similar to the training trials, except that the platform was lowered for the first 60 s. After the platform was raised, rats were given up to 1 min to escape the water before being guided to the platform by the experimenter. Rats remained on the platform for 30 s before being returned to their home cage. Performance was

measured as the percentage of time that each rat spent in the circular zone directly above the platform location during the first 60 s of the probe trial (chance = 4%).

Watermaze acquisition. Rats learned a single platform location across five days of training. There were five trials/day: a reinforced probe trial followed by four training trials (as described above for DMP training) with delays between the trials of about 10 min. As with the DMP task, the start point was changed for each trial and counterbalanced across animals during training. Performance was measured as the percentage of time that each rat spent in the circular zone directly above the platform location during the first 60 s of the probe trial at the beginning of each day (chance = 4%).

Histology

At completion of testing, the rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by 10% formaldehyde solution (in 0.1 M phosphate buffer). The brains were removed and cryoprotected in 20% glycerol/10% formaldehyde. Coronal sections (50 um) were cut with a freezing microtome beginning at the level of the anterior commissure and continuing caudally through the length of the hippocampus. Every fifth section was mounted and stained with thionin to assess the extent of the lesions.

Images of the thionin-stained tissue sections were acquired using a DM6000 microscope (Leica Microsystems, Inc.). The images from every other mounted section were then analyzed using Stereo Investigator software (mbf Bioscience;

MicroBrightField, CA, USA). The volumes of spared tissue in CA1, CA3, and DG were calculated using the Cavalieri method (as in Hales et al., 2014). This analysis was conducted for all lesion rats and 8 sham rats (4 from the WM First condition, 4 from the DMP First condition).

RESULTS

Neurohistological Findings

In the CA1 groups, all animals sustained significant damage to both the dorsal and ventral regions of area CA1. Figure 8A shows two sections from a sham animal, and Figure 8B shows the extent of a representative CA1 lesion. The mean percent damage to area CA1 was 76.1%. Sparing occurred most frequently in the posterior-most extent of CA1. There was also typically some extra-CA1 damage in area CA3 (24.3%) and the DG (12.6%). Additionally, there was some damage to structures immediately adjacent to area CA1, which included the normal and unavoidable cortical damage often observed above the dorsal hippocampus. In three rats, there was also mild damage in the posteromedial, amygdalohippocampal, and amygdalopiriform transition areas near ventral CA1. However, this damage did not seem to affect performance as these three rats performed similarly to the other rats in their respective groups.

Figure 8D shows the mean percent damage in each hippocampal subregion for the CA1 groups and for the H groups. In the H groups, all animals sustained significant damage to both the dorsal and ventral regions of the hippocampus. Figure 8C depicts the extent of a representative hippocampal lesion. The mean percent damage to the total

hippocampus was 75.2%. The damage was most complete in area CA1 (85.8%) and in area CA3 (86.9%). Sparing occurred most frequently in the dorsal-most and ventral-most extents of the DG, although DG damage was still substantial (57.6%). Sparing also often occurred in the posterior-most extent of the hippocampus. Additionally, there was some damage to structures immediately adjacent to the hippocampus, which included cortical damage above the dorsal hippocampus. In two rats, there was also mild damage to the primary and secondary auditory cortices near the intermediate hippocampus, and in one of these two rats, this damage extended to the temporal association, ectorhinal, and perirhinal cortices. Again, this extra damage did not seem to affect performance as these two rats performed similarly to the other rats in their respective groups.

Behavioral findings.

Watermaze Acquisition. In the WM First condition, where rats had no prior training, a repeated-measures ANOVA revealed differences in acquisition rate between the H, CA1, and control groups ($F[2,21]=7.1$, $p<0.005$; Figure 9A, left). Post hoc, pair-wise comparisons using the Tukey-Kramer test (alpha=0.05) showed that the two lesion groups learned the platform location at a similar rate, and both groups were impaired relative to controls. A repeated-measures ANOVA also demonstrated differences between the three groups when DMP training was given prior to the watermaze task (DMP First condition; $F[2,20]=6.5$, $p<0.01$; Figure 9A, right). However, in contrast to the findings for the WM First condition, in the DMP First condition the CA1 group acquired the watermaze at the same rate as controls. The H group was impaired relative to the other two groups (Tukey-Kramer test, alpha=0.05).

Together, these results indicate that prior DMP training substantially improved watermaze acquisition, but only in rats with CA1 lesions. A repeated-measures ANOVA showed that the CA1 group in the DMP First condition outperformed the CA1 group in the WM First condition ($F[1,14]=15.4$, $p<0.005$). A similar, but smaller benefit of prior DMP training appeared for the H and sham groups, but the effects were marginal ($F[1,14]=3.6$, $p=0.08$ for the H group; $F[1,13]=3.8$, $p=0.07$ for the sham group).

DMP Performance. In the DMP First condition, where rats had no prior training, a one-way ANOVA revealed differences in performance between the H, CA1, and control groups during phase one on the first day of DMP training ($F[2,20]=7.1$, $p<0.005$; Figure 10A, left). Post hoc, pairwise comparisons showed that the two lesion groups were impaired relative to controls (Tukey-Kramer test, alpha=0.05), and that the two lesion groups performed similarly to each other. A one-way ANOVA also demonstrated differences in performance on the first day of DMP training between the three groups when watermaze training was given prior to the DMP task ($F[2,21]=6.8$, $p<0.01$; Figure 10A, right). However, in contrast to the findings for the DMP First condition, in the WM First condition the CA1 group performed as well as controls. The H group was impaired relative to the other two groups (Tukey-Kramer test, alpha=0.05).

These results for rats with CA1 lesions indicate that prior watermaze training improved initial DMP performance. That is, on the first day of training on the DMP task, the CA1 group in the WM First condition outperformed the CA1 group in the DMP First condition ($t[14]=5.2$, $p<0.0005$). A similar benefit of prior watermaze training was also observed for the H and sham groups (all $ts>3.4$, all $ps<0.005$), although the H group

remained impaired. This benefit of watermaze training on DMP performance may have resulted from rats learning and remembering certain features of the task structure during their previous experience in the watermaze (i.e., searching for a platform to escape the water, swimming in the middle of the pool instead of along the edges, and using distal spatial cues as navigational guides).

During the remaining four days of phase one testing, the CA1 group was no longer impaired, regardless of prior experience (data not in Figure 10). In the DMP First condition, mean distance swum across days 2-5 was 3.3 ± 0.5, 7.1 ± 1.0, and 1.8 ± 0.3 m for the CA1, H, and sham groups, respectively. A repeated-measures ANOVA demonstrated differences in performance between the three groups ($F[2,20]=16.2$, $p<0.0001$). Post hoc, pairwise comparisons showed that the CA1 group performed similarly to controls, and the H group was impaired relative to the other two groups (Tukey-Kramer test, alpha=0.05). The results were the same in the WM First condition (1.7 ± 0.2, 6.0 ± 0.8, 1.4 ± 0.07 m for the CA1, H, and sham groups, respectively; $F[2,21]=25.9$, $p<0.0001$; Tukey-Kramer test, alpha=0.05).

Similar findings were obtained during phase two testing. In the DMP First condition (Figure 10B, left), a repeated-measures ANOVA revealed differences in performance between the three groups across the three delays ($F[2,20]=17.8$, $p<0.0001$). Post hoc, pairwise comparisons showed that the CA1 group performed as well as controls, and the H group was impaired relative to the other two groups (Tukey-Kramer test, alpha=0.05). The same findings were obtained when watermaze training was given prior to the DMP task ($F[2,21]=18.3$, $p<0.0001$; Tukey-Kramer test, alpha=0.05; Figure

10B, right). There were no main effects of delay (DMP First: $F[2,20]=1.4$, $p>0.1$; WM First: $F[2,21]=0.5$, $p>0.1$).

During phase three testing, CA1 lesions also did not impair performance. In the DMP First condition (Figure 10C, left), a one-way ANOVA demonstrated differences between the three groups in the percent time spent in the platform location during the probe trial ($F[2,20]=9.7$, $p<0.005$). Post hoc, pairwise comparisons showed that the CA1 group performed similarly to controls ($p=0.19$), and the H group was impaired relative to the other two groups (Tukey-Kramer test, alpha=0.05). The results were the same in the WM First condition ($F[2,21]=9.2$, $p<0.005$; Tukey-Kramer test, alpha=0.05; Figure 10C, right).

Watermaze 43d Post Surgery. In the watermaze task, rats with CA1 lesions performed much better when they were given prior DMP training than when they had no prior training (Figure 9A, right). In this group, the interval between surgery and watermaze training was 43 d. By comparison, the surgery-watermaze interval for the CA1 group not given prior DMP training was only 15 d (Figure 9A, left; timelines in Figure 7). To determine if improved performance in the CA1 group was related to the extended surgery-watermaze training interval (43 d vs. 15 d), we assessed the performance of rats with H lesions or CA1 lesions on the watermaze task 43 d after surgery and without prior DMP training (timeline in Figure 11).

Watermaze acquisition was impaired in both lesion groups 43 d after surgery. A repeated-measures ANOVA showed that the H group was slightly more impaired than the H group in the WM First condition, where the watermaze task was given only 15 d after

surgery ($F[1,14]=6.9$, $p<0.05$; Figure 11A, left). The CA1 group was impaired similarly to the CA1 group in the WM First condition ($F[1,14]=0.7$, $p>0.1$; Figure 11A, right). Together these findings indicate that the extended surgery-training interval was not the cause of the good watermaze performance of CA1 rats that received prior DMP training (Figure 9A, right).

DISCUSSION

We trained rats with either complete hippocampal lesions, CA1 lesions, or control surgeries in two different tasks in the watermaze (a conventional watermaze task and delayed match-to-position, DMP). In one condition, rats were trained in the DMP task first, followed by the watermaze task. In another condition, rats were trained in the watermaze task first, followed by the DMP task. Ordinarily, hippocampal lesions and CA1 lesions impair performance in both tasks. Yet, with CA1 lesions, rats were intact in the watermaze task when they had prior DMP training, and they were intact in the DMP task when they had prior watermaze training. In contrast, rats with hippocampal lesions were impaired in both tasks regardless of prior training.

It is perhaps not surprising that rats with large hippocampal lesions did not benefit from prior experience. In earlier work, rats with hippocampal lesions did not benefit from prior training, even when they had been trained on the same kind of task (Bannerman et al., 1995; Moser and Moser, 1998; Steele and Morris, 1999). In contrast, in our study, rats with CA1 lesions exhibited striking benefits from prior training, even when the prior training involved different tasks. Thus, rats with CA1 lesions acquired the watermaze

(after prior DMP training) as well as controls. And they acquired the DMP task (after prior watermaze training) as well as controls. To our knowledge, such a substantial benefit of prior training on the learning of a different task has not been demonstrated in rats with lesions in the hippocampus.

We considered, in the case of the watermaze, that performance might have benefited from the extended interval between surgery and watermaze training (43 days) that was needed in order to interpose DMP training. However, watermaze acquisition was not improved in rats with either CA1 or hippocampal lesions when rats were given the same extended surgery-watermaze interval but without prior DMP training (Figure 11).

We also noted that rats altered their swim pattern as they performed, which might have helped them learn a second task in the watermaze environment. For example, when DMP training was scheduled first, all three groups subsequently spent less time swimming along the edges of the pool during watermaze training than they did when watermaze training was scheduled first (see heat maps in Figure 9). This effect could have contributed to the finding that all three groups performed at least marginally better on the watermaze task if watermaze training was preceded by DMP training (compare Figure 9, left and right). However, after DMP training, rats with large hippocampal lesions were still severely impaired at the watermaze in comparison to the other two groups, even with the potential benefit of this swimming strategy. Accordingly, the development of a swim strategy does not readily account for the advantage of prior training found selectively in animals with CA1 lesions.

Why was there a difference between the effects of hippocampal and CA1 lesions when both lesions would be expected to disrupt hippocampal function? One possibility is

that large hippocampal lesions caused remote effects, such as volume loss in the cortex (Anagnostaras et al., 2001; Jarrard and Meldrum, 1993). Because CA1 lesions spared the majority of the projections to the hippocampus, such lesions could have reduced remote effects and preserved function in adjacent structures important for learning. Another possibility turns on the descending projection from area CA3 to the septum (Witter, 2007), which remains intact after CA1 lesions but not large hippocampal lesions. The septum projects to the thalamus (Swanson and Cowan, 1979) and might thereby provide an alternative pathway for hippocampal output to reach neocortex. A third possibility is that spared CA1 tissue was able to support hippocampal output to neocortex. CA1 lesions did leave nearly 24% of area CA1 intact, somewhat more than the 14% that was spared with large hippocampal lesions ($t[46]=3.1$, $p<0.005$).

Note that rats with CA1 lesions were impaired at watermaze acquisition and at the early stage of DMP training when they had no prior training but were intact when they had prior training. Accordingly, we suggest that prior experience is the critical factor in understanding the effect of CA1 lesions. As rats gained experience in one of our tasks, conventional watermaze or DMP, they formed memories of many features common to both tasks: a platform is to be found, the platform is not at the edges, distal cues are important. These memories may gradually organize into a coherent framework, a schema (Morris, 2006), which facilitated the learning of new, but related information (see Inglis et al., 2013). Thus, when rats were given DMP training (or watermaze training), they developed a schema from their experience that subsequently facilitated learning in the other task.

We suggest that experience with the training environment and with features common to the two tasks were sufficient to form a useful schema. If so, schemas can be useful even when a previously learned task and a new task are quite different. In earlier work with rats, impairment in a visual discrimination task following visual cortical lesions was reduced by prior experience with a conceptually similar task trained in a different modality (Clark and Delay, 1991). Our finding that prior experience benefited rats with CA1 lesions, but not large hippocampal lesions, suggests that hippocampal function (in areas upstream of CA1) may be important for schema formation.

In summary, rats with large hippocampal lesions or restricted CA1 lesions were impaired in the watermaze task and in the DMP task. However, when given prior training with one task, CA1 lesions had no effect on performance in the other task. By contrast, rats with hippocampal lesions did not benefit from prior training. The concept of schema may be useful for understanding the benefits of past experience. Because experience with one task can benefit subsequent learning in a different task, we suggest that features common to the two tasks are required to form a functional schema. This idea leads to the prediction that benefits of prior experience should not be expected across two tasks that are fundamentally different (see Wiltgen et al., 2011).

Acknowledgments

Chapter 3, in full, has been submitted for publication of the material as it may appear in Learning & Memory, 2018, Ocampo, Amber C.; Squire, Larry R.; Clark, Robert E. The dissertation author was the primary investigator and author of this paper.

CHAPTER 4:

CONCLUSIONS

Decades of research have shown that damage to area CA1 causes a broad array of memory impairments. However, it remains unclear if these impairments are caused by the loss of CA1-specific function or the disruption of hippocampal output to neocortex. The experiments in this dissertation have examined if memory requires area CA1 to send hippocampal output to neocortex using a novel lesion that encompasses the entire CA1 subfield. Specifically, we characterized the effects of this lesion on retrograde and anterograde memory with a variety of memory tasks and compared them against the effects of large hippocampal lesions.

Chapter 2 presented the first study, which examined the effects of the complete CA1 lesion on retrograde memory at recent and remote time points. We considered the possibility that because the CA1 lesion is more discrete than large hippocampal lesions, it might spare remote memory in tasks like the watermaze or context fear conditioning, where large hippocampal lesions have been known to cause ungraded retrograde impairments (Bolhuis et al. 1994; Clark et al. 2005a; Mumby et al. 1999; Sutherland et al. 2001; Broadbent and Clark 2013; Lehmann et al. 2007; Sparks et al. 2011; Sutherland et al. 2008). Such a finding would have supported the principles of systems consolidation. However, we found that CA1 lesions caused ungraded impairments in the watermaze, context fear conditioning, and trace fear conditioning tasks. The findings provide no support for systems consolidation, but they do show that the CA1 lesion impairs memory

similarly to large hippocampal lesions. Therefore, the findings support the idea that memory depends on CA1 output to neocortex.

In chapter 3, we compared the effects of complete CA1 lesions and large hippocampal lesions on anterograde memory using two watermaze-based tasks: the delayed match-to-position task (DMP) and the standard watermaze task. We found that the two lesions normally impair performance in both tasks. However, when rats had prior experience in one task, CA1 lesions had no effect on the other task. In contrast, rats with hippocampal lesions were impaired in both tasks regardless of prior experience. These experiments demonstrated an intriguing difference in the way prior experience affects learning in rats with CA1 and hippocampal lesions - namely, that rats with CA1 lesions benefit from prior experience, but rats with hippocampal lesions do not.

This difference between the two lesions suggests that hippocampal output through area CA1 is not always needed to support memory. As discussed in Chapter 1, it is possible that under certain conditions, memory-related information can leave the hippocampus through descending CA3 efferents. Area CA3 projects to the septum (Witter, 2007). The septum, in turn, projects to the thalamus (Swanson and Cowan, 1979), which can direct information to the neocortex. Thus, descending CA3 efferents may provide an alternative pathway for hippocampal output to reach neocortex. Alternatively, the septum may be able to process input from area CA3 and play a more direct role in spatial memory formation. Apart from its role in regulating hippocampal theta oscillation (Buzsaki, 2002), little is known about septal function in the memory system. There are a few studies, however, that report spatial memory impairments

following septal lesions (Decker et al., 1995; Noonan et al., 1996; Rashidy-Pour et al., 1996).

We also considered the possibility that the difference between the two lesions could be explained by a difference in remote lesion effects. Large hippocampal lesions might indirectly disrupt the function of neighboring regions that project to the hippocampus, similar to the disruption observed in area CA1 after excitotoxic lesions of the entorhinal cortex (Miettinen et al. 1998). Accordingly, cortical volume loss is sometimes reported after large hippocampal lesions (Anagnostaras et al. 2001; Jarrard and Meldrum 1993). Because CA1 lesions spare the majority of the projections to the hippocampus, such lesions might have reduced possible remote effects and preserved function in adjacent structures important for learning.

Additionally, we cannot rule out the possibility that our findings may have resulted from CA1 sparing. In the experiments in Chapter 3, CA1 lesions left nearly 24% of area CA1 intact (whereas hippocampal lesions only left 14% intact). It may be the case that with CA1 lesions, information processed in the DG and area CA3 could still reach neocortex through this small amount of spared CA1 tissue.

Still, the CA1 lesion impaired performance in the watermaze and DMP tasks when rats were experimentally naive. Therefore, the previous explanations cannot account for the entirety of our findings. In particular, they cannot explain why prior experience benefited learning in rats with CA1 lesions. Similar beneficial effects have been shown in both humans (Bransford & Johnson 1972; Maguire et al. 1999; van Kesteren et al. 2013; Race et al. 2015) and animals (Bannerman et al., 1995; Dragoi and Tonegawa 2013; Inglis et al., 2013; Otnaess et al., 1999; Tse et al., 2007; Wiltgen et al.

2011), and are thought to support the idea that preexisting knowledge structures, or schemas, can easily incorporate new, but related information (Bartlett, 1932; Morris, 2006). Thus, the formation of schemas might be important for understanding our findings with the CA1 lesion. Briefly, we suggest that rats gradually formed a schema as they gained experience in one of our two tasks (conventional watermaze or DMP), and this schema subsequently facilitated learning in the other task. By this account, our finding that prior experience benefited rats with CA1 lesions, but not large hippocampal lesions, suggests that hippocampal function (in areas upstream of CA1) may be important for schema formation. We considered that the hippocampus may also be important for incorporating new information into relevant schemas, but the current findings do not exclude the possibility that learning might occur without the hippocampus if a schema already exists.

Interestingly, we observed one other behavioral difference between CA1 and large hippocampal lesions, specifically in the DMP task when rats had no prior training. We found that CA1 lesions no longer impaired DMP performance once one day of training (4 trials) had been given, whereas hippocampal lesions impaired DMP performance throughout training. It is unclear why CA1 lesions impaired DMP performance only for one day, even when rats were experimentally naive. However, we suggest that the findings might reflect spared function in area CA3. Area CA3 has been proposed to be important for one-trial learning (Rolls and Kesner, 2006; Rolls, 2013; Nakazawa et al., 2003).

Considering our findings as a whole, one question that might arise is why we observed a benefit of prior experience in the watermaze task with anterograde memory

(Chapter 3), but not with retrograde memory (Chapter 2). In our retrograde experiments, rats were given 10 days of watermaze training before receiving CA1 lesions and a subsequent probe test to assess their memory for the platform location. Presumably, rats should have formed a schema for the watermaze during the 10-day training period that could later enhance performance in the probe test. Yet, probe test performance was severely impaired after CA1 lesions.

Other groups have considered similar discrepancies between anterograde and retrograde amnesia following hippocampal damage, as anterograde amnesia is often less severe than retrograde amnesia in rodents. To explain these findings, it has been proposed that normally, the hippocampus is used to support memory in a given task, but when the hippocampus is removed, the brain can adaptively make use of its remaining systems to support learning, albeit with alternative learning strategies (Anagnostaras et al., 2001; Frankland et al., 1998; Maren et al., 1997). Similarly, we suggest that in our anterograde experiments, rats with CA1 lesions were able to use the remaining hippocampal circuit to support hippocampal function (i.e., through descending CA3 efferents or through spared CA1 projections to neocortex) or were able to use other systems (which may be indirectly disrupted by large hippocampal lesions) to support alternative learning strategies. Although these conditions were suboptimal and caused impairments in watermaze acquisition, performance was improved with prior experience and with the development of a watermaze schema. In the case of our retrograde experiments (where rats were intact during watermaze training) we suggest that the memory for the platform location was supported normally in the hippocampus, through CA1 output to neocortex. However, once area CA1 was removed, the memory trace was lost, and there was no opportunity

before or during the probe test to relearn the platform location. Thus, prior experience and an existing watermaze schema could not benefit retrograde memory.

Together, the experiments in this dissertation have characterized the behavioral effects of a novel CA1 lesion in order to test the idea that memory requires area CA1 to send hippocampal output to neocortex. The findings show that retrograde and anterograde memory normally depend on CA1 output to neocortex. However, with prior experience, rats can form new anterograde memories without CA1 output. While it is possible that the small amount of spared CA1 tissue left by our CA1 lesions might still have been able to support hippocampal output, the fact remains that anterograde memory benefited from prior experience in rats with CA1 lesions. This finding adds to a growing body of work that supports the concept of the schema and highlights the importance of preexisting knowledge and experience in learning and memory.

APPENDIX: FIGURES

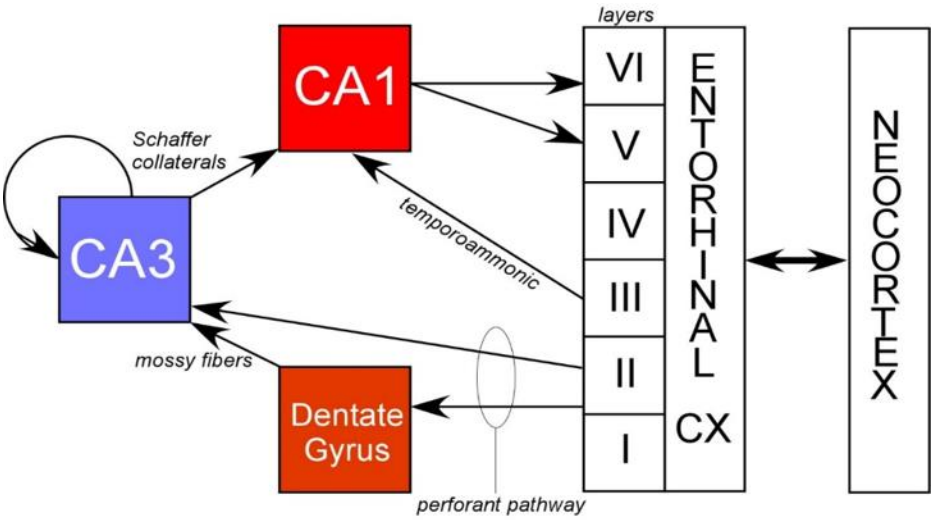


Figure 1. Hippocampal circuitry.

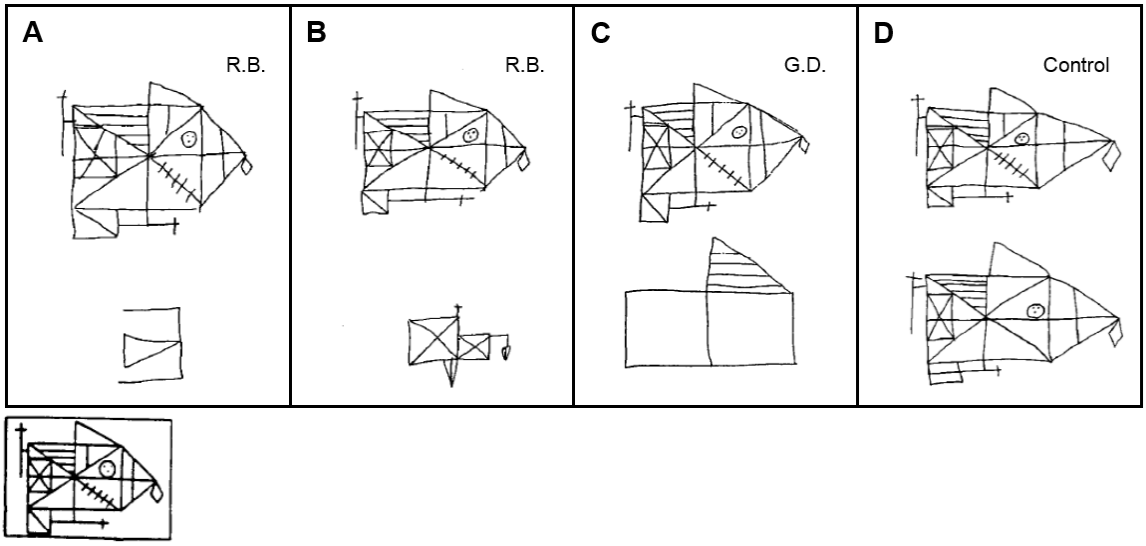


Figure 2. Performance of patients with CA1 lesions on the Rey-Osterreith complex figure test. Patients were asked to copy the image in the small box on the bottom left and then to reproduce the figure 10-15 mins later from memory. The copies are shown on the top and the reproductions are shown at the bottom of each box. Performance by Patient R.B. 6 months (A) and 23 months (B) after the onset of amnesia are shown in the left two boxes (Zola-Morgan et al., 1986), followed by Patient G.D. in the middle, right box (C; Rempel-Clower et al., 1996). Control performance is shown in the last box on the right (D; Zola-Morgan et al.

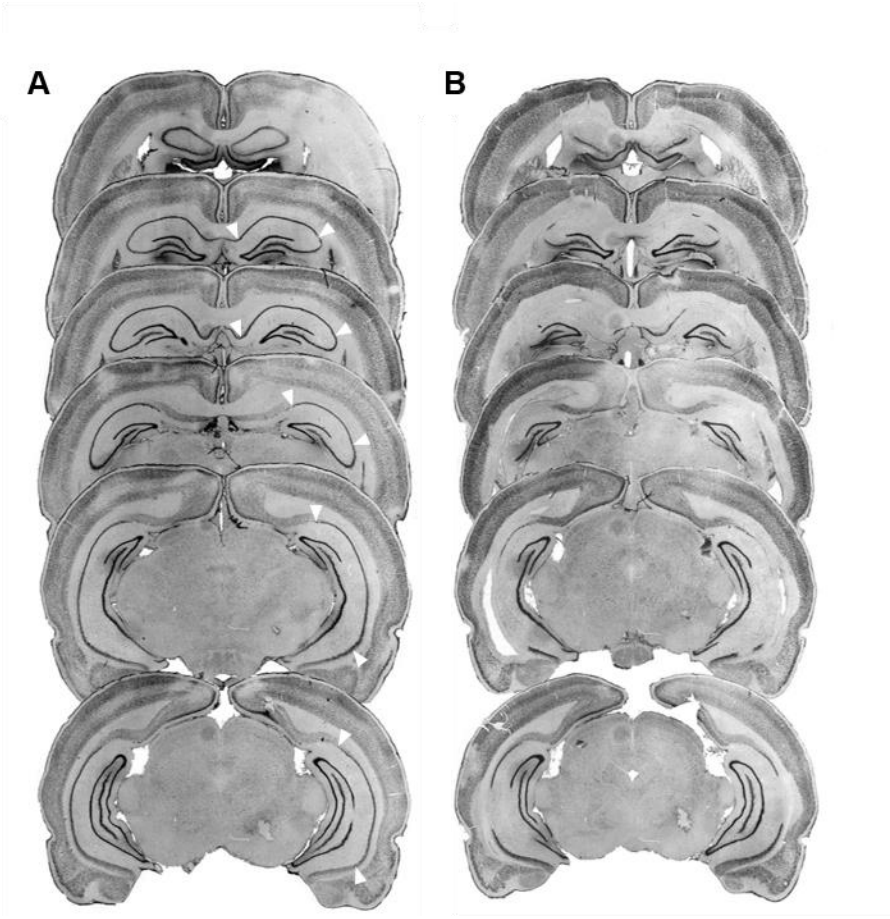


Figure 3. Lesions targeted both dorsal and ventral regions of CA1. Photomicrographs of six coronal histological sections through the hippocampus of a representative (A) sham brain and (B) a brain with a CA1 lesion. The sections are arranged from anterior (top) to posterior (bottom). Arrows indicate the CA1 borders in each section for the sham animal.

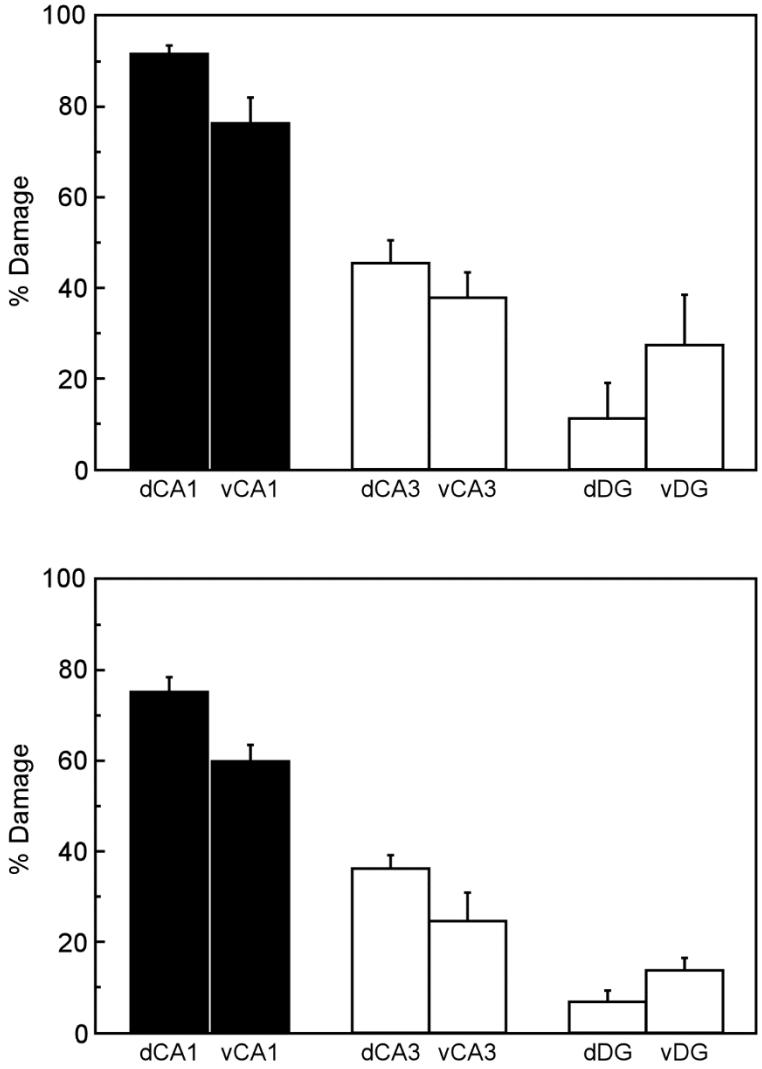


Figure 4. Lesions encompassed the majority of area CA1, while leaving the rest of the hippocampus largely intact. The black bars show the mean percent damage to the CA1 subregion of the hippocampus for animals in the recent condition (top, n=16) and remote condition (bottom, n=16). Extra-CA1 damage is shown in white.

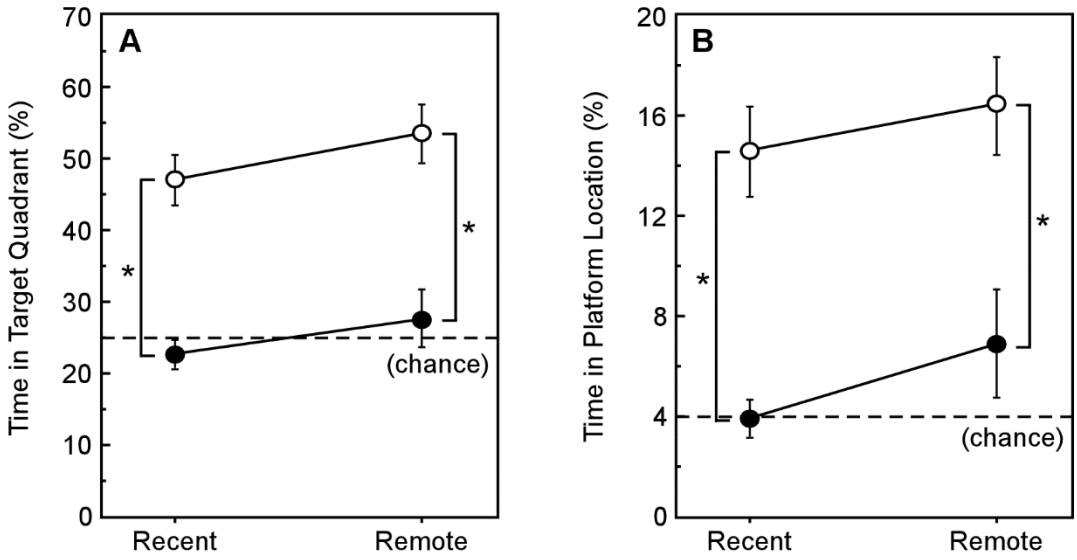


Figure 5. Spatial memory retention in the watermaze was impaired following CA1 lesions in both the recent and remote conditions. (A) Test probe performance measured as the mean percent time spent in the target quadrant. The dashed line indicates chance performance (25%). (B) Test probe performance measured as the mean percent time spent in the platform location. The dashed line indicates chance performance (4%). Performance after CA1 lesions is shown in black (recent n=16, remote n=16). Sham performance is shown in white (recent n=16, remote n=16). Error bars indicate SEM; sham scores are well above chance, $p < 0.0001$; * denotes $p < 0.005$.

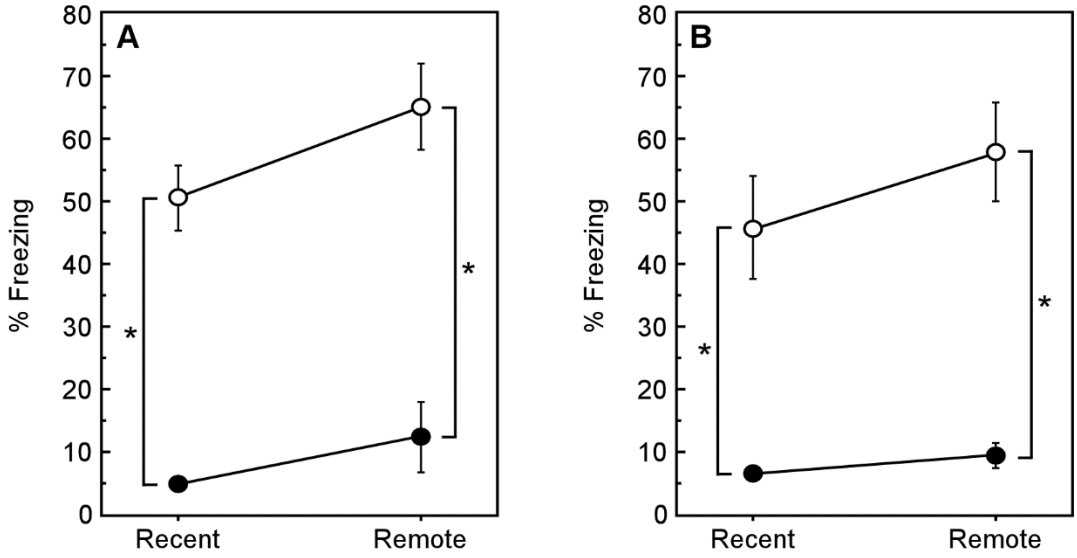


Figure 6. Context and trace fear memory were impaired following CA1 lesions in both the recent and remote conditions. (A) Context fear retention measured as mean percent freezing during the 8-min context test. (B) Trace fear retention during the tone test measured as mean percent freezing during the 240 s after the onset of the tone. Performance after CA1 lesions is shown in black (recent n=16, remote n=16). Sham performance is shown in white (recent n=16, remote n=16). Error bars indicate SEM; * denotes $p < 0.0001$.

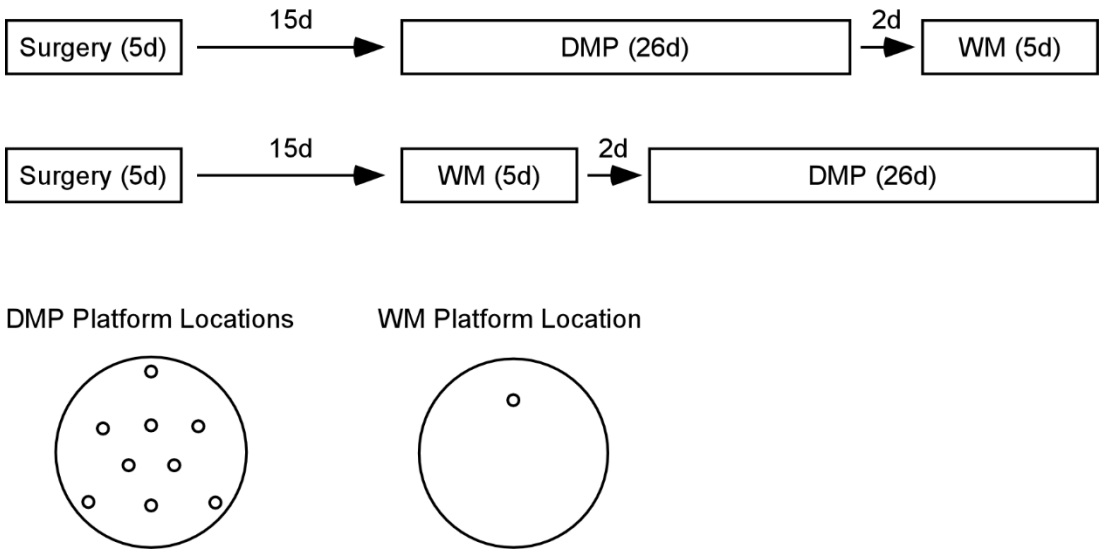
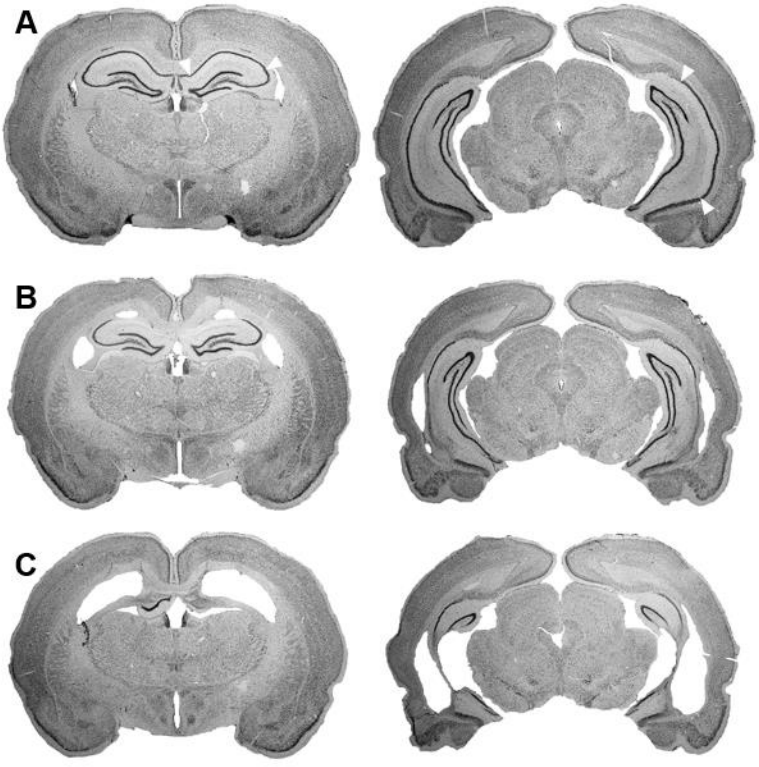
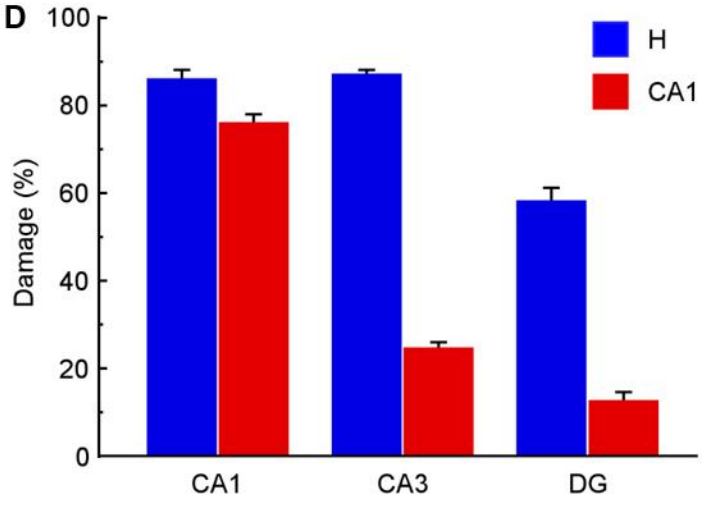


Figure 7. Experimental design. Approximately 15 d after receiving H lesions, CA1 lesions, or sham surgeries, rats were trained in the delayed match-to-position (DMP) task, followed 2 d later by the watermaze (WM) task (top timeline). Different animals with the same lesions were trained on the WM task first, followed 2 d later by the DMP task (bottom timeline). The diagram at the bottom left shows the different platform locations used for the DMP task (where a new platform location was used each day). The diagram at the bottom right shows the single platform location used for the WM task.

Figure 8. CA1 lesions were selective to area CA1, while hippocampal lesions encompassed the majority of the hippocampus. Both lesions included the entire dorsoventral extent of the hippocampus. Photomicrographs at two coronal levels of a representative (A) sham brain, (B) a brain with a CA1 lesion, and (C) a brain with a hippocampal lesion. From left to right, the sections are -2.80 and -5.40 mm posterior to bregma. White arrows indicate the CA1 borders in each section for the sham animal. (D) Mean percent damage to area CA1, area CA3, and the DG for animals with CA1 lesions (n=24) and hippocampal lesions (n=24). Error bars indicate SEM.


A
B
C


D
100
80
60
40
20
0
Damage (%)
H
CA1
CA1
CA3
DG

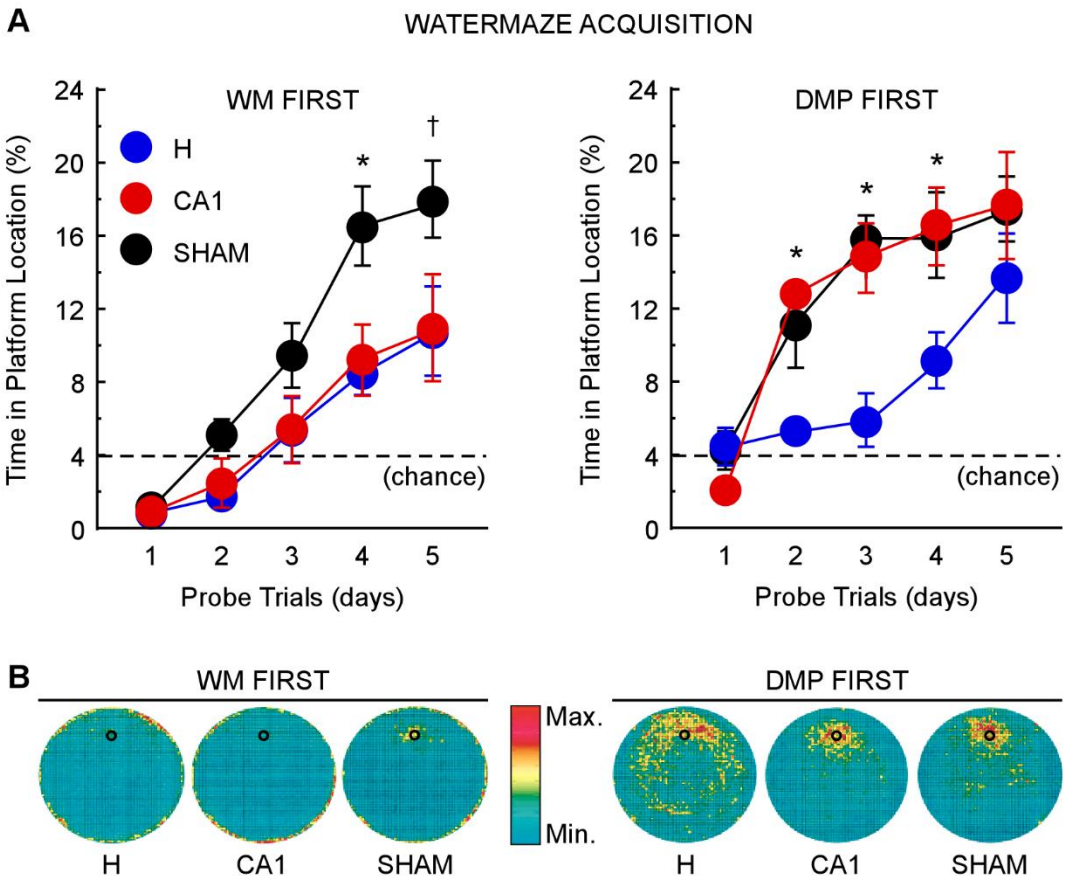
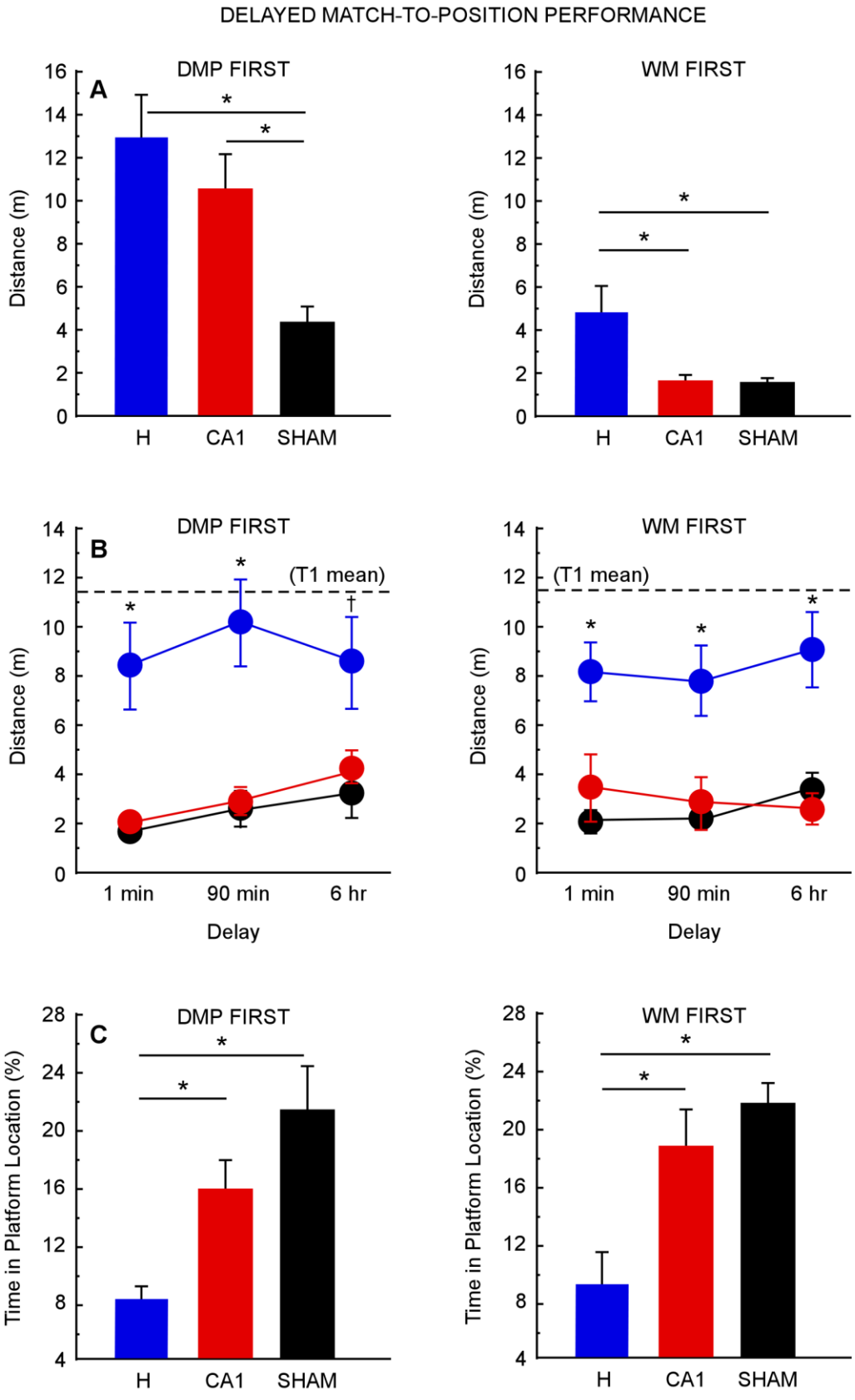


Figure 9. CA1 lesions impaired WM acquisition similarly to H lesions when rats had no prior experience (WM First, left). However, the lesions had no effect on WM acquisition when rats previously had DMP training (DMP First, right). H lesions impaired WM acquisition regardless of prior training. (A) Performance of H (WM First n=8; DMP First n=8), CA1 (WM First n=8; DMP First n=8), and sham (WM First n=8; DMP First n=7) groups across 5 days of WM training, measured as the mean percent time spent in the platform location during a probe trial at the beginning of each training day. The dashed line indicates chance performance (4%). (B) Heat maps represent the time spent in different parts of the watermaze on probe trials during acquisition by the two H groups, the two CA1 groups, and the two sham groups. For the color scale, red corresponds to the most frequently visited areas and turquoise to the least visited areas. Small black circles indicate platform location; Error bars indicate SEM; * denotes $p<0.05$ between one group and the other two groups; † denotes $p<0.05$ between sham and H and $p<0.07$ between sham and CA1.

Figure 10. DMP performance after H lesions (DMP First n=8; WM First n=8), after CA1 lesions (DMP First n=8; WM First n=8), and for sham animals (DMP First n=7; WM First n=8). CA1 lesions impaired early DMP performance when rats had no prior experience (DMP First, left), but the lesions had no effect when rats had prior WM training (WM First, right). H lesions impaired DMP performance regardless of prior training. (A) Performance on the first day of DMP testing during phase one (four trials separated by 15-s delays), measured as the mean distance traveled to reach the platform on each trial. * denotes $p<0.05$. (B) Performance during the second phase of DMP testing (two trials separated by 1-min, 90-min, or 6-hr delays) across the three delays, measured as the mean distance traveled to reach the platform during trial 2. Dashed lines indicate the distance traveled to reach the platform in trial 1 (T1) averaged across all three groups at all three delays (DMP First = 11.4 m; WM First = 11.5 m; the three groups in each condition performed similarly in T1); * denotes $p<0.05$ between the H group and the other two groups; † denotes $p<0.05$ between H and sham and $p<0.06$ between H and CA1. (C) Performance during the last phase of DMP testing (one trial and one test probe separated by a 1-min delay), measured as the mean percent time spent in the platform location during the test probe. Chance performance was 4%. * denotes $p<0.05$. Error bars indicate SEM.


DELAYED MATCH-TO-POSITION PERFORMANCE
A
DMP FIRST
WM FIRST
Distance (m)
H
CA1
SHAM
B
(T1 mean)
1 min
90 min
6 hr
Delay
C
Time in Platform Location (%)

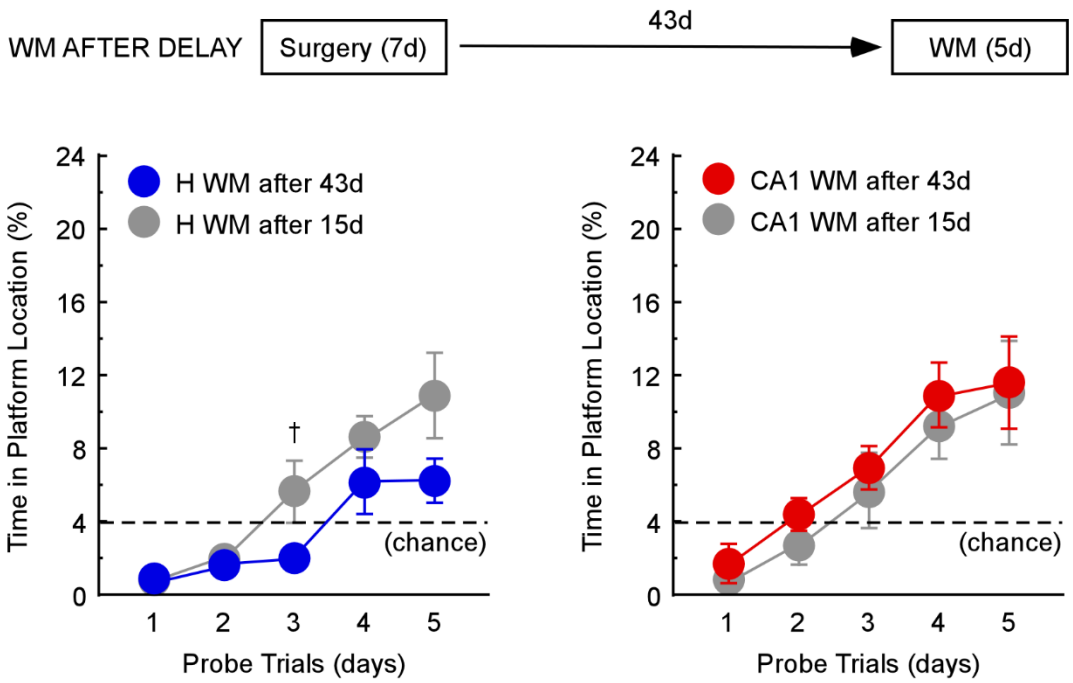


Figure 11. Rats with H lesions or CA1 lesions were trained on the WM task 43 d after surgery (and without prior DMP training). WM acquisition was impaired after H lesions (blue, left; n=8) as well as after CA1 lesions (red, right; n=8). For comparison, WM data are included in each panel from Figure 9 (left) for the WM First condition (gray; H n=8; CA1 n=8), where animals were given the WM task 15 d after surgery (also without prior DMP training). Note that controls given the watermaze task without prior DMP training attained scores above 16% by day 4 (Figure 9, left). The dashed line indicates chance performance (4%); Error bars indicate SEM; † denotes $p<0.07$.

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