UNIVERSITY OF CALIFORNIA,
IRVINE

Investigation of the Neural Basis of Order and Item Memory: Roles of Hippocampus, Prelimbic Cortex, and Perirhinal Cortex

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

Leila Mangan Feinberg

Dissertation Committee:
Associate Professor Norbert J. Fortin, Chair
Professor Craig E. L. Stark
Associate Professor John F. Guzowski

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DEDICATION

To

my wonderful husband, loving parents, caring family, and dear friends

in recognition of their lasting support of my endeavors

"Looking back now on the long path my life has followed, on the lives of my peers and colleagues, and on the briefer ones of the young recruits who have worked with us, I have become persuaded that, in scientific research, neither the degree of one's intelligence nor the ability to carry out one's tasks with thoroughness and precision are factors essential to personal success and fulfillment. More important for the attaining of both ends are total dedication and a tendency to underestimate difficulties, which cause one to tackle problems that other, more critical and acute persons instead opt to avoid."

- Rita Levi-Montalcini

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CURRICULUM VITAE

LEILA M. FEINBERG

Address: 202 Bonney Research Laboratories
University of California, Irvine
Center for Neurobiology of Learning and Memory
Irvine, CA 92697-3800
Tel: (714) 625-6935
E-mail: leilamf@uci.edu

EDUCATION

Doctorate, Neurobiology & Behavior
June 2015
University of California, Irvine

Master of Science, Neurobiology & Behavior
December 2011
University of California, Irvine

Bachelor of Arts with Honors, Psychology
Minor in Biology
August 2005
University of Texas at Austin

WORK AND RESEARCH EXPERIENCE

January 2014 – Present: Graduate research, UC Irvine. Advisor: Dr. Norbert Fortin. Thesis: "The role of the hippocampus, perirhinal cortex, and prelimbic cortex in recognition memory and memory for sequences of events."

August 2012 – December 2013: Lecturer, California State University Long Beach.

April 2012 – December 2012: Junior Specialist, UC Irvine. Primary Investigator: Dr. Norbert Fortin. Perform surgical procedures related to projects within the lab; fabrication of head stages; behavioral assessment of memory in rats.

March 2009 – June 2011: Graduate research, UC Irvine. Advisor: Dr. Norbert Fortin. Thesis: "Recognition memory for odors: Differential roles of the hippocampus and perirhinal cortex." Assessing the roles of structures within the Medial Temporal

Lobe (MTL) using a novel olfactory-based behavioral paradigm to elucidate neurobiological mechanisms contributing to episodic memory in rodent models.

January 2010 – January 2011: Graduate research, UC Irvine. Collaborating Advisor: Dr. John Marshall. Neuropharmacological effects of methamphetamine on long-term recognition memory in rats.

April 2007 – March 2009: Graduate research, UC Irvine. Advisor: Dr. Carl Cotman. *in vitro* cell culture and *in vivo* behavioral analyses of transgenic mouse models of Alzheimer's Disease; effects of neurotrophins on neuropathology and cognition.

August 2006 – January 2007: Graduate rotation, UC Irvine. Advisor: Dr. John Marshall. Long-term memory effects of variable doses of cocaine on conditioned place preference and
object recognition memory in rats.

December 2005 – August 2006: Laboratory Manager, University of Texas at Austin. Primary Investigator: Dr. Timothy Schallert. Managed grant funding and renewals, edited grant applications and articles for submission, trained new staff, and maintained laboratory supply inventories.

January 2004 – August 2006: Undergraduate research, University of Texas at Austin. Advisor: Dr. Timothy Schallert. Developed a novel behavioral paradigm to assess olfactory recognition memory in rodents following administration of pharmacologic agents including pentylenetetrazol, caffeine, and ethanol.

TEACHING EXPERIENCE, MENTORSHIP, AND VOLUNTEER WORK

Mind, Memory, and Brain (BioSci 38), UC Irvine
Administrative TA: Spring 2014
Guest Lecturer: Fall 2014

Introductory Statistics (HDEV 250, SOC 250), California State University Long Beach
Lecturer: Spring 2013, Fall 2013

Data Analysis (SOC 260), California State University Long Beach
Lecturer: Fall 2013

Psychobiology (PSY 241), California State University Long Beach
Lecturer: Fall 2012, Spring 2013
Guest Lecturer: Fall 2014

Everyone's A Reader Volunteer Program, Magnolia Elementary School, Carlsbad, CA
Tutor: November 2011 – March 2012

Brain Dysfunction (BioSci 37), UC Irvine
Lecture TA: Spring 2011

NeuroBlitz Weekly Seminar Series, Neurobiology & Behavior Department, UC Irvine
Coordinator: 2009-2010

Howard Hughes Medical Institute (HHMI) Teacher Training Program, UC Irvine
Fall 2009

DNA to Organisms (BioSci 93), UC Irvine
Administrative TA/Discussion Leader: Fall 2009

Brain Awareness Week, Carson High School and Mesuda Middle School; Sowers Middle School
Volunteer Lecturer: Spring 2008-2010; Spring 2015

Neurobiology Lab (BioSci N113L), UC Irvine
Instructing TA: Spring 2007 and Winter 2008

Undergraduate students mentored:
Rachel Lesyshyn (5/13-present), UC Irvine, CSULB research assistant.
Collin Fuhrman (8/14-3/15), UC Irvine, Bio 199 student.
Clare Quirk (3/12-8/14), UC Irvine, Bio 199 student, now a UCSD graduate student.
Denise Ly (8/09-9/11), UC Irvine, Bio 199 student.
Ani Hakobyan (8/09-3/10), UC Irvine, Bio 199 student.
John Stewart (6/09-8/09), UC Irvine Summer Research Program student.
Akhila Kothapalli (01/08-08/08), UC Irvine, Bio 199 student.
Preetham Kumar (01/08-08/08), UC Irvine, Bio 199 student.

GRANTS AND AWARDS

Ralph Waldo Gerard Award for Excellence in the History of Neuroscience, UC Irvine
January 2010

Howard Hughes Medical Institute (HHMI) Teaching Award, UC Irvine
December 2009

Institute for Brain Aging and Dementia Training Grant Fellowship, UC Irvine
2008-2009

Graduate Student Representative, Department of Neurobiology & Behavior, UC Irvine
2007-2009

College Scholar, University of Texas at Austin
May 2005

Collegiate Honors Program, University of Washington, Seattle
Academic year 2002

PEER-REVIEWED PUBLICATIONS

Feinberg LM, Allen TA, Ly D, Fortin NJ (2012) Recognition memory for social and non-social odors: Differential effects of neurotoxic lesions to the hippocampus and perirhinal cortex. *Neurobiology of Learning and Memory*, Jan; 97(1): 7-16.

Poon, WW, Blurton-Jones M, Tu CH, **Feinberg LM**, Chabrier MA, Harris JW, Jeon NL, Cotman CW (2011) Beta-Amyloid impairs axonal BDNF retrograde trafficking. *Neurobiology of Aging*, May; 32(5): 821-33.

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POSTERS, ABSTRACTS, AND PRESENTATIONS

Allen TA, Jacobs N, **Feinberg LM,** Bharadwaj KR, Wang MX, Fortin NJ. Prefrontal cortex neurons code for sequences of events. Society for Neuroscience Conference, 2011, Washington D.C.

Poon WW, Aguilar B, **Feinberg LM,** Seow J, Tu T, Cedeno A, Carlos A, Cotman CW (2011) Neural stem cells rescue deficits in Alzheimer's transgenic mice. *Alzheimer's and Dementia,* 7(4): 7-8.

Feinberg LM, Allen TA, Black, YD, Ly D, Fortin NJ. Differential effects of hippocampal and perirhinal cortex excitotoxic lesions on recognition memory for social and non-social odors. Society for Neuroscience Conference, 2010, San Diego, CA.

O'Dell SJ, **Feinberg LM,** Marshall JF. A neurotoxic regimen of methamphetamine impairs novelty recognition measured by a social odor-based task. Society for Neuroscience Conference, 2010, San Diego, CA.

"The life of Rita Levi-Montalcini & the discovery of Nerve Growth Factor." **Leila M. Feinberg.** Ralph Waldo Gerard Award Ceremony for Excellence in the History of Neuroscience, 2010, UC Irvine.

Poon WW, Blurton-Jones M, Tu CH, **Feinberg LM**, Jeon N, Harris JW, Cotman CW. The use of microfluidics chambers to study BDNF-TrkB retrograde signaling in CNS neurons. Society for Neuroscience Conference, 2007, San Diego, CA.

Spinetta MJ, Woodlee MT, **Feinberg LM,** Heymann J, O'Connell W, Parent J, Lichtenwalner R, Schallert T. Alcohol and retrograde amnesia: can rats have blackouts and does caffeine help? Society for Neuroscience Conference, 2006, Atlanta, GA.

Spinetta MJ, Woodlee MT, Hester NW, Heymann JC, **Feinberg LM,** Rajagopalan KN, Lichtenwalner R, Parent J, Schallert T. A simple and sensitive odor recognition task for rats and mice detects retrograde amnesia caused by ethanol or other drugs that interfere with memory consolidation. Society for Neuroscience Conference, 2005, Washington D.C.

MEMBERSHIPS

Center for the Neurobiology of Learning and Memory (CNLM) at UC Irvine
Society for Neuroscience
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ABSTRACT OF THE DISSERTATION

Investigation of the Neural Basis of Order and Item Memory: Roles of Hippocampus, Prelimbic Cortex, and Perirhinal Cortex

By

Leila Mangan Feinberg

Doctor of Philosophy in Biological Sciences

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Assistant Professor Norbert J. Fortin, Chair

The field of research devoted to studying the neural basis of episodic memory is relatively new, and only very recently has been operationalized for study in non-human species. In this dissertation, episodic memory is operationally defined as memory for sequences of events in the spatial and/or temporal context in which they occurred. In a series of experiments, I examined memory for items and the order in which odor events occur over varying temporal domains. I used these approaches to help elucidate the roles of HC, PL, and PER in episodic memory. First, I used a novel odor recognition paradigm to probe item memory for social and non-social odors using varying retention intervals from 5 min to 48 hr. In this study, I found that HC lesions do not affect odor recognition memory for both odor types at all retention intervals. However, I found that PER lesions induced long-term memory deficits for social odors. These data implicate a role for PER in mnemonic processes for highly overlapping, but not distinctive, stimuli. Second, I designed a new behavioral paradigm to assess memory for sequences of odors. This ethologically-relevant task is a non-rewarded, incidentally encoded, trial-unique paradigm that can

assess both order and item memory following the presentation of a sequence of odors. Using this method, I found that lesions to HC, PL, and PER, but not V2, induced specific deficits for order, but not item memory. Altogether, these data contribute to a larger understanding of the roles these structures play in temporal context, suggesting a critical role for HC, PL, and PER in sequence memory and ultimately episodic memory.

INTRODUCTION

Episodic memory is defined as the memory for personal events or experiences. In recent years, operational definitions of episodic memory have been developed, such as memory for "events in context" (e.g., memory for what, where, and/or when), so that it may be more rigorously studied in a laboratory setting. Although the neural basis of episodic memory remains poorly understood, a conceptual framework is emerging. Current theoretical models propose that item (e.g., 'what') and contextual (e.g., 'where') information is processed in distinct cortical streams through perirhinal cortex (PER) and postrhinal cortex, respectively. These streams converge on the hippocampus (HC), where they are integrated to form a unique item-in-context representation that characterizes an episode (e.g., 'what-where').

Although this model has been useful in organizing a large body of data, recent evidence indicates that it is overly simplistic. First, the contribution of PER appears to extend beyond simply representing specific items. Notably, PER has been implicated in the perceptual/mnemonic ability to remember certain types of visual objects, indicating that the nature of the item in question is consequential. There is a need to understand the role of PER in item memory using a different modality, such as olfactory memory, to better delineate how this structure contributes to recognition memory. Furthermore, the role of PER in order memory remains unknown. Second, the HC has a well-characterized role in spatial contextual memory, but under which circumstances it is necessary for non-spatial tasks is a subject of debate. Finally, although the prefrontal cortex (PFC) is known to be important for episodic memory, it is unclear what specific function it performs within this framework.

Recently, a number of labs have used memory for the sequence in which events occurred (memory for 'what-when') as a model of episodic memory. In these paradigms, rats are trained to learn sequences of events, in which an event corresponds to the presentation of an individual item (e.g., odor, object). The overall objective of this dissertation is to further examine the distinct contributions of HC, PER, and PFC (specifically, the prelimbic area; PL) to episodic memory. Here, I used a novel odor recognition paradigm with social and non-social odors to assess the roles of HC and PER in item memory over time. Additionally, I have designed a non-rewarded paradigm to probe order memory for a unique sequence of odors by taking advantage of rats' natural tendency to preferentially investigate novel or older stimuli. This task models the incidental nature of episodic memory encoding, and is therefore a more ethologically relevant method in assessing memory for sequences of events. Using this approach, I will directly compare the performance of animals with excitotoxic *N*-methyl-D-aspartate (NMDA) or sham lesions to PER, HC, or PL on memory for individual items as well as memory for sequences of items.

Aim 1: Elucidate the time-dependent involvement of HC and PER in memory for distinct versus overlapping items.

In Chapter 3, I address Aim 1. On each trial, rats were presented a wooden bead scented with a unique odor in the study phase. Following a retention interval (5 min, 20 min, 1 h, 24 h, or 48 h), rats were administered a test phase in which they were presented with two odors: the previously studied odor and a novel odor. Intact memory was demonstrated by preferential exploration of the novel odor. These experiments extend traditional recognition memory paradigms by directly comparing odors with little overlap

(nonsocial household odors, e.g., basil) and odors with a high degree of overlapping features (social odors from conspecifics). Because PER is implicated in memory for conjunctive, highly overlapping stimuli, I predicted that rats with PER lesions would have severe deficits in recognition for social odors, but little to no impairments on nonsocial odors. In light of evidence that HC is important for social odor recognition, but not for object recognition, I hypothesized that HC lesions will have no effect on memory for nonsocial odors, but would result in a modest impairment in social odor recognition memory at longer retention intervals.

Aim 2: Elucidate the roles of HC, PER, and PL in memory for sequences of items.

Aim 2 is addressed in Chapter 4. On each trial, rats were presented with a unique sequence of five wooden beads, each scented with an individual household odor. The items were presented at varying inter-exposure intervals (20 min for the extended version, 45 s for the rapid version). Following a 60 min retention interval, rats were presented with two odors from the sequence (order probe). Based on previous work, we expected that memory for the order in which events occurred would be demonstrated by significantly greater investigation toward the item that appeared earlier in the sequence. Following the order probe, rats were also given an item probe to test memory for the odors that appeared in the sequence. Since HC is thought to be important for associating events with the temporal context in which they occurred (Fortin et al., 2002; Manns et al., 2007; Jacobs et al., 2013), I predicted that rats with HC lesions would have deficits at both rapid and extended timescales. PL has also been implicated in temporal order memory, and models of episodic

memory suggest PL is critical for supporting retrieval and generating episodic memory-based actions. Therefore, I predicted that PL lesioned rats will exhibit moderate deficits on the extended version of the sequence task, since retrieval of temporally disparate items may be more reliant on an intact PL. Given that PER circuitry can associate events that are separated by short time durations (<1 min), PER lesioned animals may also show impairments in the order task. However, I predicted that lesions to all three regions would spare item memory, demonstrating a selective effect of lesions on memory for the order of events.

CHAPTER 1. BACKGROUND AND SIGNIFICANCE

Though philosophers and physicians studying patients with various brain insults have long surmised where in the brain higher order cognitive mechanisms are generated, the scientific study of the neural basis of memory in the laboratory is a relatively new field. Henry Molaison was possibly the most influential patient to modern memory research. In 1957, Henry (famously referred to by his initials, H.M.) received a bilateral ablation of large areas of his medial temporal lobe in order to treat his severe epilepsy. After his surgery, physicians noticed that Henry suffered from a curious memory deficit. Brenda Milner, a doctoral student with Dr. Wilder Penfield, was brought in to characterize his impairment. She had Henry perform several types of memory paradigms and discovered that he was able to learn procedural motor skills (e.g., mirror drawing task), but he had severe deficits in declarative memory, or the conscious recollection of previous experiences and information. This finding brought forth the idea that there are multiple memory systems within the brain, and because Henry's deficit was specific to declarative, but not procedural, memory, this evidence implicated the medial temporal lobe in declarative memory.

Declarative memory can be divided into two categories: episodic memory, which is memory for personal experiences, and semantic memory, which is memory for facts and concepts (Tulving, 1972). Long thought to be a faculty unique to humans, episodic memory has now been operationalized for study in animals. Here, I will present a background on both human and animal models of episodic memory, as well as shed light on current understanding of the neurobiology of episodic memory. In my proposed experiments, I will

be examining the roles of specific medial temporal lobe structures, as well as prefrontal cortex, in a rat model of episodic memory.

1.1. EPISODIC MEMORY IN HUMANS

Endel Tulving, a prominent psychologist, coined the term "episodic memory" in 1972. He describes episodic memory as "mental time travel" through which one can re-experience a personal event with a richness of detail approximating that of the original experience (Tulving, 1972; 2002). This definition involves subjective time and autonoetic consciousness (having a sense of self). This is an impossible requisite to achieve in non-human species. This view restricted early studies of episodic memory to human subjects.

More specific distinctions between episodic and semantic memory offered the ability to test these faculties in the laboratory. Toward this end, Tulving distinguished between the capacities of 'knowing' and 'remembering' (Tulving, 1985). *Knowing* is a feature of semantic memory, referring to the ability to recall general facts and concepts, irrespective of the personal link from which that information was obtained. *Remembering* is a feature of episodic memory, characterized as the conscious recall of memory for personal episodes from the past. In "remember/know" paradigms, subjects are shown images of items in the study phase and are later given a recognition memory test, where they indicate if the image is old (was given during the study phase) or new. During recall, a subject's response is qualified as a 'remember' judgment when they can identify having previously seen an old object and give additional information about the experience (e.g., "I am certain I saw that lion in the study phase because I remember thinking about my cat when I first saw the picture"). A 'know' response is obtained when the subject identifies the

object as being old, but cannot identify any details associated with its encoding. Different interpretations about memory function arose from results of remember/know paradigms. A major issue, however, was regarding the introspective nature of the task, as subjective measures of episodic recall rely on subjects' verbal reports.

To address these issues, mathematical models became tools to aid interpretation of remember/know tasks. Single-process models view the remember/know responses as varying degrees of confidence on a linear scale of recognition (Donaldson, 1996). Alternatively, dual-process models argue that recognition memory is comprised of two distinct processes of memory retrieval: recollection, which corresponds with episodic 'remembering,' and familiarity, which corresponds with semantic 'knowing' (Knowlton & Squire, 1995; Yonelinas, 2002). Both single- and dual-process accounts pose that recognition judgments can be resolved using different mathematical signal detection analyses to characterize the nature of the recognition memory. Dual process models have successfully used receiver operating characteristic (ROC) analyses to quantify remember/know responses. Used in conjunction with imaging, ROC analyses have supplied dual-process theorists with an arsenal of data implicating distinct neural systems in recollection and familiarity. Though ROC analyses were developed for use in human subjects, they have been adapted for animal studies. Using this technique, Fortin, Wright, and Eichenbaum (2004) demonstrated a striking similarity in recognition and familiarity judgments between humans and rats, and corroborated human research showing that hippocampal lesioned rats had a specific deficit for recollection-based judgments.

Though mathematical models using signal detection theory and ROC analyses have provided insight into what brain systems are important for episodic memory, they are very

difficult to implement and interpret. Thus, scientists acknowledged the need for more rigorous operational definitions of episodic memory in order to facilitate the transition into animal models, which offer more invasive testing methods.

1.2. ANIMAL MODELS OF EPISODIC MEMORY

The operationalization of episodic memory was driven largely by animal work. Although several psychologists, including Tulving, agree that episodic memory is a faculty unique to humans, considerable evidence suggests that this is not the case. Not only is there a large body of behavioral evidence for episodic memory in numerous species, but structures implicated in episodic memory are well conserved across mammalian species and birds (Allen & Fortin, 2013).

Episodic memory involves remembering an episode that was incidentally encoded, so tasks in which no explicit training occurs are valuable tools (DeVito and Eichenbaum, 2011). One-trial learning tasks are useful to satisfy incidental learning criteria, but do not alone satisfy the criteria for episodic memory (Morris, 2001). For example, conditioned taste aversion is acquired in a single-trial, but does not require episodic retrieval of the food or illness for associative conditioning to occur (Clayton, Bussey, and Dickinson, 2003). Recognition memory paradigms are also commonly used one-trial behavioral measures, however they are not sufficient to demonstrate episodic memory because recognition alone does not necessarily distinguish semantic from episodic memory.

In 1998, Clayton and Dickinson coined the term 'episodic-like' memory to refer to the animal equivalent of episodic memory, defined by behavioral exhibition of 'what-where-when' information. They were the first to demonstrate what-where-when memory

in scrub jays, a species of food caching birds. In this study, birds exhibited episodic-like memory by identifying what had been cached (earthworms or peanuts,), where (the location inside the cage), and when (4 or 124 hours ago). Clayton, Bussey, and Dickinson (2003) outlined three critical features of episodic-like memory to model in animals: content, structure, and flexibility. First, the *content* recalled from a specific episodic memory should contain 'what-where-when' information of a specific episode. Second, the *structure* of the 'what-where-when' components making up the representation should be inextricably linked, such that bringing up one aspect of the episode unintentionally leads to retrieval of the others. Third, episodic memories should be able to be used *flexibly* in novel circumstances (Clayton, Bussey, & Dickinson, 2003).

Episodic-like memory has been demonstrated in several other species using what-where-when paradigms, including several mammalian species (rats, mice, voles, pigs, and nonhuman primates; Martin-Ordas et al., 2010; Allen & Fortin, 2013), as well as several species of birds, and even honeybees (Henderson, et al., 2006; Pahl et al., 2007). Collectively, these experiments have provided strong evidence that episodic memory capacity is conserved across species. Also, they have shown that several species have the capacity to demonstrate what-where-when memory in a single paradigm. However, it is unknown whether all episodic memories are always composed of what-where-when information. Also, because the criteria are so stringent, results from these studies are difficult to interpret. With so many criteria that need to be satisfied, it is difficult to attribute specific functions to structures. Thus, it is imperative to develop tasks that closely model episodic memory encoding and retrieval, and are amenable to widespread study in many different species, so that the roles of specific brain structures can be isolated.

Recently, approaches to test memory for "events in context" have emerged. In these paradigms, an event ('what') is an item or stimulus presentation, and the context can be either spatial ('where') or temporal ('when'), or a combination of both (e.g., 'what-where', 'what-when', 'what-where-when'). Manipulating fewer variables offers a more powerful means of studying the underlying neurobiological mechanisms for a given task.

1.3. THE ANATOMY OF THE MEDIAL TEMPORAL LOBE SYSTEM

The medial temporal lobe (MTL) system includes the hippocampus (HC) as well as the adjacent entorhinal cortex, perirhinal cortex (PER) and postrhinal cortex (parahippocampal cortex in primates; Burwell et al., 2004). Overall, sensory and perceptual information enters the MTL system through PER and postrhinal cortex (POR). Information is transmitted from PER and POR through the lateral and medial entorhinal cortex (Kerr et al., 2007), respectively, which send efferents directly to the hippocampus (Allen & Fortin, 2013; Knierim et al., 2014). The hippocampus is thus the first major site of anatomic convergence for the PER and POR pathways.

Based on anatomical features, each structure is thought to uniquely contribute to components of memory. The PER and LEC cortex receive information from all sensory regions, with only slight species-specific profiles in unimodal sensory weights, and is thus thought to contribute representations of multimodal items and objects (Furtak et al., 2007; Kerr et al., 2007; Allen & Fortin, 2013). The POR and MEC receives visual-spatial inputs similarly across species, and is thus thought to contribute to the representations of landmarks and spatial contexts (Furtak et al., 2007; Kerr et al., 2007; Furtak et al., 2012; Allen & Fortin, 2013). Lastly, the anatomy of the hippocampus is highly conserved across

mammalian species (Manns & Eichenbaum, 2006), wherein intrinsic connectivity of the subregions of the hippocampal formation and the classic trisynaptic circuit (entorhinal cortex-dentate gyrus-CA1-CA3), such as massive CA3 recurrents, are thought to contribute to the integration of information from the two PER-LEC and POR-MEC pathways.

Neurophysiological studies have provided strong evidence for this division of labor in the MTL. For example in rats, single-unit recordings from PER reflects object information (Allen et al., 2007; Furtak et al., 2007), whereas in POR spatial information is represented (e.g., Furtak et al., 2009). By contrast, single-unit and ensemble activity in the hippocampus encodes many features of an experience including items, places, time and rewards (Wood et al., 199; McKenzie et al., 2014). Thus, the increasingly rich neural representations across the MTL structures, reaching its highest level of conjunctive representations in the hippocampus, are consistent with hierarchical anatomical account of the intrinsic organization.

1.4. NEUROBIOLOGY OF EPISODIC MEMORY

It is well established that the medial temporal lobe (MTL) is critical to declarative memory (Eichenbaum, 2001; Suzuki & Eichenbaum, 2000; Teyler & Rudy, 2007; Squire, 2009). Damage encompassing these brain areas leads to deficits in episodic and semantic memory (O'Keefe & Nadel, 1978; Fortin et al., 2002; Kesner et al., 2002; Squire et al. 2004; Eichenbaum et al., 2007; DeVito & Eichenbaum, 2011). However, the specific function of individual MTL structures, including the nature of their contribution to episodic memory, remains unclear.

Episodic memory encoding is thought to occur when item-in-context representations are formed in the HC. One prominent theory proposes that item ('what') and context ('where') information are processed in segregated parallel streams through PER and postrhinal cortex, respectively, and converge onto the HC to contribute to the formation of episodic memories (Brown & Aggleton, 2001; Diana, Yonelinas, & Ranganath, 2007; Eichenbaum, Yonelinas, & Ranganath, 2007; Teyler & Rudy, 2007; **Figure 1.1**). Episodic recall is thought to occur upon reactivation of the item-in-context representation. This process is presumed to be initiated in the HC upon cue-elicited reactivation, which subsequently reactivates the representations in the parahippocampal region and other associated areas.

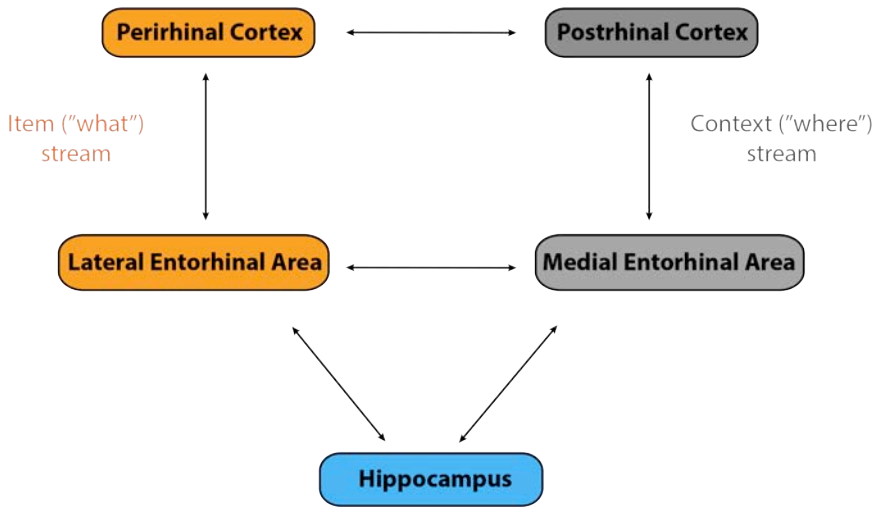

Figure 1.1. Proposed circuitry underlying the division of labor for episodic memory in the hippocampal system. Adapted from Eichenbaum, Yonelinas, &, Ranganath 2007.

This theory suggests that the HC is critical for episodic memory, but not for item recognition memory, a notion that remains a subject of debate (Brown & Aggleton, 2001; Fortin et al., 2002; 2004; Winters et al., 2008; Albasser et al., 2009; Broadbent et al., 2010).

The same model proposes that PER is crucial for item memory, in part based on observations that PER plays an important role in object recognition memory (Brown & Aggleton, 2001; Eichenbaum et al., 2007; Winters et al., 2008; Albasser et al., 2010; Aggleton et al., 2010). However, recent evidence suggests that the role of PER in item memory is more complex than originally thought. For instance, several studies have shown that PER is necessary when objects contain a high degree of overlapping features (Wan et al., 1999; Buckley et al., 2001; Eacott et al., 2001; Bussey et al., 2002; Norman & Eacott, 2005; Albasser et al., 2009; for a review see Winters et al., 2008). PER-lesioned animals demonstrate greater levels of impairment as the number of overlapping features increases, resulting in more feature ambiguity (Bussey et al., 2002; Buffalo et al., 2006; Bartko et al, 2007). PER lesions also cause impairments in distinguishing simultaneously presented stimuli, suggesting that PER might mediate the perceptual disambiguation of overlapping stimulus representations, in addition to serving aspects of recognition memory (for a review see Bussey et al, 2006; Baxter, 2009; but see Suzuki 2009, 2010).

An important facet of episodic memory that is not well understood is temporal context ('when') memory. HC has recently been implicated in sequence order memory (Chiba, et al., 1994; Michell & Laicona, 1998; Fortin, et al., 2002; Kesner, et al., 2002; Jacobs, et al., 2013; Allen, et al., 2014; Allen, et al., 2015). In addition, several studies suggest that prefrontal cortex (PFC) may contribute to the memory for sequences of events (Barker, et al. 2007; DeVito & Eichenbaum, 2011). PFC is thought to be involved with retrieval and guiding the appropriate behavioral response (Allen & Fortin, 2013). Prior lesion studies in rodents have not been able to isolate damage to discrete areas of PFC, so our understanding of the roles of subregions within PFC is limited. For example, prelimbic

cortex (PL), a region within PFC, does not appear to be necessary for item recognition (Barker, et al., 2011), yet may be important for temporal context memory given its role in trace fear conditioning and delayed variable-response tasks (Gilmartin & McEchron, 2005; Delatour & Gisquet-Verrier, 2005; Brito, et al., 1982).

Below is a simple schematic of the proposed roles of HC, PL, and PER in order and item memory, based on what is currently known about these structures (**Figure 1.2**). There are direct reciprocal connections from HC to PER, and PL to PER, and unilateral connections from HC to PL demonstrating clear anatomical evidence of their interactions and potential complementary roles in episodic memory (DeVito & Eichenbaum, 2011; Jenkins & Ranganath, 2010). However, there is a need to develop a better understanding of the roles of HC, PL, and PER, and build on the current working model about how these structures contribute to item and order memory, as no one study has looked specifically at the interactions of all three regions on the same task.

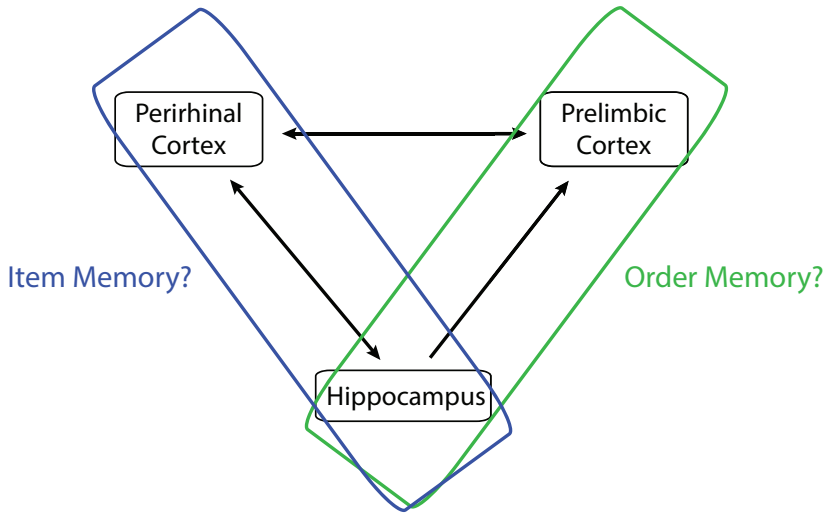

Figure 1.2. Current working model of the roles of HC, PL, and PER in item and order memory. PER and HC are predicted to be important for item memory, while PL and HC are predicted to be important for order memory.

1.5. Memory for Sequences of Events as a Model of Episodic Memory

There are different types of temporal integration in the brain including elapsed time, response timing, and the order of events (Mauk & Buonomano, 2005; Buhusi & Meck, 2005; Terrace 2005; Jacobs et al., 2013; Merchant et al., 2013; MacDonald et al., 2014). Sequence memory is a type of temporal information specifically related to the order of events. Generally, sequence memory is a broad term that can be used to describe explicit or implicit recollection of a sequence on a variety of timescales and functions. For example, sequences can be encoded and retrieved over a variety of timescales from milliseconds to hours (Buhusi & Meck, 2005). Sequence memory can also refer to perception, motor coordination, the setting of an internal clock, and/or episodic memory recall (in addition to other tasks; for review see Terrace, 2005; Eichenbaum, 2012). It is important to establish both the timescale and cognitive function when studying sequence memory in the laboratory.

Here, we examine memory for sequences of events (herein referred to as "sequence memory" for simplicity—not to be confused with motor or perceptual sequence memory). Memory for the sequence of events as they occurred during an experience is a critical requisite for episodic memory (e.g., Tulving, 1972; Eichenbaum & Fortin, 2005). Specifically, the episodes considered here consist of a single series of items that occurs in minutes-to-hours timescales. Episodic memory capacities retain memory for the items and also for the order of items as they occurred during an experience. Herein, we use the term order memory to describe the memory for the order in which items occurred on a timescale of minutes-to-hours. Our operational definition of memory for sequences of

events requires both memory for the items that are present within a given sequence, and the memory for the order in which the items occurred.

Our lab uses the memory for sequences of events as a model of episodic memory ('what-when'). The ability to organize memories by the order of occurrence is a fundamental aspect of episodic memory (Tulving & Markowitsch, 1998). This approach allows us to investigate the neurobiology underlying the memory for events that occur in a particular temporal context, controlling for any spatial component. In these tasks, events correspond to presentations of odors. Rats are trained to identify the order in which events occurred, or indicate whether the items are in- or out-of-sequence. We are able to probe the neurobiological mechanisms important for these tasks by disrupting various regions and assessing subsequent effects on performance. These paradigms translate well to human tasks of memory for sequences of events, in which the spatial context is minimal.

Incorporating spontaneous preference into episodic memory tasks is extremely useful toward modeling the incidental nature of episodic encoding. These tasks take advantage of rats' natural tendency to preferentially explore novel or older stimuli. The basic approach has been used to determine the relative recency of two items, but very few studies have implemented a spontaneous paradigm to test memory for longer sequences of events. We have developed a spontaneous sequence task using five odor items to probe memory for sequences of events. After sampling a sequence of five odors, rats are given an order probe, in which two odors from the sequence are presented simultaneously. Intact memory for sequences of events is demonstrated by preferential exploration toward the odor that came earlier in the sequence (e.g., B>D). We can use this paradigm in conjunction with region-specific lesions to elucidate the mechanisms underlying sequence memory.

Because this task is not rewarded, it may best recruit the normative neural mechanisms activated in episodic memory encoding without the confound of goal-directed or motivation-based decision making.

1.6. SUMMARY

The neural basis of episodic memory is not well understood. In particular, the HC, PER, and PL are known to be important for episodic memory, but their respective roles in memory for individual items and memory for the order in which those items occur in a sequence remain unclear. Here, I propose a more refined understanding of the roles of HC and PER in item memory by examining their roles in memory for distinct versus overlapping odors over time. In addition, I directly compare the involvement of HC, PL, and PER using a single paradigm to test both item and memory for sequences of items to help answer long standing questions regarding their contributions to episodic memory. This innovative approach captures the incidental nature of episodic encoding and requires no training, making it a valuable assay for episodic memory. The experiments in my proposal will contribute to a better understanding of the involvement of HC, PL, and PER in item and order memory, and ultimately the neural basis of episodic memory.

CHAPTER 2: GENERAL RESEARCH DESIGN AND METHODS

2.1. ANIMALS

Subjects were male Long Evans rats (weighing 250-300 g on arrival). Rats were individually housed in clear rectangular polycarbonate cages and maintained on a 12hr light-dark cycle (lights off at 8:00 am). Naïve rats were briefly handled for 3 - 5 days after initial arrival. Access to food and water was unrestricted before surgery. Following surgery, rats were mildly food restricted to maintain 85% of their free-feeding body weight with free access to water throughout testing (for Chapter 3). All behavioral test sessions took place during the dark phase (active period) of the light cycle under ambient red lighting conditions. One hour prior to behavioral testing, food hoppers and water bottles were removed to acclimate rats to testing conditions. All surgical and behavioral methods were in compliance with the University of California Irvine Institutional Animal Care and Use Committee guidelines.

2.2. SURGERIES

Lesions were induced by infusions of of *N*-methyl-D-aspartate (NMDA; Sigma, St. Louis, MO), in order to produce excitotoxic neural damage. General anesthesia was induced (5%) and maintained by isoflurane (1 – 2.5%) mixed with oxygen (800 ml/min). Rats were then placed into the stereotaxic apparatus (Stoelting Instruments, Wood Dale, IL) and the scalp was anesthetized with Marcaine® (7.5 mg/ml, 0.5 ml, s.c.). The skull was exposed following a midline incision and adjustments were made to ensure bregma, lambda, and sites ± 0.2 mm lateral to the midline were level. During surgery, all rats were administered

glycopyrrulate (0.2 mg/ml, 0.5 mg/kg, s.c.) to help prevent respiratory difficulties and 5 ml Ringer's solution with 5% dextrose (s.c.) for hydration. Sham-operated controls undergo the same surgical procedures as the lesion group, except no infusion was made.

Following lesion procedures for the HC, PER, PL, or V2 (details below), incision sites were sutured and dressed with Neosporin®. Rats were returned to their home cages and monitored until they awoke from anesthesia. White paper AlphaDri bedding was used for post-surgical rats to aid in identifying bleeding or discharge from the incision site. One day following surgery, rats were given an analgesic (Flunixin, 50 mg/ml, 2.5 mg/kg, s.c.) and Neosporin® was applied to the incision site. Rats were allowed to recover from surgery for approximately two weeks before behavioral testing.

2.2.1. Hippocampal Lesions

The bone overlaying the HC infusion sites was resected bilaterally and remained hydrated in sterile saline during infusions. Infusions were performed using a 33-gauge 10 µl syringe (Hamilton Company, Reno, NV) driven by a motorized infusion pump (World Precision Instruments, Sarasota, FL) that was mounted onto a manipulator arm of the stereotax. The needle remained at the injection site for at 5 mins after drug infusion to allow for diffusion. HC sites were infused with 200-225 nl NMDA 85 mM solution at 200-250 nl/min. See **Table 2.1** for HC lesion coordinates.

Table 2.1. Surgical coordinates for dorsal and ventral hippocampal NMDA infusions (mm relative to bregma). Dorsoventral coordinates are measured from the *dura mater*.

	Anteroposterior (A/P)	**Mediolateral (M/L)**	**Dorsoventral (D/V)**
Dorsal HC	-2.2; -3.0; -4.0	±1.0, ±1.8; ±2.8	-3.0; -2.8; -2.6
Ventral HC	-4.8; -4.8; -5.7; -5.7	±4.8; ±4.5; ±4.9; ±5.1	-6.5; -3.3; -2.8; -5.8

2.2.2. Perirhinal Cortex Lesions

Two holes were drilled on each hemisphere of the dorsal skull (~-4 and -7 mm A/P relative to bregma, ~1 mm medial to the temporal ridge) for anchor screws to hold a tissue spreader (Allen et al., 2007; Kholodar-Smith et al., 2008). Temporal muscles were then pulled away to expose the temporal and parietal plates of the skull until the zygomatic arch was visible. The tissue spreader was then secured between the anchor screws and the inner surface of the temporal muscles.

The bone overlaying the temporal cortex (~2 mm x 5 mm) was resected and the fragment remained hydrated in sterile saline during infusions. Infusions were performed using a 33-gauge 10 µl syringe with a non-coring needle (Hamilton Company, Reno, NV) driven by a motorized infusion pump (World Precision Instruments, Sarasota, FL) that was mounted onto the micromanipulator. The syringe was positioned at a 45˚ angle from the vertical surface of the temporal cortex, with the needle eye facing ventral and posterior to direct flow of NMDA toward PER. PER-lesioned rats received NMDA infusions (85 mM; 50 mg/mL) at approximately 7-8 sites (80 nL per infusion; 70 nL/min; equally spaced at ~0.5

mm) spanning the rostrocaudal extent of PER from -2.8 to -7.6 A/P relative to bregma (Burwell, 2001). Occasionally, only seven injections are made when a large blood vessel was present at an intended infusion site (Kholodar-Smith et al., 2008). The needle tip was inserted ~1.5 mm into the cortex, measured from the *dura mater*.

2.2.3. Prelimbic Cortex Lesions

Small holes overlying the PL infusion sites were drilled bilaterally. Infusions were performed using a 33-gauge 10 µl syringe (Hamilton Company, Reno, NV) driven by a motorized infusion pump (World Precision Instruments, Sarasota, FL) that was mounted onto a manipulator arm of the stereotax. The needle remained at the injection site for at 5 mins after drug infusion to allow for diffusion. PL sites were infused with 250 nL NMDA (85 mM) at 200 nL/min (coordinates relative to bregma: anteroposterior (A/P) 3.2 mm, mediolateral (M/L) ±0.75 mm, dorsoventral (D/V) -3.0 mm; Sharpe and Killcross, 2012). Dorsoventral coordinates were measured from the *dura mater*.

2.2.4. Secondary Visual Cortex Lesions

Small holes overlying the secondary visual cortex (V2) infusion sites were drilled bilaterally. Infusions were performed using a 33-gauge 10 µl syringe (Hamilton Company, Reno, NV) driven by a motorized infusion pump (World Precision Instruments, Sarasota, FL) that was mounted onto a manipulator arm of the stereotax. The needle remained at the injection site for at 5 mins after drug infusion to allow for diffusion. V2 sites were infused with 250 nL NMDA (85 mM) at 200 nL/min (coordinates relative to bregma:

anteroposterior (A/P) -4.5 mm, mediolateral (M/L) ±2.5 mm, dorsoventral (D/V) -0.8 mm). Dorsoventral coordinates were measured from the *dura mater*.

2.3. Olfactory Stimuli

All odor stimuli were presented on 1" round wooden beads (Woodworks Ltd., Haltom City, TX). Experimental rats were familiarized with wooden beads prior to testing by placing a number of unscented beads in their cages (beads were removed before testing begins; Spinetta et al., 2008; O'Dell et al., 2010; Feinberg et al., 2012). This general familiarity with wooden beads ensures that, during testing, animals focus their investigation on the odor added to the experimental beads (see below). Importantly, the natural odor of the wooden beads serves as a familiar background odor, which is consistent across all odor stimuli.

2.3.1. Non-social Odors

Non-social odors were presented to the rats on wooden beads, each scented with an individual household spice. Beads were scented by being placed in a container of a mixture of playground sand and a single household spice (e.g., rosemary) for 48 hours. Sand was included to dilute odorants and serve as a consistent background odor for all non-social odor beads.

2.3.2. Social Odors

Social odors were presented to the rats on wooden beads, each scented with the odor of a single conspecific animal. Beads were scented by being placed in the cage of an

individually-housed odor donor rat for one week (Spinetta et al., 2008; O'Dell et al., 2010; Feinberg et al., 2012). The conspecific odor donor rats were free to interact with the wooden beads during this period.

Odor donor conspecifics were healthy adult male Long-Evans rats completely segregated from experimental rats, which were housed in a separate vivarium space. Considerable effort was made to ensure that the experimental rats had no prior experience with the odor donor rats. Thus, the social odor beads contained a mixture of both familiar odors present in all rats' cages (e.g., bedding, food) and the unique combination of odors of an unfamiliar conspecific rat (e.g., saliva, urine, feces). Beads from conspecifics were "preference tested" using an independent cohort of naïve rats to help ensure equal levels of innate preference/aversion to individual odor donors. Additionally, upon arrival, experimental rats were also individually-housed in cages with specialized filter tops to help isolate the experimental rats from the odors of neighboring experimental rats.

2.4. Odor Recognition Paradigm

Details of the experimental approach for this task are outlined under Approach in Chapter 3 (Section 3.2.).

2.5. Data Analysis of Recognition Memory Paradigm

Two measures of discrimination were calculated from the exploration data (Ennaceur & Delacour, 1988; Aggleton et al., 2010). The difference in seconds of exploration toward the novel odor (N2) minus seconds of exploration toward the familiar odor (N1) in the test phase is the unadjusted discrimination score (*DI*).

$$DI = sec_{N2} - sec_{N1} \quad (1)$$

The second discrimination measure (*DI'*) was calculated by dividing *DI* by the total exploration time and multiplying that number by 100, providing a percent difference score between exploration toward the novel odor (N2) and the familiar odor (N2). The *DI'* values range from +100 to -100%. Positive values correspond to a preference toward the novel odor. Negative scores correspond to a preference toward the previously encountered odor. A score of zero indicates no preference for either odor. *DI'* scores significantly different from zero are interpreted as recognition memory for the previously encountered odor.

$$DI' = (DI/sec_{N2} + sec_{N1}) \times 100 \quad (2)$$

Each animal was tested twice on every retention interval for both social and non-social odor stimuli. Discrimination scores for the same retention interval and stimulus type were averaged for each rat.

Statistics were performed using SPSS 17 and custom-written MATLAB (R2009a) scripts. Group data were analyzed using analysis of variance (ANOVAs) and t-tests. Group data is expressed as the mean ± standard error. The family-wise α-error rate was maintained at 0.05. Significant trends are noted when $p \leq 0.10$, but > 0.05. Lesion effect sizes (*d)* on discrimination was computed as (Cohen, 1988):

$$d = (mean_{Controls} - mean_{Lesion})/(\sqrt{(SD^2_{Control} + SD^2_{Lesion})/2}) \quad (3)$$

The numerator is the difference between the mean discrimination index of control and lesioned animals, and the denominator is the standard deviation of pooled estimates from control and lesioned animals.

2.6. SPONTANEOUS SEQUENCE TASK

Details of the experimental approach for this task are outlined under Approach in Chapter 4 (Section 4.2.).

2.7. DATA ANALYSIS OF SPONTANEOUS SEQUENCE MEMORY PARADIGM

Two measures of discrimination were calculated from the exploration data (Ennaceur & Delacour, 1988; Aggleton et al., 2010). For the order probe, the difference in seconds of exploration toward the earlier odor (e.g., B) minus seconds of exploration toward the later odor (e.g., D) is the unadjusted discrimination score (DI_{Order}). For the item probe, the difference in seconds of exploration toward the novel odor (e.g., X) minus seconds of exploration toward the familiar odor (e.g., C) in is the unadjusted discrimination score (DI_{Item}).

$$DI_{Order} = (\text{sec}_{\text{Earlier}} - \text{sec}_{\text{Later}})$$

$$DI_{Item} = (\text{sec}_{\text{Novel}} - \text{sec}_{\text{Familiar}}) \quad (4)$$

The second discrimination measure (DI') is calculated by dividing DI by the total exploration time and multiplying that number by 100, providing a percent difference score between exploration toward the earlier odor and the later odor (DI'_{Order}), as well as the novel odor and the familiar odor (DI'_{Item}). The DI' values range from +100 to -100%. Positive values correspond to a preference toward the earlier odor in the order probe, and the novel odor in the item probe. Negative scores correspond to a preference toward the later odor in the order probe, or the previously encountered odor in the item probe. A score of zero indicates no preference for either odor. DI'_{Order} scores significantly different from zero are interpreted as memory for order of sequences of events. DI'_{Item} scores

significantly different from zero are interpreted as recognition memory for the previously encountered odor.

$$DI'_{Order} = (DI_{Order} / \sec_{\text{Earlier}} + \sec_{\text{Later}}) \times 100$$

$$DI'_{Item} = (DI_{Item} / \sec_{\text{Novel}} + \sec_{\text{Familiar}}) \times 100 \quad (5)$$

Each animal was tested three times on every retention interval for both rapid and extended versions of the sequence. Discrimination scores for the same retention interval and stimulus type will be averaged for each rat.

Statistics were performed using SPSS 21 and custom-written MATLAB (R2009a) scripts. Group data are analyzed using analysis of variance (ANOVAs) and t-tests. Group data is expressed as the mean ± standard error. The family-wise α-error rate will be maintained at 0.05. Significant trends are noted when $p \leq 0.10$, but > 0.05. Lesion effect sizes (*d)* on discrimination were also computed as (Cohen, 1988):

$$d = (\text{mean}_{\text{Controls}} - \text{mean}_{\text{Lesion}})/(\sqrt{(\text{SD}^2_{\text{Control}} + \text{SD}^2_{\text{Lesion}})/2}) \quad (6)$$

The numerator is the difference between the mean discrimination index of control and lesioned animals, and the denominator is the standard deviation of pooled estimates from control and lesioned animals.

We flagged significance at $p<0.05$, and significant trends at $p<0.10$.

2.8. HISTOLOGY

Rats were administered an overdose of sodium pentobarbital (Euthasol, 390 mg/ml, 150 mg/kg, i.p.) and were transcardially perfused with 100 ml PBS followed by 200 ml of 4% paraformaldehyde (pH 7.4; Sigma-Aldrich, St. Louis, MO). Brains were post-fixed overnight in 4% paraforlmadehyde and afterwards placed in a 30% sucrose solution for

cryoprotection. For Specific Aim 1 (Role of HC and PER in Recognition Memory), frozen brains were sectioned on a sliding microtome (60 μm; coronal orientation) into four sets of immediately-adjacent sections for a cell body-specific Cresyl Violet stain, a neuron-specific NeuN stain, a myelin-specific gold chloride stain and a spare set. Exact methods for each stain are described in detail elsewhere (see Supplementary Materials from Kholodar-Smith et al., 2008). For Specific Aims 2 & 3 (Spontaneous Sequence Memory), frozen brains were sectioned on a sliding microtome (50 μm; coronal orientation) into four sets of immediately-adjacent sections.

2.9. Lesion Reconstructions

Using Image J software and Photoshop v.C4 (Adobe Systems Incorporated), the extent of neurotoxic damage to the HC and PER, as well as lateral entorhinal cortex, will be estimated on the basis of serial NeuN-stained sections for Chapter 3 (Paxinos & Watson, 1998; PER localization based on Burwell, Witter, & Amaral, 1995). Gold-chloride sections were qualitatively assessed with a light microscope for damage to major fiber bundles. For Chapter 4, the extent of neurotoxic damage to the HC, PER, PL, and V2, as well as lateral entorhinal cortex, infralimbic cortex, and anterior cingulate cortex was estimated on the basis of serial NeuN-stained sections.

CHAPTER 3: THE TIME-DEPENDENT INVOLVEMENT OF HIPPOCAMPUS AND PERIRHINAL CORTEX IN MEMORY FOR DISTINCT VERSUS OVERLAPPING ITEMS.

3.1. RATIONALE

As described previously in the background section, current theoretical models propose that item (e.g., 'what') and contextual (e.g., 'where') information is processed in distinct cortical streams through perirhinal cortex (PER) and postrhinal cortex, respectively. These streams are then proposed to converge on the hippocampus (HC), where they are integrated to form a unique item-in-context representation that characterizes an episode (e.g., 'what-where'; Ranganath, Yonelinas, and Eichenbaum, 2006). However, recent evidence suggests that these models are overly simplistic. Because the PER has a particular role in mnemonic representations of highly overlapping stimuli, the nature of when it is necessary for representing item information needs to be refined. In addition, the HC has a known role in spatial paradigms, but its contribution to non-spatial recognition memory is unclear.

The overall objective of this study was to extend these influential theories by directly comparing the roles of the HC and PER in odor recognition memory. Specifically, we used olfactory stimuli to determine whether these theories extend to another modality, since a majority of studies have been done using visual stimuli, and directly compare the use of social and non-social stimuli. Rodents are capable of rapidly learning and remembering odors over long periods of time, and have particularly sensitive olfactory discrimination abilities (Linster et al., 2002; Schellinck et al., 2008). Here, we contrast the use of highly overlapping social odors and relatively distinctive non-social odors.

Olfaction is a critical modality for mammals, guiding numerous aspects of their daily lives including food preference, reproductive status, maternal bonding, and identification of conspecific allies and predators (Doty, 1986; Schellinck et al., 2008; Sanchez-Andrade & Kendrick, 2009). Furthermore, olfactory inputs are highly interconnected with numerous mnemonic structures. In particular, the olfactory bulbs have direct projections to a number of putative memory structures in the MTL (Brennan & Kendrick, 2006; Kay, 2008).

Importantly, social odors are processed differently and have a unique composition compared to non-social odors. The rodent olfactory system is comprised of two distinct pathways, the main olfactory pathway and the accessory (vomeronasal) olfactory pathway, which are thought to transmit differential information about volatile and non-volatile olfactory stimuli, respectively (Martinez-Marcos, 2008). Social odors from conspecifics are composed of a complex assortment of various molecules with components shared between individuals, conveying information about the age, sex, health status, and relatedness (Brennan & Kendrick, 2006). These social odors are processed through both olfactory pathways, while non-social odors are processed through the main olfactory pathway.

Here, we use odor-based stimuli in an adaptation of the spontaneous novel object recognition paradigm (Ennaceur & Delacour, 1988; Spinetta et al., 2008; O'Dell et al., 2010; Monaghan et al., 2010) to elucidate the effects of pre-training lesions to the HC and PER on odor recognition memory. Additionally, we tested five retention intervals (5 min, 20 min, 1 hr, 24 hr, or 48 hr) because of known time-dependent contributions of the HC (Zola-Morgan & Squire, 1990; Rolls, 1996; Anderson, 2007) and PER (Mumby et al., 2007; Sacchetti et al., 2007) to other memory tasks. In particular, these retention intervals

allowed assessment of short- and long-term odor recognition memory. See **Figure 3.1** for a diagrammatic representation of the recognition memory paradigm used.

We also tested the effects of HC and PER lesions on recognition memory for both conspecific social odors and non-social odors (household spices). Conspecific and household odor stimuli represent an ecological and arbitrary approach, respectively, to the study of odor recognition memory (Domjan et al., 2004). Considering that HC lesions have been shown to impair various aspects of social memory (Alvarez et al., 2002; Kogan et al., 2000; Countryman et al., 2005), it is possible that the HC plays a general role in social odor memory. Also, because PER has been implicated in the learning of social stimuli (Petrulis & Eichenbaum, 2003; Furtak, et al., 2007; Kholodar-Smith et al., 2008), we sought to investigate whether PER lesions differentially affect recognition memory for social odors versus non-social odors.

3.2. APPROACH

In the study phase, a single bead scented with a novel odor (Novel Odor 1; N1) was placed in the center of the front-most quadrant (most accessible to the experimenter) of the cage. Upon initiation of exploration (defined as sniffing and whisking within ~1 cm of the bead), rats were given 1 min to investigate the bead. Exploration times were recorded on a laptop computer using ODLog software (www.macropodsoftware.com). Beads were discarded at the end of each presentation. The experimenter changed gloves each time a new bead was used to prevent cross contamination. All odors were counterbalanced between rats and retention delays.

Following a variable retention interval (5 min, 20 min, 1 hr, 24 hr or 48 hr), each rat was presented with two beads: one bead scented with the odor presented in the study phase (N1), alongside one bead scented with a novel odor (Novel Odor 2; N2). During testing, novel odors were always paired with the same odor type (social or non-social) that the rat had sampled during the study phase. Test beads were placed in the same cage quadrant as the sample bead and were positioned approximately 3 cm apart. Upon initial exploration, rats were given 1 min to investigate the beads. Bead position (right or left) was counterbalanced for all rats and presentations. Exploration time for each bead was recorded in ODLog. See **Figure 3.1** for a diagrammatic representation and video still of the spontaneous odor recognition task.

Rats were tested on the odor recognition task over many days. Odor type and retention interval were counterbalanced across rats (within a session) and across sessions so each rat was tested twice on each combination.

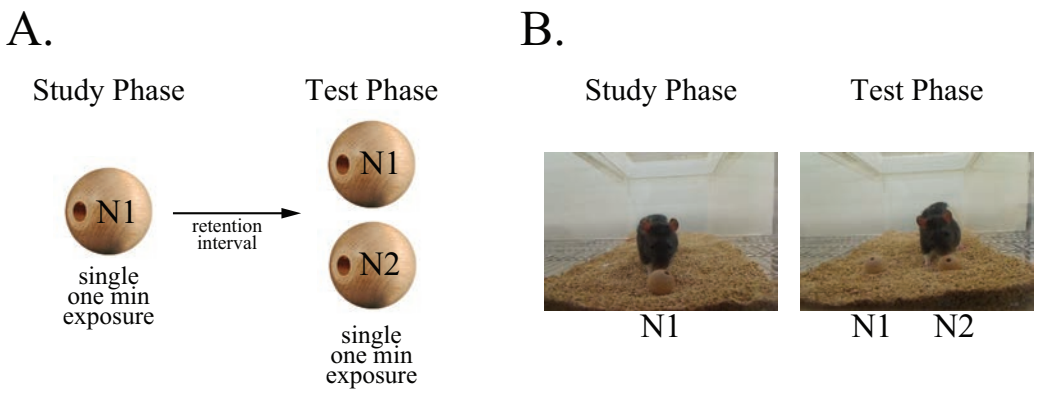

Figure 3.1. Spontaneous novel odor recognition memory paradigm. During the study phase, a single wooden bead was presented (Novel Odor 1; N1) to a rat in his home cage, scented with either a non-social or social odor not previously encountered by the rat. Upon initiation of olfactory exploration (active sniffing, whisking, nose within 1 cm) toward the bead, the rat was given 1 min to investigate the odor. Following a retention interval (5 min, 20 min, 1 hr, 24 hr, or 48 hr), the test phase occurred, in which two odor beads were presented simultaneously to the rat in his home cage. One bead was scented with the previously encountered odor from the study phase (N1) and the other bead was scented with new completely novel odor (Novel Odor 2; N2). Panel (A) shows a diagrammatic representation of the study and test phase. Preferential exploration toward N2 indicates recognition memory for N1. Panel (B) shows video stills of a rat during the study and test phases.

3.3. RESULTS

3.3.1. Histology of Control Subjects

HC and PER Controls had no noticeable evidence of brain damage as assessed with Nissl, gold-chloride and NeuN histological stains. Both HC and PER Controls are interpreted as having full and normal neural capabilities during all behavioral experiments, and were combined for subsequent analyses. See **Figure 3.2** for sample histology from a Control subject.

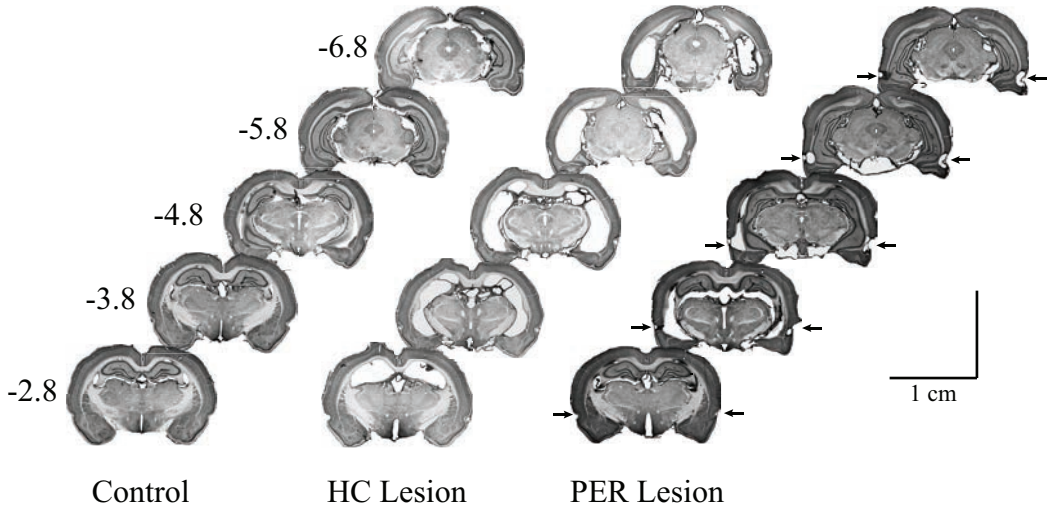

Figure 3.2. Sample control and lesion brains. The photomicrographs shows coronal histological sections covering the anterior-posterior extent from -2.8 to -6.8. Sections are were stained for NeuN. A sample brain is presented from each of the three experimental groups (Controls, HC lesions, and PER lesions). The localized neurotoxic lesions were induced with multiple injections of NMDA, and resulted in neuronal loss and atrophy of the target regions. The arrow indicated the central location of the PER lesions.

3.3.2. Histology of Hippocampus Lesioned Subjects

HC-lesioned subjects had large and complete lesions to the entire hippocampus while surrounding fibers were spared. There was a clear lack of hippocampal tissue throughout the rostral-caudal extent of the brains that was evident in all 3 stains. Two-dimensional

lesion area analysis was performed using the NeuN-stained sections. Overall, 90.3 ± 0.2% of the hippocampus was lesioned. There was no difference in damage produced in the left hemisphere (90.0 ± 0.03%) compared to the right hemisphere (90.6 ± 0.01%) $t_{(8)} = -0.26$, $p = 0.799$. Using the gold-chloride stained sections, we visually confirmed that the major fiber bundles surrounding the hippocampus, such as the corpus callosum, were intact. See **Figure 3.2** for an example of a HC-lesioned brain.

3.3.3. Histology of Perirhinal Lesioned Subjects

In PER-lesioned subjects, damage was centered in the cortical tissue surrounding the mid-posterior rhinal sulcus. These rats had large lesions to PER, and, to a lesser extent, a region of lateral entorhinal cortex (LEC) situated immediately ventral to area 35 of PER. There was very minor damage to the ventral HC (vHC).

PER, LEC and the vHC were included in a quantitative two-dimensional lesion area analysis. A Brain Region x Hemisphere repeated-measures ANOVA was run to examine differential damage to these regions and any potential laterality. There was a main effect of Brain Region, with PER being the most damaged (60.2 ± 0.4%), followed by LEC (23.8 ± 0.06%) and very little damage to vHC (1.4 ± 0.004%), $F_{(2, 18)} = 95.15$, $p < 1 \times 10^{-5}$. The amount of damage is similar to what has been previously found with a similar lesion technique (Kholodar-Smith et al., 2008). Additional examination of gold-chloride sections showed that the major fiber bundles surrounding the lesion area, such as the external capsule, remained intact. See **Figure 3.2** for an example of a PER-lesioned brain.

3.3.4. Study Phase Odor Exploration

During the study phase, rats were allowed to explore the novel odor bead for up to 60 s. Overall, rats spent 12.53 ± 0.48 s actively investigating the bead during the study phase. Exploration time during the study phase was compared between HC, PER, and Control rats using a Retention Interval x Odor Type x Lesion Group repeated-measures ANOVA to examine any potential differences in exploration times between conditions. Importantly, there were no main or interaction effects of the Lesion Group (HC, 12.98 ± 0.89 s; PER, 12.63 ± 0.85 s; and Controls, 11.99 ± 0.67 s), p's > 0.10. Thus neither the HC lesions, nor the PER lesions, significantly affected the exploration time of the rats during the study phase and cannot account for any differences in memory-based performance during test phases.

There were some differences in investigation time depending on the type of odor stimulus and the retention interval. Overall, rats investigated social odors (14.44 ± 0.71 s) more than non-social odors (10.63 ± 0.41 s), seen in a main effect of Odor Type, $F_{(1,32)}$ = 31.27, $p < 0.001$. This 36.8% increase in exploration time for social odors likely reflects a real difference in spontaneous investigation of social odors compared to non-social odors, and suggests that rats prefer social odors in general, or may need to sample social odors longer to fully perceive and/or encode their multifaceted composition. There was also a significant main effect of Retention Interval, $F_{(4,128)} = 4.45$, $p < 0.05$. The difference in exploration time was relatively small, with the mean difference of 1.00 ± 0.18 s in exploration times between the groups, representing a modest 7.9% change from overall mean levels. The retention intervals were randomly assigned and the study phases were

identically presented for each retention interval, and thus we do not make any strong interpretations from this effect.

3.3.5. Novel Odor Discrimination During the Test Phase

Exploration behavior during the spontaneous novel odor recognition task was quantified using a difference score in seconds (*DI*, Eqn. 1) and a discrimination index (*DI'*, Eqn. 2) as the measures of behavioral performance. Both measures yielded the same pattern of results. Here we are presenting the more commonly used *DI'* when reporting data from the memory-based test phase performance.

3.3.6. Control Subjects: Odor Type and Retention Intervals

We found that control rats demonstrated odor recognition memory for both non-social and social odors at all retention intervals. Using one-sample t-tests tested against no odor preference (*DI'* = 0), we found that control rats showed a significant preference for the novel odor (N2; see **Figure 3.3**) under all conditions (all p's < 0.001).

However, there were differences in performance levels based on the odor type and retention interval. A repeated-measures ANOVA was used to examine differences in Odor Type x Retention Interval. Control rats had larger *DI'* scores for non-social odors (47.92 ± 8.30) compared to social odors (33.80 ± 7.15), revealed by a main effect of Odor Type, $F_{(1,15)} = 7.87$, $p < 0.05$. This difference may reflect a need for longer investigation times to identify or recognize social odors, which would result in smaller *DI'* scores. This possibility is consistent with the longer search times found during the study phase for social odors compared to non-social odors. In addition, there was a main effect of Retention Interval in

Controls ($F_{(4,60)} = 3.50$, $p < 0.05$), indicating a moderate decline in the *DI'* scores as retention intervals increased. There was no significant Odor Type x Retention Interval, $F_{(4,60)} = 0.17$, $p = 0.95$.

3.3.7. Hippocampal Lesioned Subjects versus Control Subjects: Non-social Odors

HC-lesioned rats showed no detectable impairments in recognition memory for non-social odors compared to Controls. There was no significant main effect of Group, $F_{(1,23)} = 0.87$, $p = 0.36$, nor a significant interaction effect of Retention Interval x Group, $F_{(4,92)} = 0.50$, $p = 0.73$. There was a significant trend for a main effect of the length of the Retention Interval, $F_{(4, 92)} = 2.32$, $p = 0.06$. The *DI'* of HC rats are plotted against Control rats, for all five Retention Intervals (**Figure 3.3a**).

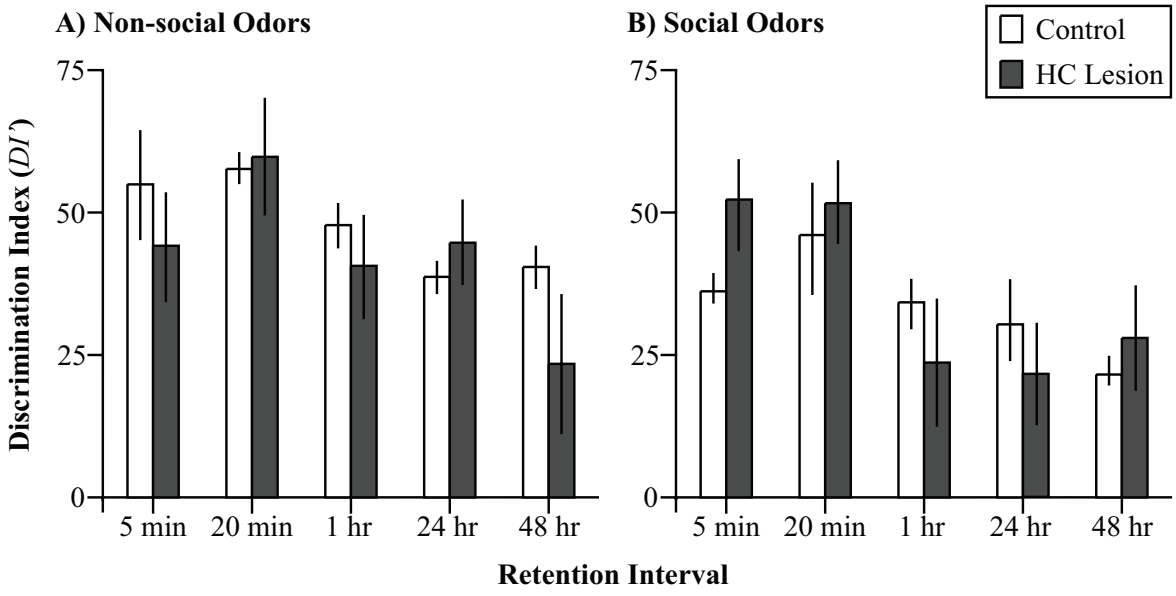

Figure 3.3. Hippocampal lesions do not impair recognition memory for either non-social or social odor stimuli (see Results). HC lesion (open bars) versus Control (filled bars) performance on non-social (panel A) and social (panel B) spontaneous novel odor recognition test at five retention intervals. HC lesioned rats demonstrate normal recognition memory (significant preference for novel odor during test phase) at all retention intervals for both odor types.

3.3.8. Hippocampal Lesioned Subjects versus Control Subjects: Social Odors

HC-lesioned rats showed no detectable impairments in recognition memory for social odors compared to Controls. There was no significant main effect of Group, $F_{(1,23)} = 0.37$, $p = 0.55$, nor a significant interaction effect of Retention Interval x Group, $F_{(4,92)} = 0.43$, $p = 0.79$. There was a significant trend for a main effect of the length of the Retention Interval, $F_{(4, 92)} = 2.12$, $p = 0.08$. The *DI'* of HC rats are plotted against Control rats, for all five Retention Intervals (**Figure 3.3b**).

3.3.9. Perirhinal Lesioned Subjects versus Control Subjects: Non-social Odors

PER-lesioned rats showed no detectable impairments in recognition memory for non-social odors compared to Controls. There was no significant main effect of Group, $F_{(1, 24)} = 0.86$, $p = 0.36$, nor a significant interaction effect of Retention Interval x Group, $F_{(4,96)} = 0.64$, $p = 0.64$. There was a main effect of the length of the Retention Interval, $F_{(4, 92)} = 3.74$, $p < 0.01$. The *DI'* of PER rats are plotted against Control rats, for all five Retention Intervals (**Figure 3.4a**).

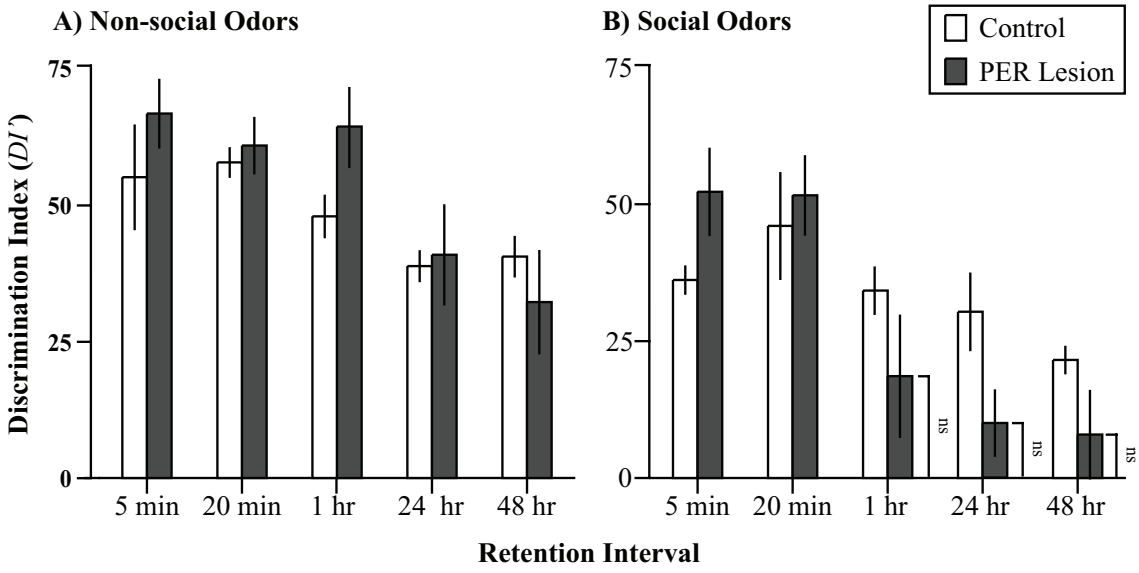

Figure 3.4. Perirhinal cortex lesions significantly impair recognition memory for social, but not non-social, odor stimuli (see Results). PER lesion (open bars) versus Control (filled bars) performance are plotted for non-social (panel A) and social (panel B) spontaneous novel odor recognition test at various retention delays. A) PER lesioned rats demonstrate normal recognition memory (significant preference for novel odor during test phase) at all retention intervals for non-social odor stimuli. B) PER lesion rats have a significant deficit for social odor recognition memory at 1 hr, 24 hr, and 48 hr compared to Controls, suggesting specific long-term memory deficits for social odors. Abbreviation: ns, not significantly different from no odor preference (DI' = 0).

3.3.10. Perirhinal Lesioned Subjects versus Control Subjects: Social Odors

PER lesions significantly impaired the ability of rats to demonstrate recognition memory for social odors following long retention intervals (≥ 1 hr), but not after short retention intervals (≤ 20 min). There was a significant interaction effect of Retention Interval x Group, $F_{(4, 96)} = 2.68$, $p < 0.05$. Post-hoc one-sample t-tests were performed against no odor preference (DI' = 0) in order to identify the specific retention intervals affected by PER lesions. The t-tests showed that PER lesioned rats only showed significant preference for the novel odor in the 5 min condition ($t_{(9)} = 6.70$, $p < 0.001$) and the 20 min condition, ($t_{(9)} = 7.39$, $p < 0.001$). However, there was no significant preference for the novel odor (N2) in the 1 hr condition ($t_{(9)} = 1.66$, $p = 0.13$), 24 hr condition ($t_{(9)} = 1.72$, $p =$

0.12), nor the 48 hr condition ($t_{(9)}$ = 1.01, p = 0.34; see **Figure 3.4b**); thus, given lesions to PER, rats fail to demonstrate significant long-term memory for social odors. These findings strongly suggest that the PER is critical for long-term, but not short-term, memory for social odors. Notably, the interaction effect rules out the possibility that PER is necessary to perceive social odors given the lack of effect at short retention intervals (5 and 20 min).

There was no main effect of Group, $F_{(1, 24)}$ = 0.63, p = 0.44, but there was a main effect of Retention Interval, $F_{(4,96)}$ = 10.11, p < 0.001. The social odor *DI'* of PER-lesioned rats are plotted against Control rats, for all five retention intervals (**Figure 3.4b**).

The magnitude of the behavioral impairment was determined by calculating effect sizes (Cohen's d; Eqn. 3) of PER lesion against Control performance. By convention, there was a large effect of PER lesions on the 24 hr retention interval (*d* = 1.08; large effect ≥ 0.8), a medium effect size was observed at the 1 hr time point (*d* = 0.53; medium effect ≥ 0.5), and a small effect size observed at the 48 hr time point (*d* = 0.44; small effect ≥ 0.3; **Figure 3.5**).

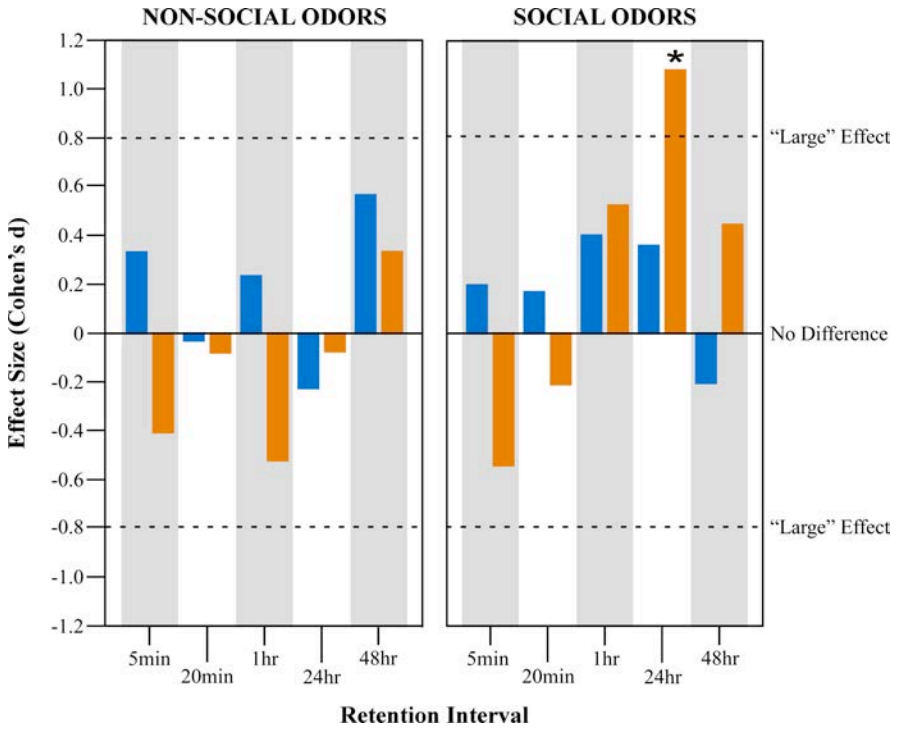

Figure 3.5. Magnitude of behavioral impairment in HC (blue) and PER (orange) lesioned animals determined by effect sizes based on Cohen's d, 1988.

3.4. DISCUSSION

3.4.1. Summary of Main Findings

The present study assessed recognition memory for olfactory stimuli in rats with neurotoxic NMDA lesions to either the HC or PER. Stimuli included both non-social and social odors. Non-social odors consisted of household spices that served as relatively distinct, minimally overlapping stimuli that are commonly used in olfactory-based tasks (Fortin et al., 2002; Kesner et al., 2002; DeVito & Eichenbaum, 2011). Social odors were obtained from individually-housed conspecifics and served as ethologically-relevant stimuli with highly overlapping constitutive olfactory features. The present experiments address three important issues pertaining to olfactory recognition memory: HC and/or PER

dependence, effects of non-social versus social odors, and effects of short and long retention intervals.

We first sought to determine the necessity of the HC and PER in odor recognition memory. Despite complete focal lesions to the entire extent of the HC formation, there were no significant differences in discrimination indices for the HC-lesioned rats compared to Controls. In contrast, precisely-targeted PER lesions yielded interval-dependent and stimulus-type specific deficits.

Second, we wanted to investigate differences in behavior when using non-social versus social odors. Controls showed significant differences in their performance on non-social compared to social odors, spending more time investigating social odors in sample phases and having smaller, though significant, *DI'* scores in test phases. We found no effect of HC lesions on subsequent recognition memory for odors of either type (**Figure 3.3**). PER lesions did not affect recognition memory for non-social odors, but was found to impair recognition memory of social odors at long retention intervals (≥ 1 hr; **Figure 3.4**).

Third, we tested rats at several retention intervals from sample to test phase to investigate whether recognition performance decays over time following a single exposure to an odor. Rats demonstrated a significant decrease in preference indices over retention intervals from 5 min to 48 hr. We did not find any effects of stimulus type on decay rate, as both non-social and social odors yielded similar gradients over time.

3.4.2. Spontaneous Social Odor Recognition Task

Intact item recognition memory is experimentally characterized by correct identification of a previously encountered stimulus. The behavioral paradigm employed in

the present study was adapted from Spinetta et al. (2008), in which spontaneous novelty preference was used to assess social odor recognition memory in rats (O'Dell et al., 2011; Monaghan et al., 2010). In contrast to Spinetta et al. (2008) in which rats habituated to a novel odor over three 1 min exposures, rats here were given a single 1 min exposure. We demonstrate that a single encoding trial is sufficient to yield novelty preference during the subsequent test phase, at similar preference levels. Using a rapid one-trial learning paradigm enabled us to investigate recognition memory for singly encountered, incidentally encoded events. Furthermore, study phases consisting of a single exposure trial afford the opportunity to assess recognition memory at shorter retention intervals from the initial encounter to test (e.g., 5 min). Albasser et al. (2009) demonstrated that PER lesion-induced deficits in novel object recognition are not reversed by extending the sample duration, further justifying our use of a single-trial exposure.

Additionally, a single sample trial decreases encoding time, which should result in a weaker memory trace and be more likely to decline over time, reducing ceiling and overtraining effects. Here, we observed a significant gradient of preference indices as the retention interval increased, suggesting a significant decrement in recognition memory over time. Alternatively, this may reflect a change in novelty preference over time.

We also expanded the stimulus set used by Spinetta and colleagues (2008), using both social and additionally non-social odors, in order to probe for differential recognition memory profiles as a result of odor type. Rats demonstrated greater exploration for social compared to non-social odors in the study phase. Despite longer exploration for social odors during the study phase, we found smaller *DI'* scores during the test phase for social compared to nonsocial odors. Given longer explorations times for the social odors during

the study phase one might expect greater familiarity for the social odors and thus larger *DI'* scores for the social odors during the test phase due to stronger memories. Interestingly, we observed smaller *DI'* scores for social odors during the test phase. It is possible that the multifaceted and overlapping composition of social odors results in the need for longer exploration times for full encoding and recognition, compared to more distinctive non-overlapping non-social odors. This latter possibility would predict both longer search times in the study phase and smaller *DI'* scores in the test phase for social odors, compared to non-social odors, as we observed.

3.4.3. Neural Basis of Social and Non-social Recognition Memory

Rats with HC and PER lesions revealed differences in the neural processing of recognition memory for social and non-social odors. PER-lesioned rats had a recognition memory deficit for social odors at retention intervals greater than or equal to 1 hr. By contrast, HC lesioned rats showed no detectable recognition memory deficits.

The retention-interval dependent impairment in odor recognition memory following PER lesions is consistent with the literature on visual recognition memory in different species. It is important to note that, although the same pattern of findings is observed across studies, the specific length of the retention interval at which deficits are detected in PER-lesion subjects varies across studies. For example, in human patients with medial temporal lobe damage that includes PER, Buffalo et al. (1998) reported normal visual recognition memory at short retention intervals (0 – 2 sec), but found deficits at longer retention intervals (6 – 10 sec), with the most severe impairments at the longest interval tested (25 – 40 sec). In non-human primates, memory for visual stimuli in a

delayed-nonmatching-to-sample task is impaired following PER lesions at 30 sec intervals, with the most severe impairments at 120 sec intervals. In rats, performance on simultaneous feature-ambiguous visual object discrimination is unimpaired by PER lesions (Clark et al., 2011). However, Clark et al. (2011) further demonstrated that the same PER lesions cause impairments in a novel object recognition memory version of the task at 24 hrs. Overall, these studies suggest the effects of PER lesions on recognition memory become evident and/or more severe as retention intervals increase (see also Mumby & Pinel, 1994; Bussey et al., 1999; Mumby et al., 2007). These findings are consistent with our results, demonstrating that PER is necessary for long-term, but not short-term, social odor recognition memory.

Additionally, these data argue against the necessary role of the HC in recognition memory, consistent with several previous studies using olfactory (Fortin et al., 2002; Kesner et al., 2002; DeVito & Eichenbaum, 2011) and visual stimuli (Murray & Mishkin, 1998; Brown & Aggleton, 2001; Mumby, 2001; Mumby et al., 2007; Albasser et al., 2010). The inputs to, and recurrent circuitry within the HC implies that it may be more involved with integrating information about items and contexts from upstream brain systems, generating representations that allow for declarative and episodic memory (Teyler & DiScenna, 1986; Squire, 1992; Rudy & Sutherland, 1995; O'Reilly & Rudy, 2001; Eichenbaum et al., 2007; Teyler & Rudy, 2007).

The findings that the PER lesion deficits are specific to the social odors used here highlights the qualitatively distinct nature of these stimuli. Not only are social odors comprised of greater numbers of overlapping features compared to non-social odors, they are processed through anatomically different systems and impart a great deal of

ethologically-relevant information to the rat (Sanchez-Andrade & Kendrick, 2009). Additionally, rats may have had a different history with social and non-social odors, which could have led to differences in the neural representation of the two odor types before testing. However, we had several controls over the experiential history of the rats with the experimental odors, and both odor types would have been experienced during the life of the experimental rats (see sections 2.3.1 and 2.3.2). Furthermore, PER lesions have been shown to impair recognition memory after subjects have had a vast experience with similar stimulus sets prior to receiving lesions (Meunier et al, 1993; Eacott et al., 1994; Mumby & Pinel, 1994; Prusky et al., 2004; Clark et al., 2011), and when subjects have had no experience with stimulus sets prior to receiving lesions (Bussey et al., 1999). Thus, it seems unlikely that any differences in experiential history between odor types is the major factor in the lesion effects observed here.

The experimental approach here was to use both "ethologically-relevant" and "neutral" stimuli (Domjan et al., 2004) to examine the role of the HC and PER in odor recognition memory. Notably, both HC and PER lesions have been shown to cause deficits in various social memory paradigms (Kogan et al., 2000; Alvarez et al., 2002; Petrulis & Eichenbaum, 2003; Kholodar-Smith et al., 2008), while the same lesions can be without effect when neutral odor stimuli are used (Fortin et al., 2002; Fortin et al., 2004; Kholodar-Smith et al., 2008). The use of social and non-social odors in a single experimental design allowed us to assess whether ethologically-relevant stimuli rely upon the same neural processing as neutral stimuli. Indeed, we found differential effects of lesions on recognition memory for social versus non-social odors revealing different neural pathways in processing these different types of stimuli. However, despite our findings that PER lesions

uniquely caused significant memory deficits for long-term social odor memory, we cannot determine here whether these effects were due to the social nature of the stimuli, the degree of overlapping elements present in the stimuli, or the experiential history with the stimuli. Future experiments could specifically address these explanations by deconstructing and recombining the constituent features of social odors, mixing variable ratios of household spices and through parametric manipulations of the pre-exposure to odors.

3.4.4. Perirhinal Cortex and the Conjunctive Stimulus Hypothesis

The hypothesis that PER is necessary for visual object recognition memory is well established in humans, monkeys, and rodents (Eacott et al., 1994; Ennaceur & Aggleton, 1997; Norman & Eacott, 2005; for review see Squire et al., 2004; Eichenbaum et al., 2007; Baxter, 2009). Studies in animals have found that PER lesions induce recognition memory deficits specifically for objects with a high degree of overlapping features, but not for highly-distinct objects (Murray et al., 2000; Bussey & Saksida, 2002; Iordanova et al., 2009; Aggleton & Brown, 2010; for review see Murray et al., 2007). This led to the notion that PER serves as a "perceptual-mnemonic" structure, important not only for memory, but necessary for the ability to perceptually distinguish objects and visual representations with overlapping features (Murray et al., 2007; Baxter, 2009).

This hypothesis predicts that recognition memory will differ for olfactory stimuli containing low and high amounts of overlapping features, regardless of the retention-interval. Here, wooden beads absorbed the odor of either a household spice or conspecific rats through several days of direct exposure. In the case of non-social odorants, beads

were scented over days by being immersed in a mixture of sand and a household spice. Social odor stimuli were generated by placing beads into home cages of individually-housed rats for one week. Each social odor obtained in this manner consists of a unique ratio of multiple odorants found in saliva, hair, urine, feces, including major urinary proteins, pheromones, sulfur-containing compounds produced by intestinal bacteria, hydrogen-sulfide, and odorous metabolic waste products. Thus, social odors are comprised of overlapping shared identifiers compared to non-social odors, which are differentiated by a single unique spice (Linster et al., 2002; Brennan & Kendrick, 2006; Schellinck et al., 2008).

PER-lesioned rats had a recognition memory deficit for social odors when tested at long retention delays (≥ 1 hr). This deficit cannot be accounted for by perceptual ability alone, as these rats showed normal memory for non-social odors at all retention intervals, and showed Control-level preference indices for social odors up to 20 min. Instead, these findings support the mnemonic role of PER in processing highly overlapping stimuli (Kholodar-Smith et al., 2008; Suzuki, 2009; 2010).

3.4.5. Conclusions

These data suggest that PER is critical to long-term recognition memory for odor-based object representations containing highly overlapping features, such as the social stimuli used here. Our data do not support the hypothesis that PER is necessary for perception of such odor stimuli, nor did we find PER to be necessary for recognition memory of odors that can be distinguished by a single odorant feature, such as the non-social odors used here. Additionally, our findings argue against the necessity of the HC for

odor recognition memory. These findings motivate future experiments isolating the precise conditions that cause social odor stimuli to rely on an intact PER for long-term recognition memory.

CHAPTER 4: THE ROLES OF THE HIPPOCAMPUS, PRELIMBIC CORTEX, AND PERIRHINAL CORTEX IN ORDER AND ITEM MEMORY.

4.1. Rationale

The ability to temporally organize our personal memories is an important aspect of episodic memory. Recently, the memory for sequences of events has been used as a model of episodic memory (e.g., memory for 'what '(the item) and 'when' (the order in which it was presented); Allen, et al., 2014; Davachi & DuBrow, 2015). These paradigms test the subject's ability to accurately recall the temporal configuration of events, generally consisting of the serial presentations of items (e.g., images, odors, etc.). These models have been useful developments toward studying the temporal organization of memory, however, the neural basis of this capacity is not well understood.

In addition to its role in spatial context, there is emerging evidence that the hippocampus (HC) is also important for remembering the order in which events occurred. Lesions to HC affect temporal order memory, but not item memory, in rodents (Fortin et al., 2002; Kesner et al., 2002; Barker & Warburton, 2011; Chiba et al., 1994; DeVito & Eichenbaum, 2011; Barker et al., 2013). Furthermore, HC neurons replay spatial sequences in the order that they fired during learning, suggesting memory for sequences of spatial locations (Skaggs & McNaughton, 1996). In addition, HC neurons reliably fire during aspects of a well-learned sequence task, with specific cellular responses to in- or out-of-sequence items (Allen et al., 2015). Human fMRI data also demonstrates that HC is significantly activated during a sequence memory task (Allen, et al., 2014; Davachi & Dubrow, 2015; Lehn, et al., 2009; Tubridy & Davachi, 2010). Together these findings

suggest that HC supports sequence memory.

There is evidence in human work that implicates prefrontal cortex (PFC) in memory for sequences of events (St. Jacques, et al., 2008; Jenkins & Ranganath, 2010). In rodents, lesions and inactivations to medial PFC (mPFC) affect temporal order discrimination of objects and spatial locations (Barker, et al., 2007; Hannesson, et al., 2004; DeVito & Eichenbaum, 2011; Mitchell & Laicona, 1998; Fuster, 2001). However, studies done in rodents have not isolated the damage to discrete regions of mPFC, so techniques assessing regional contributions using more focal lesion techniques are needed. Prelimbic cortex (PL), a region within the PFC, is a good candidate for having a potential involvement in order memory. PL has been suggested to be functionally homologous to the dorsolateral PFC in primates, a structure that has been implicated in sequence order memory (Allen & Fortin, 2013; Jenkins & Ranganath, 2010; Vertes, 2004). Previous studies have shown that lesions to PL affect delayed response tasks in rats (Delatour & Gisquet-Verrier, 1996; Brito, et al., 1982). Also, single neurons in PL exhibit sustained firing during the interval of a trace fear conditioning paradigm, implicating this region in temporal associations (Gilmartin & McEchron, 2005).

Perirhinal cortex (PER) has been more traditionally associated with item memory, (Feinberg, et al., 2012; Barker & Warburton, 2011; Murray et al., 2007; 2000; Bussey & Saksida, 2002; Aggleton & Brown, 2010), but could also play a role in order memory. PER is thought to form unitized representations of events that occur across time, combining temporally discontinuous features into a single perceptual object (Allen et al., 2007; Kholodar-Smith et al., 2008; Bang & Brown, 2009). Physiologically, PER neurons exhibit persistent firing activity elicited by synaptic stimulation that lasts for >1 min after the

stimulation stops suggesting that PER is involved in unitization by linking events across temporal gaps (Navaroli et al., 2012). Despite its role in associating events in time, no study to date has looked specifically at the role of PER in sequence memory. Given the role of PER in associating items over time, it is reasonable to predict PER will be directly involved in sequence memory.

The overall objective of these experiments is to identify the involvement of HC, PL, and PER in memory for sequences of events using the five-item spontaneous sequence task. To address this issue, we directly compare the performance of animals with excitotoxic *N*-methyl-D-aspartate (NMDA) or sham lesions to HC, PER, PL, or V2 (negative control group) on memory for order and items previously experienced as a sequence of odors.

Whereas several studies have utilized well-learned sequences or reward based sequence memory tasks to model episodic memory (Chiba et al., 1994; Fortin et al., 2002; Kesner et al., 2002; DeVito & Eichenbaum, 2011; Allen and Fortin, 2015), no study has examined these brain structures in incidental and one-trial memory for sequences of events. The few studies that have used spontaneous tasks to probe sequence memory feature a very small list of items (Barker and Warburton, 2011; Mitchell and Laiacona, 1998; Hannesson et al., 2004), in most cases two objects, which introduces potential confounds that affect the interpretation of the results. To directly address this gap in the field, I have designed a paradigm to assess memory of five-item sequences using a single trial, non-rewarded, spontaneous behavioral task in rats. This task is an ethologically relevant method for assessing memory for sequences of events, satisfying more aspects of episodic memory than previous efforts.

4.2. APPROACH

4.2.1. Spontaneous Sequence Memory Task – Extended Presentation of Sequence

Odor stimuli for this task consisted of non-social odors (e.g., basil, cinnamon). In the study phase, a sequence of five odors was presented using a 20 min inter-exposure interval (IEI for extended version; **Figure 4.1**). On the first trial, a single bead scented with a novel odor was placed in the center of the front-most quadrant (most accessible to the experimenter) of the cage. Upon initiation of exploration (defined as sniffing and whisking within ~1 cm of the bead), rats were given 30 seconds to investigate the bead. The amount of time spent investigating the first odor determined how much time each rat was allowed to spend investigating each subsequent odor (e.g., if a rat spends 4 s investigating odor A, we would ensure that odors B through E are each examined for 4 s). Exploration times were recorded on a laptop computer using ODLog software (www.macropodsoftware.com). To prevent cross-contamination, beads were discarded at the end of each presentation, and the experimenter changed gloves each time a new bead was used. All odors were counterbalanced between rats and retention delays.

Following a retention interval (60 minutes), rats were given an order probe, in which they were presented with two odors from the sequence (B vs. D). Based on previous work, we expected that memory for the order in which events occurred was demonstrated by significantly greater investigation toward the item that appeared earlier in the sequence. Twenty minutes after the order probe, an item probe was administered. The item probe is an important control to ensure that rats have recognition memory for items that appeared in the sequence. Here, rats were presented with one odor from the sequence

and a novel odor (C vs. X). Preferential exploration toward the novel odor bead indicates memory for the previously encountered odor.

For both order and item probes, beads were placed in the same cage quadrant as the sample bead and positioned approximately 3 cm apart. Exploration time for each bead was recorded in ODLog. See **Figure 4.1a** for a diagrammatic representation of the spontaneous sequence task.

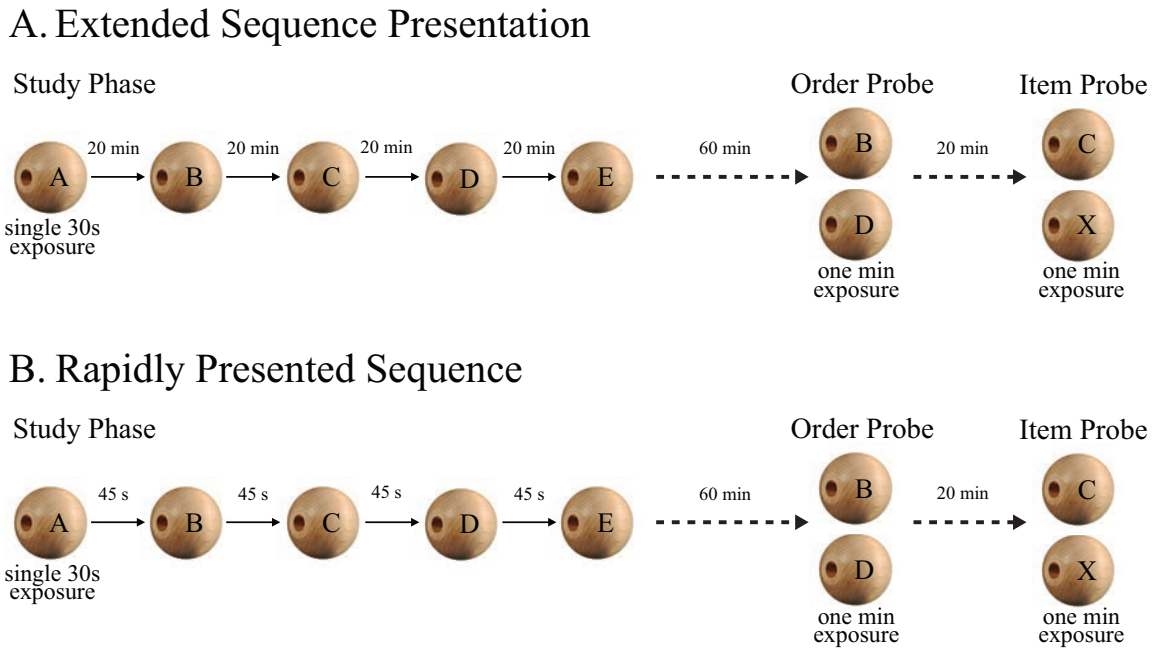

Figure 4.1. Diagrammatic representation of two versions of the spontaneous sequence memory paradigm. Panel (A) depicts the extended version of sequence task. During the study phase, a sequence of five household odors were presented individually. Rats were given 30 seconds to explore the first odor, and will be required to explore subsequent odors for the same amount of time. The inter-exposure interval (IEI) is 20 min. Following a 60 min retention interval the order probe was administered, in which two odor beads were presented simultaneously to the rat in his home cage. Beads are scented with a previously encountered odor from the sequence. Preferential exploration toward the odor that came earlier ('B') in the sequence indicates memory for order of sequences of events. Twenty minutes after completion of the order probe, the Item Probe was administered, in which two odor beads were presented simultaneously to the rat in his home cage. One bead was scented with a previously encountered odor from the sequence in the study phase ('C') and the other bead was scented with completely novel odor ('X'). Preferential exploration toward the novel odor bead indicates memory for the previously encountered odor. Panel (B) depicts the rapid version of the sequence task. The same procedures as the rapid version are followed, except the IEI was 45 s.

4.2.2. Rapid Presentation of Sequence

The same procedures as the extended version were used with the exception that the IEI was considerably shorter (45 s IEI; **Figure 4.1b**).

4.3. Results

4.3.1. Histology of Sham Subjects

HC and PL Sham (n=8) rats had no noticeable evidence of brain damage as assessed with NeuN histological stains. The HC and PL Controls are interpreted as having full and normal neural capabilities during all behavioral experiments, and were combined for subsequent analyses. PER Sham (n=2) analyses will be forthcoming, however we anticipate there being little-to-no damage in those subjects as well.

4.3.2. Histology of Hippocampus Lesioned Subjects

HC-lesioned subjects had large and complete lesions to the entire hippocampus while surrounding fibers were spared. There was a clear lack of hippocampal tissue throughout the rostral-caudal extent of the brains. Two-dimensional lesion area analysis was performed using NeuN-stained sections. Overall, 85.5 ± 2.52% of the hippocampus was lesioned. There was no difference in damage produced in the left hemisphere (85.72 ± 2.77%) compared to the right hemisphere (85.36 ± 2.26%), $t_{(10)} = 0.17$, $p = 0.87$ using a paired samples t-test .

4.3.3. Histology of Prelimbic Cortex Lesioned Subjects

PL-lesioned subjects (n=12) had large lesions to PL, and, to a lesser extent, a region of infralimbic cortex (IL) situated immediately ventral to PL. There was very minor damage to anterior cingulate cortex (ACC).

PL, IL and ACC were included in a quantitative two-dimensional lesion area analysis. PL was the most damaged (40.34 ± 3.25%), followed by IL (18.23 ± 5.85%) and there was very little damage to ACC (5.03 ± 1.60%). The amount of damage to PL is similar to what has been previously found with a similar lesion technique (DeVito & Eichenbaum, 2011), however the extent of damage to extra-PL regions was vastly reduced in this study. Thus, given specificity for which PL lesions were isolated, despite minor damage outside the region, we henceforth refer to these lesions as PL lesions (rather than mPFC or PL/IL lesions).

4.3.4. Histology of Perirhinal Lesioned Subjects

In PER-lesioned subjects (n=11), damage was centered in the cortical tissue surrounding the mid-posterior rhinal sulcus. Overall, 58.32 ± 4.27% of the PER was lesioned (A/P -2.0 to -7.2). However, a majority of the damage occurred in the posterior PER (A/P -4.0 to -7.2), where average damage overall was 80.23 ± 4.54%. Using a paired-samples t-test, we found that there was no difference in damage to posterior PER in the left hemisphere (76.34 ± 5.30%) compared to the right hemisphere (84.13 ± 5.08%), $t_{(10)} = -1.62$, $p = 0.14$. There was also minor damage to lateral entorhinal cortex (LEC) situated immediately ventral to area 35 of PER (36.71 ± 4.21%). The amount of damage is similar to

what has been previously found with a similar lesion technique (Feinberg et al. 2012; Kholodar-Smith et al., 2008).

4.3.5. Study Phase Odor Exploration

During the study phase, rats were allowed to explore the novel odor bead for up to 30 s. Overall, rats spent an average of 4.14 ± 0.98 s actively investigating the sample bead during the odor exploration phase (average of the three extended sequence presentations). Exploration time during the odor exploration phase was compared between Sham, HC, PL, PER, and V2 rats using a Retention Interval x Lesion Group ANOVA to examine any potential differences in exploration times between conditions. Importantly, there was no main effect of the Lesion Group, $F_{(4, 51)} = 1.52$, $p=0.211$. Thus NMDA lesions did not significantly affect the exploration time of the rats during the odor exploration phase and cannot account for any differences in memory-based performance during test phases.

4.3.6. Exploration and Discrimination During the Probes

Exploration behavior during the spontaneous sequence memory task was quantified using a difference score in seconds (DI_{order} and DI_{item}, Eqn. 4; Section 2.7) and a discrimination index (DI'_{order} and DI'_{item}, Eqn. 5; Section 2.7) as the measures of behavioral performance. Both measures yielded the same pattern of results. Here we are presenting the more commonly used *DI'* scores when reporting data from the memory-based test phase performance.

4.3.7. Extended Sequence Presentation: Order Probe

A one-way ANOVA was used to examine differences in DI'_{order} across lesion groups. There was a significant main effect of Lesion Group, $F_{(4, 51)} = 3.78$, p=0.01. Post-hoc comparisons using the LSD test revealed that Sham and V2 Lesion Control rats had significantly higher DI'_{order} scores (all p-values <0.03) compared to HC, PER, and PL lesion groups, with the exception of V2 Lesions compared to PL Lesions, which trended toward significance (p=0.085). The HC, PL, and PER lesions did not differ significantly from one another on the order probe. See **Figure 4.2a** for a graphical representation of these data.

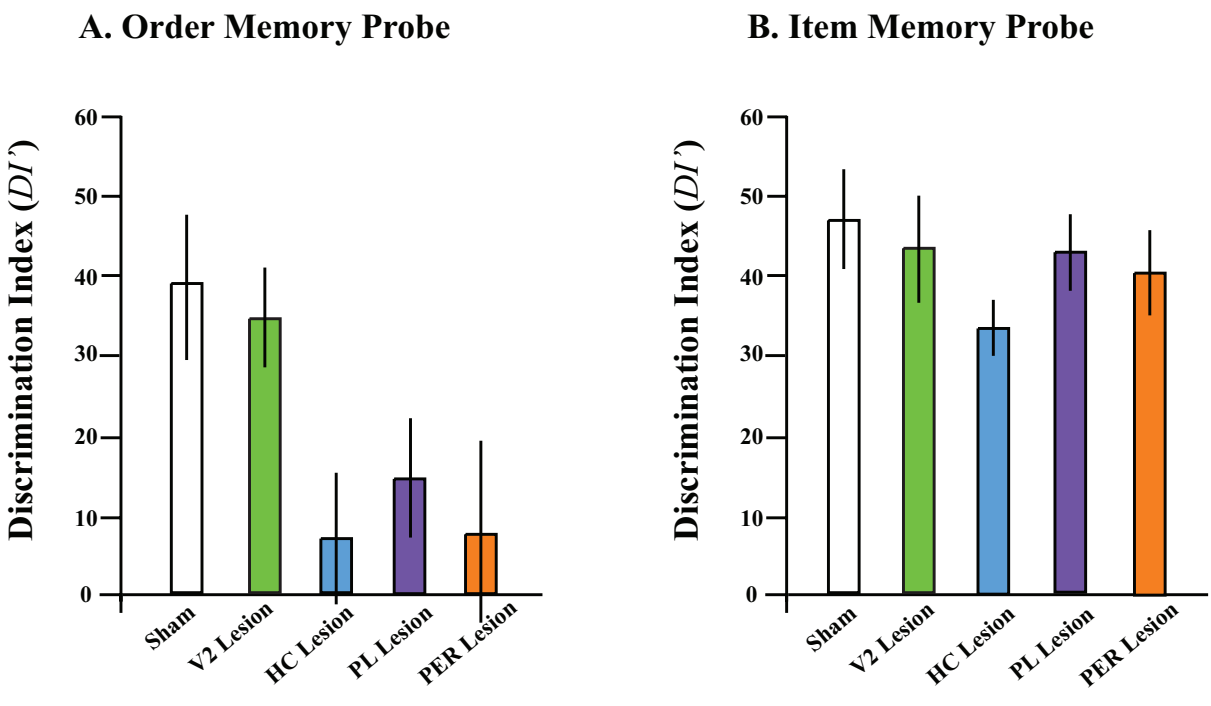

Figure 4.2. Control (Sham and V2 Lesion) and HC, PL, and PER lesioned rats' performance on order and item memory probes following extended sequence presentation. Panel (A) depicts that Control rats demonstrate intact order memory, while HC, PL, and PER NMDA Lesion groups have impaired memory for the order in which odors were presented. In panel (B), all groups show intact memory for the items that appeared in the sequence presentation.

4.3.8. Extended Sequence Presentation: Item Probe

A one-way ANOVA of DI'_{item} scores across lesions groups revealed no main effect on performance in the item probe, $F_{(4, 51)} = .904$, p=0.469. Using one-sample t-tests against chance (*DI'*=0), all groups demonstrated significant preference for the novel odor (odor X) compared to the odor presented in the sequence (odor C; all p's<0.001). See **Figure 4.2b** for a graphical representation of these data.

4.3.9. Extended Sequence Presentation: Order and Item Probes

Because there was no significant difference between Sham and V2 Lesion groups, we combined their performance on the probes into a Control Group. A two-way ANOVA comparing Lesion Groups versus Control in both Order and Item Probes revealed no significant interaction effects, $F_{(3, 48)} = 1.96$, p=0.133. However, there was a significant main effect of group, $F_{(3, 48)} = 5.80$, p=0.002. Post-hoc comparisons revealed that DI' scores were significantly lower on the Order probes than on the Item probes for the HC, PL and PER groups (all p's <0.05 using the Holm-Sidak correction for multiple comparisons) whereas the control group showed no significant difference (p's >0.05; Holm-Sidak correction). These findings strongly suggest that the deficit observed is selective to sequence memory and cannot be attributed to a secondary impairment in item memory.

4.3.10. Rapid Sequence Presentation: Order Probe

A Lesion Group x DI'_{order} for the rapid version of the sequence revealed no main effect, $F_{(3, 41)} = 1.09$, p=0.365. V2 Lesion rats were not included in this portion of the study. See **Figure 4.3a**.

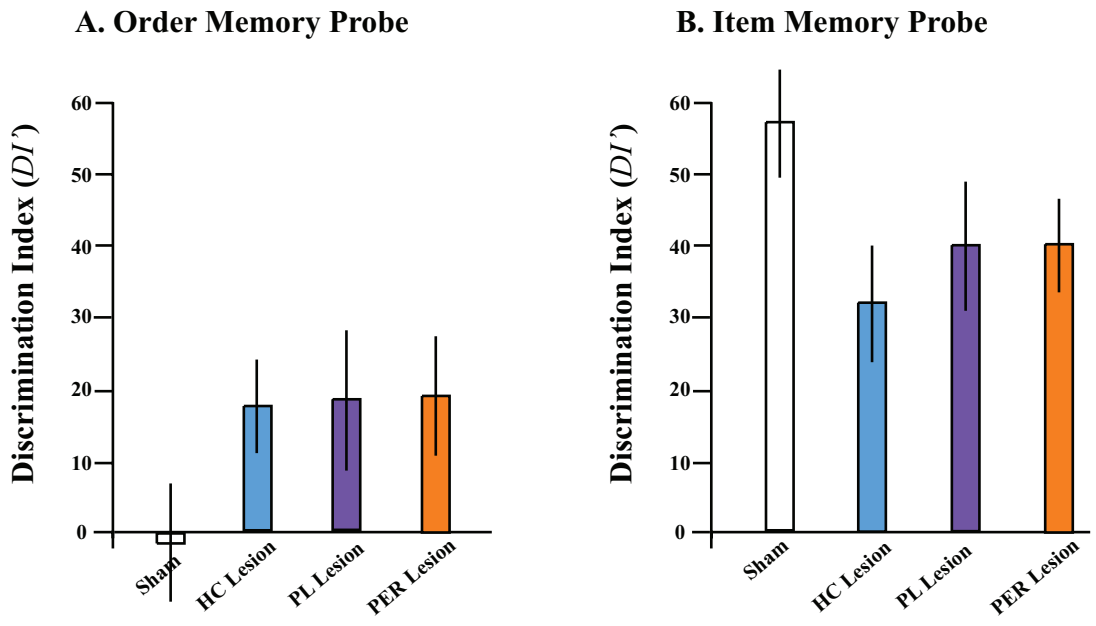

Figure 4.3. Sham and HC, PL, and PER lesioned rats' performance on order and item memory probes following rapid sequence presentation. In Panel (A), Sham and Lesion groups do not differ in their performance on the order probe following the rapid presentation of the odor sequence. In panel (B), all groups show intact item memory following rapid sequence presentation.

4.3.11. Rapid Sequence Presentation: Item Probe

Sham and lesion groups performed similarly on the item probe following the rapid sequence presentation. A Lesion Group x DI'_{item} revealed no significant main effect of group, $F_{(3, 41)} = 1.48$, p=0.24. Using one-sample t-tests against no odor preference (DI'=0), we found that all groups showed a significant preference for the novel odor (C; see **Figure 4.3b**; all p's <0.01).

4.3.12. Memory Strength Control

To account for potential alternative strategies for solving the order probe, we ran a memory strength control to test whether sequential position affected memory strength. Here, rats were given the rapid presentation version of the four-item spontaneous

sequence task (corresponding with an earlier version of the spontaneous sequence task). Subsequently, each rat was presented an odor from the sequence alongside a novel odor (e.g., A vs. W, B vs. X, C vs. Y, D vs. Z). Each rat received four sessions (in a counterbalanced fashion), in which all comparisons were made (one comparison per session).

There were no significant differences in the exploration time across odor positions, $F_{(3, 39)} = 0.42$, $p = 0.737$ (**Figure 4.4**). This suggests that all positions are remembered equally well (i.e., they have the same memory strength).

Furthermore, all odor positions were significantly greater than chance exploration times for the novel odor ($DI' > 0$). Thus, it is highly unlikely that memory strength can account for sequence position preferences when comparing two odors in our paradigm.

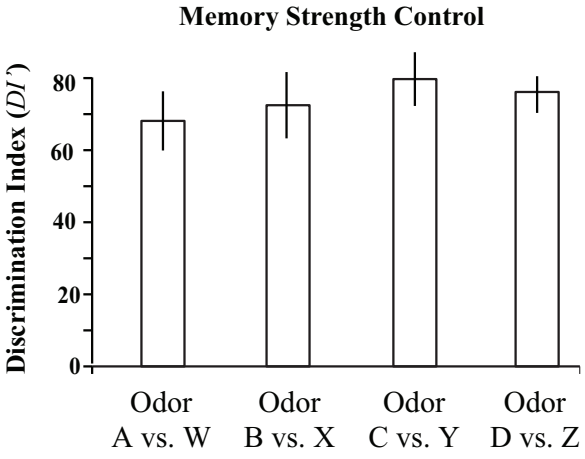

Figure 4.4. Rats were given the rapid version of the spontaneous sequence task, followed by presentation of an odor from the sequence alongside a novel odor (e.g., odors W, X, Y, or Z). Rats show significant preference for novel odors compared to odors that were in the sequence, indicating that they remember all odors regardless of the sequential position in which they were presented. This suggests that memory strength does not differ significantly across odor position. Error bars represent ± 1 SEM.

4.4. DISCUSSION

Episodic memory involves remembering a series of incidentally encoded personal experiences (Tulving, 1972). The ability to associate events into an ordered sequence as they unfolded over time is critical to having intact episodic memories (DeVito &

Eichenbaum, 2011). Furthermore, our ability to learn and remember sequences of events is important for establishing social bonds, predicting future outcomes, and navigating our daily lives (Allen and Fortin, 2013). However, the system of neural structures involved in this critical form of memory is not well understood. The objective of these experiments was to address this issue by using focal permanent lesions in four brain regions (HC, PL, PER) and testing their role in a newly developed spontaneous sequence memory task.

4.4.1. Spontaneous Sequence Memory Task

The spontaneous sequence memory task described here is an ethologically-relevant model of episodic memory, designed to assess rats' ability to remember unique sequences of events without explicit training or reward, as is the case with real world episodes. This paradigm is designed to better model memory for an incidentally encoded series of stimuli than previous models by isolating the stimuli to the olfactory domain and eliminating spatial and visual cues. Odors are presented to the rats inside their home cages using wooden beads that are all identical in visual and tactile attributes (Feinberg, et al., 2012; O'Dell, et al., 2012; Spinetta, et al., 2008). Testing occurs in the dark cycle (under ambient red lighting conditions) to reduce external visual variables, and to facilitate exploration by testing in their most active cycle.

Our odor task also contains a longer list of items presented than other spontaneous versions of sequence tasks. Specifically, our sequence consists of five items which stands in contrast to the two-item sequences that are more typical in the field (Mitchell & Laicona, 1998; Barker & Warburton, 2011). Given the length of the sequence, our task avoids primacy and recency effects by testing odors that occurred in the middle of the sequence

against one another (B vs D), or against a novel odor (C vs X).

Another useful attribute of this paradigm is the fact there are always two non-rewarded probe trials following a 60 min retention interval: an order probe, and an item probe. The order probe serves to assess rats' memory for the order in which the sequence of odors appeared. The item probe is given 20 min after the order probe, and tests memory for items that were presented previously in the sequence. The item probe serves as a built-in control to ensure that impairments are selective to order memory, and that rats meet the required criteria of remembering whether an item was presented in a sequence at all.

4.4.2. Order Memory

The order probe is designed to test rats' memories for sequences of events. In the Order Probe, rats are given two odors from the sequence (odors B and D; see **Figure 4.1**) and are allowed to explore these odors for 1 min. We show that sham and V2 lesioned rats (negative control group) demonstrate a significant preference for the odor that occurred earlier in the sequence (odor B), suggesting that they have intact memory for the sequence order. However, rats given a lesion to either HC, PER, or PL all show a lack of preference for either odor B or D, and therefore no evidence of order memory.

Previous studies using various sequence memory tasks have implicated HC as necessary for order memory (Kesner and Novak, 1982; Chiba, et al., 1994; Mitchell and Laicona, 1998; Fortin, et al., 2002; Kesner, et al. 2002; DeVito and Eichenbaum, 2011; Barker and Warburton, 2011; Barker, et al. 2013). Further evidence that HC is involved in memory for the order of events includes findings that HC is activated during recall of temporal events in humans (Lehn, et al. 2009; Hsei, et al., 2014; Eichenbaum, 2013) and in

electrophysiological recordings in rats (Manns, et al., 2007; Allen et al., 2015). Here, we expand on the role of HC in sequence memory, demonstrating its necessity in a non-rewarded, trial-unique task of using a five-item sequence of odors.

PFC has also been implicated in sequence order memory in both spatial and object discrimination tasks in rodents (Barker, et al., 2007; Hannesson, et al., 2004; DeVito & Eichenbaum, 2011; Mitchell & Laicona, 1998; Fuster, 2001) and humans (Staresina & Davachi, 2009; Jenkins & Ranganath, 2010; Tubridy & Davachi, 2010; Allen & Fortin, 2013). Our data here contributes to the growing evidence that PFC, and more specifically, PL region, is necessary for long-term memory of incidentally encoded unique sequences of odors.

This study is the first to report that lesions to PER cause a specific deficit in sequence order memory. PER has been implicated in bridging temporal memories by its necessity in trace fear conditioning (Kholodar-Smith, et al., 2008, Navaroli, 2012). Previously it was shown that rats given lesions to PER have deficits in temporal order memory (Barker, et al., 2007). However, in this study they also found significant deficits in recognition memory, so this task could not prove the selectivity of sequence memory deficits because it was confounded by rats' inability to recognize previously experienced items. Also, theirs was an object based task, which have previously demonstrated PER lesion effects (Murray & Richmond, 2001; Bussey & Saksida, 2005). In addition, the sequence used was only comprised of two-items, a very short list of stimuli, which can be confounded by primacy and recency effects. Thus the research performed here makes a significant contribution to understanding the role of PER in memory, extending it beyond complex perception to include memory for sequences of events.

Secondary visual cortex (V2) served as a negative control group in the sequence memory paradigm to demonstrate that brain damage alone does not disrupt memory for sequences of events. Here we found that V2 lesions do not affect order or item memory, a result that we expected. V2 is important for perception of visual stimuli (Lopez-Aranda, 2009; Saksida, 2009), and given that this task is olfactory in nature, we did not expect that it would have a role in spontaneous sequence memory for odors. Because V2 is located directly over the HC, it also serves to demonstrate that mechanical damage of the cortex above the HC does not itself cause sequence memory deficits.

4.4.3. Memory Strength Control

The memory strength control was performed to ensure that rats' preference for the odor that occurred earlier in the sequence (odor B) was not due to degraded memory strength for that odor compared to the later odor (odor D). Therefore, using a separate group of pilot rats, sequences of odors were presented and after a retention interval, rats were presented with an odor from the sequence alongside a novel odor (similar to the item probe; A vs W, B vs X, C vs Y, etc.; see **Figure 4.4**). All items in the sequence were tested compared to a novel odor. Here we found that levels of exploration on the familiar odors from the sequence were not significantly different from one another, and therefore memory strength for all odors is similar when tested for sequence memory. We also demonstrated previously that discrimination indices of novel versus familiar household odors at retention intervals of 5 min, 20 min, and 1 hr were not different in both sham and HC or PER lesioned animals, further supporting the notion that memory strength does not significantly decay within this timeframe (Feinberg, et al., 2012). Therefore, rats are not

simply choosing the odor that came earlier in the sequence because that memory is weaker than the later odor. Rather, we interpret this to mean that rats are demonstrating memory for the sequences of events when preferentially investigating the earlier odor.

4.4.4. Item Memory

In the Item Probe, rats are presented an odor from the sequence (odor C) along side a novel odor (odor X; see **Figure 4.1**). If memory for the items that were present in the sequence is intact, rats will prefer the novel odor. All rats, regardless of lesion site, performed the item memory probe well, demonstrating preference for the odor that was not present in the initial sequence (odor X). Unlike order memory, lesions to HC, PER, or PL did not affect item memory. This is critical to the logical design of the experiment here, as it allows sequence memory to be isolated. It has been well established that HC and PL are not necessary for novelty discriminations (Feinberg, et al. 2012; Barker, et al., 2007; Fortin, et al., 2002; Kesner, et al., 2002; Mitchell & Laicona, 1998;). Furthermore, PER lesions did not previously yield deficits in odor recognition memory for non-social odors at any time point between 5 min to 24 hr, so these data corroborate previous findings (Feinberg, et al. 2012).

4.4.5. Rapid Spontaneous Sequence Test

In an attempt to dissociate the respective roles of HC, PER, and PL in this task, we also ran a more rapid version of the spontaneous sequence memory task. In this version, retention intervals were shortened such that an inter-stimulus interval between item presentations was 45 s, as opposed to 20 min. Sham animals did not show a significant preference for the earlier or more recent odor, which suggests a lack of sequence memory.

In addition, our results did not yield a significant difference between sham and lesioned animals. Our null results may be due to the increased difficulty level of the task given the rapid nature at which stimuli were presented compounded with the fact that animals were not rewarded and the sequence was presented in a single trial. Several other studies that have utilized sequence tasks on such timescales and found significant preference for the earlier item used either very short sequences (e.g. two-items) or reward during sampling and/or probe trials, making these tasks easier to learn with fewer stimuli, or enhancing the animals' motivation to encode the sequence with reward (e.g. 3 sec IEI; DeVito and Eichenbaum, 2011; 1 min IEI - Barker and Warburton 2011; 2-2.5 min IEI - Fortin, et al. 2002; Barker, et al. 2007). In our pilot data, sham animals did show a significant preference for the odor that was presented earlier, however these data were collected using a four-item sequence rather than the five-item version adopted for this study. Thus, further piloting would be needed before this version of the task can be considered reliable. Due to these null effects, no conclusions can be made on the role of HC, PER, or PL in rapid sequence memory.

4.4.6. Conclusion

Here I described my newly developed model of episodic memory, designed to assess sequence memory in rats. This ethologically-relevant paradigm was useful for demonstrating that rats can learn and remember five-item sequences of odors without reward. Furthermore, these data demonstrate the necessity of HC, PL, and PER in sequence memory, shedding light on this critical aspect of episodic memory.

CHAPTER 5: CONCLUSION

In these experiments, I sought to develop a better understanding of brain regions involved in episodic memory. Specifically, I was interested in better elucidating the roles of HC, PL, and PER toward item and order memory. To do so, I developed two new behavior paradigms and used ethologically-relevant olfactory stimuli to test memory in rats.

5.1 Summary of Findings

5.1.1. Item Recognition Memory for Social and Non-social odors

In Chapter 3, I discuss how I used a novel behavioral paradigm to assess olfactory recognition memory for distinct (non-social) and overlapping (social) odors across retention intervals varying from 5 min to 48 hr. I found that excitotoxic lesions in HC did not impair olfactory recognition memory at any time point for both social and non-social odors. However, lesions to PER had a specific effect on recognition memory for social, but not non-social odors, at retention intervals greater than or equal to 1 hr. These data contribute to a large body of research on the role of MTL structures in recognition memory. Though it has been well established that the HC is not necessary for item recognition memory, the specific role of HC in social recognition memory is not well understood. The role of PER in recognition memory is very contentious, namely in the visual domain. However, several lines of evidence implicate PER in the perceptual/mnemonic domain of stimuli with overlapping features, rather than recognition in general. These data corroborate the notion that PER is necessary for remembering highly overlapping stimuli, but not for perception of those stimuli or memory for relatively distinct stimuli.

5.1.2. Memory for the Order of Events

In Chapter 4, I describe an ethologically-relevant paradigm that I developed to assess incidentally encoded sequences of five odors, utilized in a non-rewarded, single-trial paradigm that can probe for both order and item memory. I demonstrate that sham and rats given lesions to either HC, PL, PER, or V2 are capable of recognizing odors that appeared in the sequence, but that only animals with HC, PL, or PER lesions have a deficit for sequence memory. These data reveal the necessity of these structures in sequence memory, and further characterizes their roles in episodic memory function as, at least in part, related to computations necessary for temporal contexts.

A variant of the sequence task was performed using a more rapid presentation of odors (45 s versus 20 min), however sham rats were impaired in sequence memory on this version, and were not significantly different from lesioned animals. Therefore no conclusive data was obtained for the rapid presentation task. However, future experiments using a different rate of odor presentation are warranted since the data obtained from these results should ultimately provide useful insights into the roles of HC, PL, and PER in order memory and may reveal dissociations of the roles of each region.

5.2. Updated Working Model

Overall, with these data we are able to update the current theoretical model about the respective roles of HC, PFC, and PER in item and order memory (see **Figure 5.1)**. First, these data corroborate the evidence that HC is not critical to item recognition memory (Feinberg, et al., 2011; Fortin, et al., 2002; Kesner, et al., 2002). Second, the role of PER in

item memory is further refined, implicating it as having a specific role in long-term memory, but not perception, of highly overlapping stimuli. Third, I was able to more specifically isolate the role of PFC in order and item memory with discrete lesions to PL. Here, I found that HC, PL, and PER are all necessary for ability to successfully recall the order in which events occur. Using the data from the extended version of the task alone, and with the data we obtained, we cannot dissociate the roles each structures contribution toward memory for sequences of events. However, the data obtained here demonstrates the necessity of all three structures in order memory. Thus, future studies can be done to further assess the distinct roles that each structure plays in sequence memory.

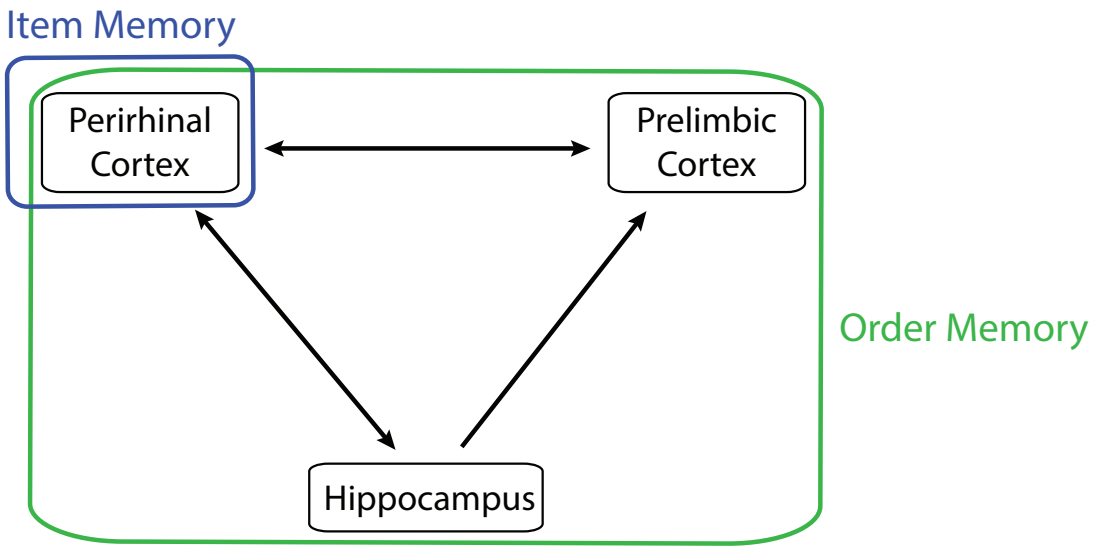

Figure 5.1. An updated working model on the functions of HC, PL, and PER. These experiments demonstrate that PER plays a role in long-term item recognition memory for highly overlapping stimuli. Furthermore, we found that HC, PL, and PER all play a role in memory for the order in which events occurred, expanding on current understanding about these structures.

5.3. The Earlier Item Preference Effect in the Sequence Order Probe

An important finding in my sequence task data is that sham and V2 control animals consistently prefer the odor that came earlier in the sequence, which has been reported in

a number of previous studies. For example, in a two-item object recognition task, sham rats showed significant preference for the object that they were exposed to earlier (Mitchell & Laicona, 1998; Barker & Warburton, 2011). Similarly, sham rats and mice preferentially explored the earlier odor from a well-learned sequence of five odors in a rewarded version of an olfactory sequence task (Fortin, et al., 2002; DeVito & Eichenbaum, 2011). This behavior is also demonstrated in spatial memory tasks, in which rats spend more time exploring an old familiar arm in a radial arm maze over a more recent familiar arm (Hannesson, et al., 2004).

One possible explanation for these early-item effects on behavior is that rats' memory for the earlier odor has weakened over time since it was encountered further back in time than the later odor. However, we performed a memory strength control experiment in which rats were given a sequence of odors (e.g. A, B, C, etc.; see **Figure 4.4**) and were later presented each odor from the sequence along with a novel odor (i.e. A vs W, B vs X, C vs Y, etc.). All rats spent equal time exploring each previously encountered odor from the sequence (odor A, B, C, etc.) relative to the novel odors (odor W, X, Y, etc.). Thus, the earlier odor preference effect is not simply due to decreased or degraded memory strength for that odor. These data are consistent with data from DeVito and Eichenabum (2011) who showed that mice did not preferentially explore odors presented in a relatively recent sequence compared to odors in a different sequence that occurred at an earlier time. Furthermore, I found that rats showed similar DI's at retention intervals of 5 min, 20 min, and 1 hr on familiar vs. novel odor probes, suggesting similar memory strengths for the previously explored odors (Feinberg, et al., 2012).

There is a possibility that the preference for an odor which occurred earlier in a

previously encountered sequence relates to foraging behavior. If a rat is given a choice between a recently foraged food site and a more temporally distant site, they prefer the area they went to further back in time because it is more likely to be restocked with food than a more recently explored location (Olton & Schlosberg, 1978). This is an innate strategy rats use in food foraging, commonly referred to as a win-shift strategy. This efficient foraging strategy may explain why rats have an innate preference for items presented earlier in a sequence, despite similar relative familiarity of the items. Similarly, rats demonstrate reliable spontaneous alternation when given the opportunity to successively explore arms on a maze (Barnett, 2007; Lalonde, 2002). Overall, these behavioral data suggest there is an innate tendency for rats to prefer regions they explored most distant in time compared to recent locations.

While the exact mechanism remains speculative, empirically I found that rats have an innate preference for odors that were encountered earlier in a sequence that is not accounted for by memory strength or novelty, clearly indicating order memory, which was the focus here. By showing preference for the earlier item, despite equivalent memory strength for both items, rats must logically be responding to the sequential contexts for the items.

5.4. Future Directions

5.4.1. Rapidly Presented Odor Sequence Task

Here I found null effects using a rapid version of the spontaneous sequence memory task. However, in the future, it would be useful to pilot a working version using short retention intervals in an attempt to dissociate the roles of HC, PL, and PER in their

contributions toward memory for the order of events.

5.4.2. Temporary Inactivations

The large effects of permanent brain lesions observed here demonstrate necessity of HC, PL, and PER in order memory, and the appropriateness of the permanent lesion approach employed. However, in future studies, inactivations of the regions in conjunction with one another might shed light on the distinct contributions of each region toward sequence memory, and may therefore be a valuable tool to further elucidate the roles of these structures in sequence memory providing a different level of inferential necessity. This study would be useful for elucidating whether these structures form a network that is necessary for order memory to occur.

5.4.3. Translational Applications of Order Memory

The spontaneous sequence task is a simple and elegant behavioral paradigm that may serve as a sensitive measure of deficits in a variety of disease models or drug states. Recent reviews identify the need for such tools to use in translational disease and drug research (Snigdha, et al., 2013; Marusich, et al., 2013). For example, in collaboration with the Marshall Lab at UCI, I found that a single binge dose of methamphetamine induced long-term memory deficits for social odors (O'Dell, et al, 2012). In future studies, it would be useful to assess the effects of the same dose of methamphetamine on memory for non-social odors and potentially for order memory as well.

5.5. Concluding Remarks

First, recognition memory for simple household spices does not critically depend on the HC, PL, nor PER. Second, when these same household spices are used in the sequence task, sequence memory depends on the HC, PL, and PER. Notably, this latter demonstration that PER is critical to sequence memory has never been shown before. In addition, the one-trial sequence memory task developed here is a novel contribution to allowing rapid assessment of sequence memory in rodents. The results are interpreted in the context of the greater literature to mean that the HC, PL, and PER form a memory system, from which sequence memory emerges, and through which episodic memory is possible.

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