BEHAVIORAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR

HIPPOCAMPAL INVOLVEMENT IN OBJECT MOTION PROCESSING IN

C57BL/6J MICE

by

Herborg Nanna Ásgeirsdóttir

A Dissertation Submitted to the Faculty of

The Charles E. Schmidt College of Science

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

Florida Atlantic University

Boca Raton, FL

December 2017

BEHAVIORAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR

HIPPOCAMPAL INVOLVEMENT IN OBJECT MOTION PROCESSING IN
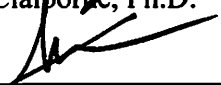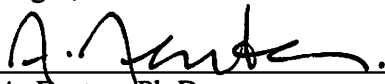
C57BL/6J MICE

by

Herborg Nanna Ásgeirsdóttir

This dissertation was prepared under the direction of the candidate's dissertation advisor, Dr. Robert W. Stackman, Department of Psychology, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
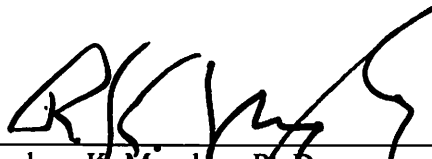
SUPERVISORY COMMITTEE:

_____
Robert W. Stackman, Ph.D.
Dissertation Advisor

_____
Brenda J. Claiborne, Ph.D.

_____
Ceylan Isgor, Ph.D.

_____
André A. Fenton, Ph.D.

_____
Rodney K. Murphey, Ph.D.
Chair, Department of Biological Sciences

_____
Ata Sarajedini, Ph.D.
Dean, Charles E. Schmidt College of Science

_____
Deborah L. Floyd, Ed.D.
Dean, Graduate College

_____
November 20, 2017
Date

iii

ACKNOWLEDGEMENTS

ABSTRACT

Author: Herborg Nanna Ásgeirsdóttir

Title: Behavioral and Electrophysiological Evidence for Hippocampal
                   Involvement in Object Motion Processing in C57BL/6J Mice

Institution: Florida Atlantic University

Dissertation Advisor: Dr. Robert W. Stackman, Jr.

Degree: Doctor of Philosophy

Year: 2017

Considerable research has been carried out to establish a rodent model for the study of human memory, yet functional similarities between the species remain up for debate. The hippocampus, a region deep within the medial temporal lobe of the mammalian CNS, is critical for long-term episodic memory. Projections from the medial entorhinal cortex convey spatial/contextual information, while projections from the lateral entorhinal cortex convey item/object information to the hippocampus. The functional significance of these parallel projections to the rodent hippocampus has been suggested to support spatial processing, while the same projections to the human hippocampus support spatial and non-spatial memory. Discharging in a location-specific manner, hippocampal place cells contribute to spatial memory; however, evidence for neuronal correlates of non-spatial object memory has not been fully defined. The current experiments were designed to address the following questions, while utilizing electrophysiology, functional inactivation during a novel behavioral task, and

immunohistochemistry. Is the memory for objects hippocampal-dependent, solely due to the location of the object, or are objects represented within hippocampal activity *independent of location*? To tease apart spatial and non-spatial processing by the hippocampus, the spatial aspects of 3D objects were enhanced by utilizing movement. A novel discriminatory avoidance task, Knowing Your Enemy, was adapted from an Enemy Avoidance task to test true object memory in mice. Current findings support the notion that object-associations acquisition depends upon a specific context. Retrieval of such object-associations is not context-dependent, yet remains sensitive to temporary inactivation of the CA1 region of the dorsal hippocampus. The avoidance impairments observed following hippocampal inactivation were shown to not be a result of reduced anxiety. Immunohistochemical marker expression suggests that the CA1 region was highly active during object exposures, yet the hippocampal system responded differentially to moving and to stationary objects. Recordings of CA1 neurons yielded non-bursting object-related activity during object exploration, and place cell activity remained unaffected in the presence of moving objects; supporting independent, yet simultaneous processing of spatial and non-spatial information within the hippocampus. Together, the current findings support the notion that the CA1 region of the rodent hippocampus processes object-related information, independent of spatial information.

DEDICATION

This manuscript is dedicated to all laboratory rats and mice. A special shout out to the mice that contributed to the data presented within this dissertation. Your lives were meaningful.

BEHAVIORAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR

HIPPOCAMPAL INVOLVEMENT IN OBJECT MOTION PROCESSING IN

C57BL/6J MICE

TABLES

FIGURES

PART I: INTRODUCTION

1.1 Why rodents are used to study Memory Systems

Memory is something that rarely comes into consideration unless it fails in some way. Investigating memory impairments following trauma or disease has provided initial discernment of the functions of human memory. Case studies looking into loss of memory function following an event have provided invaluable understanding of the anatomical and functional substrates for memory. The well-known example of Henry Gustav Molaison, or patient H.M., has unveiled and accelerated the field of human and animal memory studies. H.M. had suffered from severe epilepsy ever since he sustained a childhood head injury and as an adult had his bilateral medial temporal lobes surgically removed to alleviate his insufferable seizures. Following the surgery, he suffered severe anterograde amnesia that lasted throughout the duration of his life. Despite learning immensely about the brain regions and functions of human memory from H.M. and other case studies, we cannot experimentally replicate, manipulate or rescue the specific memory functions lost or obtained in individual human cases. In order to further our understanding of how the typical human memory functions, and to better define deficits following damage to anatomical regions of the brain associated with memory, we can experimentally carry out studies using non-human subjects. To ensure that we draw appropriate conclusions from results obtained from memory studies using animal models, we must first confirm that the explored brain regions are anatomically parallel and contribute to similar behavioral functions as they do in humans (Cave & Squire, 1991).

Throughout history, rodents have proven to be an excellent model for answering questions regarding molecular, cellular, and behavioral mechanisms that underlie mammalian long-term memory. Nevertheless, the degree to which the findings from such rodent studies can fully be applied to our understanding about human memory as well as the appropriateness of rodent models to infer knowledge regarding specific memory functions in humans has been questioned. Providing additional support for the functional similarity between the rodent and human memory system will bring the two experimental fields together and closer towards answering mechanistic questions regarding circuits of mammalian memory systems.

1.2 Memory Processes

Memory is the ability to store newly acquired information and utilize it later on. The processes of learning and remembering are referred to as encoding, consolidation and retrieval of information and involve a specific circuit or areas within the brain. Encoding refers to the creation of a mental representation of an event or an item, consolidation is when that representation gets stored in a specific location, and retrieval is when that representation is later accessed when required. Reconsolidation of a memory occurs when that representation is accessed and somehow altered, either by rendering it stronger, weaker, or the meaning of the stored information is modified.

The science of determining what brain regions are responsible for certain functions of memory processes have yielded abundant data with ambiguous conclusion. One of these studies (Lepage, Habib, & Tulving, 1998), however, conducted a meta-analysis of 54 Positron Emission Tomography (PET) experiments, suggested that both encoding and retrieval occur bilaterally within the hippocampus in human subjects, a

bilateral structure that lies deep within the medial temporal lobes. Further, the study concluded that encoding of both verbal and non-verbal novel information occurs within the more rostral portion of the hippocampus, while the retrieval of that same information is processed in more caudally in the structure (Lepage et al., 1998). Studying the consolidation phase of memory processes has proven more difficult to do using human subjects, but experiments using animal models have provided support for the notion that consolidation also occurs within the hippocampus. Furthermore, amygdala activity can enhance the emotional arousal causing a stronger association with the newly formed memory resulting in a memory that is accurately retrieved well into old age (McGaugh, 2000).

After the bilateral temporal lobotomy, and having recovered a life without seizures following his surgery, H.M. could no longer verbally discuss newly created memories for more than a few seconds. He could, however, recall memories from years earlier, but lacked any knowledge of what had happened the past three years prior to the surgery. Seemingly, it appears that H.M.'s anterograde amnesia or failure to form new memories resulted from the inability to consolidate or retrieve specific information previously encoded. According to the previous results from Lepage et al., it is likely that H.M.'s memory deficits did not stem from problems with encoding of new memories since structural Magnetic Resonance Imaging of his brain following the surgery revealed that most of his caudal hippocampal formation remained intact (Corkin, Amaral, & González…, 1997). The study of H.M's memory problems and the post mortem analysis of his brain provided sufficient evidence for the notion that human memory processing relies heavily on the hippocampal formation and surrounding structures.

Additional case studies have been reported where the patient suffered damage to smaller and more specific regions within the medial temporal lobe results in severe memory impairments. One example, patient R.B. suffered an ischemic episode following heart surgery that restricted blood flow specifically to the hippocampus, causing cell death within the structure. R.B. demonstrated severe anterograde amnesia following the stroke which was later attributed to bilateral lesions of the entire CA1 region of his hippocampus (Zola-Morgan, Squire, & Amaral, 1986). Although R.B. did not suffer from any other noticeable cognitive impairments, he did experience minor improvements in his memory performance during the first year following the stroke, but he lived with severe anterograde amnesia throughout the remainder of his life. These case studies, along with many others, demonstrate substantial evidence for the role of the hippocampus and surrounding anatomical regions required for specific types of memory.

1.3 Memory Systems

Human memory has different classifications depending on its duration or specific function. How long a newly formed memory lasts and the amount of information it processes can be broken down into short-term and long-term memory. Short-term memory is strong, can include highly detailed information but only lasts for a few minutes up to several hours at best. Long-term memory, like indicated in the name, lasts longer than several minutes and up to a lifetime following consolidation of the information. The information that gets stored within long-term memories is rarely highly detailed but can be correlated with the amygdala activation during memory encoding (McGaugh, 2000). Previous work has demonstrated that short-term and long-term memory types do not only vary in information stored or duration. The functions of the

underlying brain regions supporting these different memory systems vary as well. Evidence showing that specific medial temporal lobe regions, including the hippocampus, comprise the structures required for successful long-term memory function. Other regions of the inferior temporal lobe have been associated with functions related to short-term memory (Ranganath & Blumenfeld, 2005). In addition, these memory functions can be broken into different types. Declarative memory refers to information we've learned and can talk about such as facts and events. Information we cannot consciously recollect or talked about in detail is referred to as nondeclarative memory. These two different types of long term-memory systems will be the topic of the following sections.

1.3.1 Implicit Memory

Nondeclarative memories that guide our behavior without the ability to consciously recall or verbally discuss are referred to as implicit memory. This includes functions such as our stored knowledge of how to ride a bike, or the skills gained during mirror drawing training (Figure 1 A). The implicit memory processes have been found to depend on structures outside of the medial temporal lobe. These types of functions were spared in H.M. after his surgery, as he was observed to significantly improve at a mirror drawing following repeated training despite declaring that he had never performed the task in his life. Nondeclarative memory falls into the category of various skillful behaviors such as habits or conditioning, and is generally unaffected in amnesic patents as some of these functions are supported by regions of the neocortex (Squire, 2004). Multiple studies, including research from both animal and non-human primate studies have examined the functions of implicit memory processes and found similarities spanning across test subjects (Squire, 1992). The striatum, a subcortical region of the

5

forebrain, is believed to be responsible for functions of procedural memory whereas the cerebellum, with the support from the amygdala, provides neural substrates for simple classical conditioning.

1.3.2 Declarative Memory

The term "memory", as commonly used in everyday language, generally refers to declarative memory. Like stated above, long-term memory we consciously recollect and discuss in a representational way is believed to depend on function of regions in the medial temporal lobes and especially the hippocampus (H. Eichenbaum, 2000; Squire, 1992). Declarative memory can further be broken down into semantic and episodic memory, dissociating memories for facts from the events that occur in our lives (Figure 1 B). Memories for facts or information potentially acquired through effortful conscious recollection independent of life events are referred to as semantic memory (H. Eichenbaum, 2000). Episodic memories however, provide the ability to mentally re-experience a past life event in the original context, although this process is, in addition to the hippocampal region, thought to be highly dependent upon frontal lobe function (Squire, 2004). Long-term episodic memory that depends upon the hippocampus and surrounding structures will be the main topic of this dissertation.

1.4 Anatomy of the Hippocampal Circuit

Long-term declarative memory in humans and episodic memory in other mammals depend critically upon a circuit of interconnected structures within the medial temporal lobe. The hippocampal formation; consisting of the dentate gyrus, CA3, CA1 and the subiculum, makes up a complex pathway of circuits with input from regions of the neocortex through the entorhinal cortex (Lavenex & Amaral, 2000) (Figure 2). The

formation or encoding of new declarative memory depends on the flow of multimodal sensory experiential information through the hippocampal formation. Cortical association regions such as the perirhinal (PER) and postrhinal cortices (POR) receive the input and then differentially distribute the information through the parahippocampal region. Specifically, the PER. a region involved in visual object perception and familiarity (Brown & Aggleton, 2001) sends projections to, and receives input from the lateral entorhinal cortex (LEC) whereas the POR sends projections to, and receives input from, the medial entorhinal cortex (MEC) (Andersen, 2007). The lateral and medial entorhinal cortices are thought to receive different inputs from two distinct pathways, respectively the "what" and "where" pathways with those information streams converging in the hippocampus. These pathways receive distinct representational inputs. The LEC receives nonspatial information consisting of olfactory, auditory and visual object/item information whereas the MEC receives and processes mostly spatial and idiothetic information (Hunsaker, Chen, Tran, & Kesner, 2013). These regions then project the spatial and non-spatial information to the hippocampus. The trisynaptic loop, a well-characterized pathway projecting information through the hippocampal formation (dentate gyrus, hippocampus proper and subiculum) receives axonal projections from the medial and lateral entorhinal cortices, terminating on granule cells of the dentate gyrus (Lavenex & Amaral, 2000), sometimes referred to as the gateway to the hippocampus. The mossy fibers, the projecting axons of dentate granule cells, distribute the information onwards to pyramidal neurons of area CA3 of the hippocampus proper. The axons of the CA3 neurons, termed Schaffer collaterals distribute to pyramidal cells in the CA1 area of the hippocampus. Neurons in the CA1 region then project output from the hippocampal

formation through the subiculum onwards to the entorhinal cortex again, completing this prominent circuit within the hippocampal formation (Andersen, 2007). The presubiculum of the hippocampal formation also projects to entorhinal cortex which in turn, projects again to the dentate gyrus and the CA3 through layer II and the CA1 and subiculum through layer III. (van Strien, Cappaert, & Witter, 2009). These structures and detailed pathways have been identified as key regions for creating and processing episodic and semantic memories.

1.5 cFos as a marker for memory

Memory is thought to form when neurons create new connections or strengthen previously existing ones through long-term potentiation (LTP). Although LTP will not be discussed in detail within this dissertation, it is important to mention that forming new long-term memories in the form of synaptic plasticity is highly dependent on LTP and protein maintenance (Fonseca, Vabulas, Hartl, Bonhoeffer, & Nägerl, 2006). Although interfering with protein deletion, or the removal of proteins associated with inhibiting memory formation, during LTP negatively affects synaptic strength, more research has focused on exploring mRNA and protein synthesis contribution to neuronal plasticity resulting in long term memory (Igaz, Vianna, Medina, & Izquierdo, 2002; Kang & Schuman, 1996). Shortly following a specific event that will be consolidated, immediate early genes (IEG's) are transcribed in the neurons that were active during the event. These genes then get translated into proteins contributing to strengthening or changing synapses in a specific way to permit retrieval later on. Since these IEGs are short lived within neurons following activation, IEGs have become the ideal marker to look for in order not to just determine what brain regions were active, but what specific neurons

8

were affected by a specific behavioral task. The IEG cFos is commonly used to determine neuronal activation, whereas the Arc IEG has becomes an appropriate marker of synaptic plasticity. Much like most of the behavioral and electrophysiological tasks discussed below, staining for IEG markers such as cFos following an event is a well-established and common tool when studying the functions of rodent memory.

1.6 Spatial Memory and the Hippocampal formation

A vital attribute of episodic memory is the location where the event occurred. Spatial memory allows one to associate a location to a specific event or a feature. Learning about new environments and associating the contextual information with an occurrence or navigating around different settings is highly dependent upon proper function of the hippocampus, both in humans as well as rodents. Rodents will quickly learn the location of a specific place where they receive reward or to avoid aversive stimuli by associating the goal location with available environmental cues. Spatial memory is commonly studied by utilizing various mazes and small mammals, such as rodents. One of the more common mazes used to study spatial memory in rodents is the radial arm maze. This task tests working memory for recently visited places by measuring visits to a region containing a food reward (Olton & Samuelson, 1976). The radial arm maze consists of eight long arms radiating out from a central platform (Figure 3a). Each arm is baited with a small food reward which rodents collect while in the maze. Successful performance of the task is inferred when the rodent only enters each arm once. Repeated entrances into an arm indicate impairments in working memory for having previously visited that location. This task mostly places demand on working memory, which is short term memory for objects, stimulus or locations used within a single testing

session and does not compare performance between days (Dudchenko, 2004). Although the radial arm maze tests working memory, successful performance is dependent on spatial memory as the rodent needs to determine which locations have been visited. Previous work has demonstrated that rats do rely on extra-maze landmark cues when solving the task (Suzuki, Augerinos, & Black, 1980) indicating that the global reference frame where the arena is placed is an important factor to determining which arms have been visited. In a slightly different variation of this task, rats were repeatedly exposed to the radial arm maze with the same four arms baited and the others left empty. Rats with hippocampal lesions not only demonstrated impairments in their working memory, by repeatedly entering a previously visited arm, but also made more spatial memory errors (Jarrard, 1983). Together, experiments conducted using the radial arm maze support the notion that memory for locations is hippocampal dependent but as many other behavioral tasks; the radial arm maze has limits when it comes to assessing rodent navigation.

Another more common experimental tool used to study spatial memory and navigational strategies in rodents is the Morris water maze. The Morris water maze assesses spatial memory using a circular pool filled with opaque water where rodents must associate the location of a submerged hidden platform with escaping the water, by using external visual cues (Figure 3b). Rodents can easily be trained to find the platform location and later tested for the retention of the spatial memory by removing the platform and measuring the time spent searching in the appropriate area of the pool. Morris et al. (Morris, Garrud, Rawlins, & O'Keefe, 1982) showed that rats don't just recognize the correct location of the platform when they finally reach it, but they learn to efficiently navigate directly to it from a novel start location; essentially creating a new short cut to

the location of the platform. Morris et al. concluded that efficient performance in the water maze required a hippocampal-dependent spatial or cognitive map of the pool, containing the platform location relative to the surrounding visual cues. When the hippocampus is permanently lesioned prior to training, rats are significantly impaired at learning to use the distal cues to locate the hidden platform (Morris et al., 1982). When a selective AMPA antagonist that blocks glutamate receptors is chronically infused into the dorsal CA1 of hippocampus rats during training, rats show impaired acquisition as measured by significantly longer escape latencies (Riedel et al., 1999). The AMPA antagonist bocks fast glutamatergic synaptic transmission in order to disrupt normal hippocampal functioning, resulting in comparable behavioral performance to rats with lesioned hippocampi. These results indicate that the hippocampus is required for the encoding and/or consolidation of the spatial representation to form new spatial memories. When the hippocampus of rats successfully trained to navigate to the platform is temporarily inactivated before a water maze testing session, rats demonstrate spatially localized searching behavior but not in the appropriate location (Riedel et al., 1999), same findings have been reported when mice are tested under the experimental conditions (Nicola J. Broadbent, Squire, & Clark, 2006). Taken together, these findings indicate that a functioning hippocampus is necessary in order to retrieve information about a given location, whereas the rules about searching strategies are likely to depend on a different area of the brain. In addition, there appears to be a functional dissociation between the dorsal and ventral regions of the hippocampus when it comes to memory processes. When parts of either dorsal or ventral hippocampus of rats are lesioned, findings suggest that the dorsal region is mainly important for spatial memory acquisition in the water

maze but navigational impairments seem to be positively correlated to dorsal hippocampal volume damage, indicating impairments with spatial memory retrieval (Moser, Moser, & Andersen, 1993).

It is fairly well established that the hippocampus is necessary to encode, consolidate, and retrieve spatial memory; however, the degree of which retrieval of remote long-term memory depends on the hippocampus has been up for debate. A case study of patient E.P. exploring spatial memory following hippocampal damage revealed that although he was incapable at creating new spatial memories, he could very accurately recall the layout of his childhood neighborhood (Teng & Squire, 1999). This finding suggests that following an unknown time after a spatial memory is consolidated, retrieval of that memory is no longer depends upon the hippocampus. Furthermore, this finding could also indicate that spatial memories can be stored in a secondary region and retrieved without hippocampal contribution, or that brain activity during childhood development contributes to where spatial memory can be stored. Future research using case studies could aim at exploring sensitive periods for acquiring spatial memory during childhood, spared following hippocampal damage in adults.

Many tasks, other than the famous Morris water maze, have been developed to study spatial memory, both using rodents and other species. The Hebb-Williams maze was designed to test navigational memory in rodents and Kveim et al. (Kveim, Setekleiv, & Kaada, 1964) utilized it to test rats with partial or nearly total hippocampal volume loss. Their results indicated that rats with cortical control lesions significantly outperformed the rats with total or partial hippocampal lesions. The authors did however note, that rats with total hippocampal lesions made significantly more errors than the rats

with partial lesions of the hippocampus. Taken together, these findings provide additional support for the positive relationship between spatial memory performance and proportion of the hippocampus functionally intact. Furthermore, differences between the specific regions of the hippocampus responsible for encoding and retrieving long-term spatial memories may be anatomically distinct.

1.6.1 Spatial Memory Neuronal Correlates

Neuronal correlates of spatial memory were first discovered in 1971 by O'Keefe and Dostrovsky while recording action potentials of single hippocampal cells in freely-moving rats. Place cells are specific pyramidal neurons within the hippocampal formation that fire action potentials at a high frequency corresponding to animal's physical location within an environment. These cells remain silent when the animal is out of the cell's so called "place field" but when the animal returns to that specific area the neuron generally fire at same location at similar Hz, which is commonly referred as the cell demonstrating a stable place field (J. O'Keefe & Dostrovsky, 1971). Place cells demonstrate complex-spike activity and generally fire in correspondence to the most prominent cue in the environment (Muller, Kubie, & Ranck, 1987). A common way of determining if a hippocampal complex-spiking neuron contains a place field within an environment is by conducting *cue-card rotations*. This is done by recording activity from the cell within an environment containing a prominent cue. The animal is then removed for a short duration and the cue rotated to a certain degree before a second recording of the same cell. This process is then repeated for a final recording, in which the cue is returned to its original position. If the cue exerts stimulus control over the position of the firing field for all three sessions, the neuronal activity is commonly referred to as place cell firing. These

replicable and well-defined characteristics of hippocampal place cells provide robust support for the role of hippocampal neurons in spatial memory, (John O'Keefe & Nadel, 1978). For example, it is likely that when the dorsal hippocampus is temporarily inactivated prior to Morris water maze testing, as in Reidel et al., (Riedel et al., 1999), the resulting navigational impairments are a consequence of compromised place cell function. Hippocampal place cell recordings are commonly carried out after surgically implanting micro-array tetrodes into the hippocampus of freely moving rodents. Following recovery the wires can extracellularly detect action potentials from cell bodies of hippocampal principal neurons and along with location tracking, heat maps can be calculated showing the location of the animal where the place cell is most active (Figure 3c).

The development of the tetrode permits simultaneous recordings of many individual neurons and such ensemble activity recordings have yielded abundant support for the notion that place cells are the main component of spatial mapping within the hippocampus (M. Wilson & McNaughton, 1993). The discovery and research of place cells have provided a sound explanation for the mechanism supporting successful spatial navigation, and a justification for impaired navigation in hippocampus-lesioned rodents.

The cognitive map theory states that hippocampal place cells provide an internal map of the environment and offer the ability to create novel routes to familiar locations as long as the map remains stable The proposed theory suggested that the hippocampus and the place cell system represent the global record of experiences, including spatial and non-spatial elements (John O'Keefe & Nadel, 1978). When a rodent enters a novel recording arena; different from a familiar one, the hippocampal representation transforms

in an unpredictable manner, creating a new spatial map resulting from differences in the active subset and characteristics of individual hippocampal neurons (Bostock, Muller, & Kubie, 1991). The process of remapping occurs when a separate set of place cells fire within a different environment in a different pattern (Fyhn, Hafting, Treves, Moser, & Moser, 2007). As an example, the combination of place cells recorded in a cylindrical environment "remap" when the animal is placed into a square arena or a linear track. Some of the cells previously active in the cylinder may fire in an unpredictable location in the square arena while other cells become silent and other cells previously silent in the cylinder might be engaged. It is of interest to determine how long place cells can remain consistent firing in a given space with repeated exposures as this could give some insight to how spatial memories develop or change over time. Although some have debated whether stable long-term place field activity in rodents is observable, stable place fields have been demonstrated to last up to 153 days when recorded from rats (Thompson & Best, 1990). Furthermore, new technology has provided researchers with the tools to monitor stable place cell activity for over 11 weeks at a time using Ca^{2+} sensitive dyes and a head mounted camera (Ziv et al., 2013).

Place cells have been studied extensively over the years, but they are not the only cell type contributing to successful spatial navigation. Recordings from other limbic regions have shown neuronal activity with spatial correlates which collectively are likely to support spatial navigation. Hippocampal place cell activity is influenced by grid cells found within the medial entorhinal cortex (Brun et al., 2008; Fyhn et al., 2007), as well as by head direction cells found in the postsubiculum (Taube, Muller, & Ranck, 1990), anterior thalamus (Taube & Burton, 1995; Yoganarasimha & Knierim, 2005) and the

15

lateral mammillary nuclei (Stackman & Taube, 1998). Grid cell firing properties resemble place cell activity except they fire in multiple locations in a hexagonal pattern within a given environment. Lesions of the entorhinal cortex have been shown to disrupt firing fields of established place cells (Brun et al., 2008). Further, simultaneous recordings from the entorhinal cortex and hippocampal place cells in rats have demonstrated direct input from the medial entorhinal cortex to CA3 and CA1, with high modulation of grid cell activity on that of place cells. Although hippocampal place cells are only one synapse upstream from the entorhinal grid cells via a direct pathway and, grid cells in the entorhinal cortex also depend on reciprocal connections from the intact hippocampus in order to maintain stable place fields. Infusion of the GABA-A agonist muscimol into the CA1 region of the rat hippocampus, impaired the structured spatial firing properties of grid cells for minimum of 150 min (Bonnevie et al., 2013). When a rodent is placed in a novel environment; their place cells are observed to remap, and grid cell activity correspondingly shifts to represent the spatial differences within the environment. Taken together, these findings indicate that grid cells and place cells provide the basis for location-specific information processing to maintain a stable spatial map of the environment.

Although grid cells and place cells provide information for a spatial map, directional information is not highly represented within their firing properties. Head direction cells fire in accordance to the rodent's head direction in the horizontal plane independently of the animal's specific location within an environment. These neurons are controlled by a complex combination of proximal and distal environmental cues and have been shown to mediate the control of distal landmarks of place fields. Both head direction

cells (Stackman & Taube, 1998) and hippocampal place cells (Stackman, Clark, & Taube, 2002), are highly influenced by input from the vestibular system and repeatedly disorienting the animal can promote place cell remapping even in a familiar environment (Knierim & Kudrimoti, 1995). Both the presubiculum and the anterior thalamic nuclei project to and modulate hippocampal CA1 place cells, and likely that information contains head direction information (Calton, Stackman, & Goodridge, 2003). Taken together, place cells, grid cells and head direction cell activity contributes to the animal's ongoing perception and recognition of space in order to maintain a single representation of location and direction while moving around an environment.

Although we have yet to fully appreciate the spatial navigational system, recording from the dorsal CA1 of the hippocampus is the ideal location for various reasons. First of all, this region is the main output of the hippocampus; projecting directly to the subiculum as well as other cortical areas. Secondly, the rodent dorsal hippocampus is positioned close to the skull's surface, allowing for relatively minor cortical damage to surrounding areas (George Paxinos, 2004). Less than 1 mm layer of somatosensory cortex is between the dorsal surface of the hippocampal formation and the skull and only a small region of that endures damage from recording wires during surgical implantation. Recording from other regions such as CA3, entorhinal cortex and surrounding areas of the medial temporal lobe are likely to result in more damage to cortical areas due to the electrode implant. Damage to surrounding brain tissue should be kept minimal in order to limit the potential effects on the natural behavior of the animal and potential communications with the neurons within the recoding area of interest.

1.7 Non-Spatial Object Memory and the Hippocampus

To fully justify using rodent models to study functions for the basics of human memory, we must define the role of the rodent hippocampus in non-spatial memory to ensure that the structure functions in similar manner to the human hippocampus (Cave & Squire, 1991). The novel object recognition task (NOR) tests episodic memory for objects, through a well-established protocol that can easily be modified to answer specific questions (Antunes & Biala, 2012). The NOR test is typically carried out in a simple, familiar environment, using objects that the animal gets to freely explore for a relatively short period of time, and then later tested for recall or recognition of those objects. An initial object session (Sample) is often carried out with two identical objects in separate locations within a familiar arena. When the animals explore the two objects equally, we infer that they recognize them both as novel and interesting. The following session (Test) is conducted in the same environment with one of the previously encountered objects present, while the other one is replaced with new object; importantly, object placement within the arena remains consistent. The animal freely explores both objects and the amount of time exploring each one is carefully recorded (For typical NOR protocol see Figure 4). Rats and mice are naturally curious and they tend to explore new objects more than ones they are already familiar with (Ennaceur & Delacour, 1988). An animal that fully encoded and consolidated a memory of the sample objects should be able to retrieve that object memory during the test session and spend more time exploring the unfamiliar item, whereas an animal that did not successfully learn about the sample objects cannot then retrieve that memory and will explore both objects equally during the test session.

The novel object recognition task has narrowed down two main brain regions believed to play an important role in object memory. The perirhinal (PER) cortex has been identified as being critical when it comes to object memory in monkeys (Buffalo, Ramus, Squire, & Zola, 2000) and rodents (Winters & Bussey, 2005), but many disagree and have concluded that the hippocampus plays that role (N. J. Broadbent, Gaskin, Squire, & Clark, 2010; J. R. Clarke, Cammarota, Gruart, Izquierdo, & Delgado-Garcia, 2010; Cohen et al., 2013). There is a possibility that these contradicting conclusions are due to differences in experimental protocols, but the reality is that likely both previously mentioned brain regions play a vital role in creating and recollecting object memories. The "what" pathway carries unimodal sensory information (object feature) from the visual cortex to the PER cortex, that information then is conveyed to the hippocampus through two distinct pathways that will not be discussed in detail here. It is very possible that when the PER cortex is temporarily inactivated, such as in Winters & Bussey (Winters & Bussey, 2005), rodents lose the ability to perceive visual information about the explored objects, and therefore are unable to match and retrieve the hippocampal dependent memory of whether this is a previously seen object or not. The observed behavior will therefore be comparable to an animal exploring a novel object. It is of great importance to identify the specific functional contribution of a region to the memory process at hand in order to draw the appropriate conclusions from observed animal behavior during cued memory retrieval. Extracellular activity has been recorded from the rat PER cortex demonstrating that approximately 38% of neurons respond to objects, although this activity is not object specific or modulated by experience (Burke et al., 2012). Since new memories cause changes in neuronal firing properties these results

indicate that it is not the PER cortex alone that contributes to object memory, but rather may be vital for object perception.

Over 200 studies have explored hippocampal role in object memory using both permanent lesions and temporary inactivation of the structure. Although roughly half of these studies don't support rodent hippocampal involvement in object memory, the other half does provide support and there appear to be some emerging trends that indicate when the hippocampus is involved. It is beyond the scope of this dissertation to specifically evaluate the papers published regarding the matter, but review papers by Squire et al. (Squire, Wixted, & Clark, 2007) and Cohen et al (Cohen & Stackman Jr, 2015) have provided some excellent theories. Squire et al. (Squire et al., 2007) proposed that methods that separated familiarity and recollection were in fact separating weak memories from strong memories. Memory strength can determine what medial temporal lobe region processes the information at hand. Stronger memory is associated with increased hippocampal activity, whereas the weaker memory does not engage the hippocampus and is likely supported by surrounding cortical areas, such as the PER. Strong Memory that engages the hippocampus is suggested to be recollection based, where recalling previously experienced stimuli without it being cued at that time is possible. Weak memory has been suggested to be recognition based, where the memory would not be retrievable without the cued stimuli. This type of familiarity based memory has been suggested to be dependent on the adjacent PER cortex (Brown & Aggleton, 2001). What determines memory strength is yet to be clearly defined but there is strong evidence that time spent acquiring information regarding the to-be-remembered item is greatly important. Cohen & Stackman (Cohen & Stackman Jr, 2015) suggest that a

minimum time criterion should be implemented for active exploration during the NOR task to ensure proper amount of information is obtained in order for the memory to become recollection based rather than just a familiar one. This notion is further supported by human fMRI data collected during encoding and retrieval of specific memories of various strengths (Squire et al., 2007). Taken together, it is likely that both the hippocampus and the PER cortex are critical for object memory, although their functional contributions may vary greatly.

Although abundant research has been carried out where object discrimination has been impaired following temporary inactivation of the dorsal CA1 region of the hippocampus in rodents (Julia R. Clarke et al., 2008; Cohen et al., 2013) enhanced performance has been reported as well (Oliveira, Hawk, Abel, & Havekes, 2010). The underlying difference that may be contributing to these conflicting results is how familiar the rodents are with the testing environment. The enhanced object memory following hippocampal inactivation was only observed when mice had only once experienced the testing environment, but object discrimination was at chance when they had become familiarized with the context for 5 consecutive days (Oliveira et al., 2010). It is possible that the lack of object memory impairments following hippocampal inactivation in the 5-day habituation group is because of low time spent exploring during the sample, and therefore the familiarity memory of the objects would've been PER cortex dependent, hippocampal inactivation would therefore not be expected to impair object discrimination. The authors conclude that contextual information processing can interfere with object memory consolidation and the observed enhanced object discrimination following a one day context habituation and hippocampal inactivation immediately after

21

the sample session, is a result of object memory not being hippocampal dependent (Oliveira et al., 2010). These findings could also be explained by the idea that the weak object memory did not become hippocampal dependent, and the enhanced object discrimination of hippocampal muscimol treated mice is a result of PER cortex familiarity signals for the familiar object. With adequate contextual habituation and criteria on minimum object exploration during the sample session, hippocampal inactivation would be expected to interfere with object memory retrieval during the test session.

The results from the studies discussed above all seem to indicate that non-spatial object memory seems to be processed by cortical regions before becoming hippocampal dependent. The duration for which this type of memory is dependent on hippocampal memory has not been well established but as mentioned above, patient E.P., who was able to recall spatial memories acquired 50 years earlier; hippocampal dependent object memory is perhaps relocated to other brain areas when it has been fully consolidated. Recent work exploring the duration of hippocampal object memory investigated NOR performance following lesions performed much later. Rats received hippocampal lesions 1 day, 4 weeks, or 8 weeks following a sample session and were tested two weeks after the surgery. During the test session, only the 1 day and the 4 week consolidation groups were significantly impaired compared to their control counterparts. Rats that had their hippocampus intact for 8 weeks following the sample session performed as well as control rats. To ensure these results weren't due to spared or functional hippocampal tissue, the rats were tested again 3 hours following another sample session. All rats with hippocampal lesions were significantly impaired (N. J. Broadbent et al., 2010). These

data support time-specific dependence of both spatial and non-spatial object memory within on hippocampus. Although it is not yet clear whether spatial and non-spatial memory depends on the hippocampus for the same duration or specifically what that duration is, it's likely that hippocampal dependent memories only require the structure for a time sensitive period, likely longer than several weeks in rodents, before being permanently stored in other regions.

1.7.1 Object-in-Context Representation

Teasing apart non-spatial items from spatial ones can be very difficult in research. Objects do contribute to our surroundings –landmarks can be difficult to mentally separate from the environment (i.e., the context), yet recognition of items seems effortless regardless of the environment. Attempting to test non-spatial object memory specifically proves difficult since objects can never be encountered without context. One of the largest critiques of the NOR task is that rodents usually encounter the objects in the same familiar arena, and some would argue that it is simply the "object in location" or "object in context" memory that is impaired when the CA1 area of the hippocampus is inactivated or lesioned (Hardt, Migues, Hastings, Wong, & Nader, 2010). When designing experiments to test hippocampal dependence of non-spatial object memory, it would be expected that the optimal testing arena would contain limited environmental cues, in order to minimize hippocampal activation due to the spatial information. Cohen et al., (Cohen et al., 2013) took a different approach and examined hippocampal dependent object memory independent of context by presenting mice with the same sample session objects each day for 10 min in distinct environments for 3 consecutive days. During the test session, presented in another distinct context on the 4th day, control

mice preferentially explored the novel object. When the hippocampus was temporally inactivated via muscimol infusion into the dorsal CA1 region prior to the test session, mice did not preferentially explore the novel object (Cohen et al., 2013). This result indicates that even when object memory is dissociated from a specific context (i.e., truly non-spatial), it remains dependent upon the hippocampus.

Previous work has demonstrated that changing the location of a familiar object within a familiar arena results in its increased exploration (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002), supporting the notion that the rodent notices that something has changed regarding that object and since they are curious creatures, they explore the novelty further. In addition, a novel object in a familiar location would also be explored widely, whereas that same novel object in a novel location would be explored more extensively within a familiar environment (Manns & Eichenbaum, 2009; Mumby et al., 2002; Save, Poucet, & Foreman…, 1992). Experiments specifically aimed at dissociating object memory from the memory of the context found that CA1 place cell activity is not altered when objects are placed into a familiar arena, yet when the region is temporarily inactivated, impairments in NOR are observed (Ásgeirsdóttir et al., *in prep*). These findings indicate that although both spatial and non-spatial object information are processed by the hippocampus, the location of a given object is likely processed independently, but perhaps simultaneously, to the object identity itself. Rodents have the ability to learn to associate stable landmarks/objects with a rewarded location and successfully navigate to that location when only one of three landmarks is present (Collett, Cartwright, & Smith, 1986). Despite this result, rodents seem to lack the ability to associate a location with a specific object when it's presented in multiple locations

within a context. Rats were successfully trained to search for food reward in a location predicted by two distinct objects, but were unable to correctly navigate to the goal location when the objects were presented in a different location within the geometric reference frame (Biegler & Morris, 1993). Taken together, these findings indicate that it is the stable geometric organization of landmarks, and not their specific identity that contributes to successful navigation. Despite the notion that landmark identity is not crucial to goal driven navigation, identity of the landmarks is processed by the hippocampal formation.

1.7.2 Object Related Neuronal Activity

Previous work has aimed to identify hippocampal neuronal activity for non-spatial items, much like place cell code for location within an environment. Several studies have recorded place cell activity in the presence of 3D objects. Cressant et al., (Cressant, Muller, & Poucet, 1999) concluded that the place cell system must be capable of using object identity to anchor the location of specific place fields. They recorded from place cells within the dorsal CA1 in rats in a cylindrical arena containing 3 objects positioned in an isosceles triangle along periphery. Their findings indicated that objects only influence the place cell system when placed near the periphery of the arena and not towards the center (Cressant, Muller, & Poucet, 1997). A different study recorded place cells within the dorsal CA1 region of rats running on a circular track, with or without several objects; found no significant place field remapping between sessions, with or without an object. Place fields close to an object remained stable even when the object was exchanged for a different one; however, some of the CA1 neuron firing patterns were sensitive to a particular object, or to the combination of certain objects in a specific

location (Burke et al., 2011). Although this activity was limited to complex spiking location-specific pyramidal neurons, it is possible that their firing properties were affected by other neuronal types within the CA1 region. Consistent with the previous findings, Manns & Eichenbaum found no specific object activity within the dorsal CA1, independent of location, but they did find variable activity due to different objects in the same location (Manns & Eichenbaum, 2009). A different study from the same lab reported finding increased item-location firing frequencies following repeated training during a behavioral task; however, this report only included activity from complex-spiking neurons. Notably, throughout training, no differences in the firing properties of stable place cells were detected. This finding would further suggest that object-specific activity within the dorsal CA1 region modulates the spatial map over time, or across training (Komorowski, Manns, & Eichenbaum, 2009).

More recently, landmark-vector cells recorded from dorsal CA1 and CA3 regions of the rat hippocampus have been reported (S. S. Deshmukh & Knierim, 2013). These pyramidal cells develop fields at a specific distance and direction from objects introduced into a familiar arena. Remarkably, landmark-vector cells can retain their firing fields after the object is removed or relocated between sessions as well as follow the object to a new location while continuously firing at locations where it was previously found. This unique discovery supports the notion that object memory is retained within hippocampal neurons for some time with location as a significant contribution to its identity. Notably, the above mentioned studies explicitly report activity from complex-spiking neurons where other activity is not discussed. Taken together, these findings indicate that there are likely neurons within the rodent hippocampus that process object identity distinct

from the objects location, those neurons may demonstrate different firing properties than what we expect to see from location-specific firing neurons such as place cells.

1.8 Movement and the hippocampus

Movement is inherently spatial in nature, organisms depend on moving around for food and mating, but we must also navigate around other moving entities. In the simplest terms, movement can be defined as *velocity = distance* (location change) */time*. By these components, movement should heavily engage the hippocampus.

The hippocampus is believed to be a structure where time, space, and items are integrated into an episodic memory. Previous work has shown that rats can be trained to memorize the temporal order of presented odors in return for a treat, but following a hippocampal lesion trained rats lose the ability to perform the task (Kesner, Gilbert, & Barua, 2002). Rats with hippocampal lesions also lose the ability to recall the order of specific odors when presented in specific locations (Ergorul & Eichenbaum, 2004). Time sensitive cells have been recorded from the CA1 region of the rat hippocampus. Similar to place cells, thee neurons fire relative to sequence memories and "retime" much like place cells "remap" (MacDonald, Lepage, Eden, & Eichenbaum, 2011). Although "time" processing and the sequence preferring time-cells have been shown to play an important role when it comes to hippocampal dependent memory of item-order or the sequence stimuli is presented in (Eichenbaum, 2014); the specifics of time information processing will not be discussed in further detail within this dissertation. Taken together, previous findings have indicated that the rodent hippocampus organizes experiences of spatial and temporal elements of episodic memories, but we have yet to clearly define how non-spatial aspects get integrated into those memories. Teasing apart time and space within

rodent memory research can be just as difficult as trying to isolate an object memory from the context it's presented in.

All of the behavioral and electrophysiological work discussed above has been carried out in stationary environments, measuring only the rodents' movement through space and/or neurological data. Although we have gained valuable knowledge about long-term memory and how the rodent hippocampus functions from such experiments, the attempt to separate non-spatial object memory from contextual memory by isolating the two factors, instead of asking if or how they are integrated within the structure. As mentioned above, it is difficult to show a to-be-remembered object to a rodent without presenting it within a specific location in a testing arena. Presumably, during those situations, a memory of the object-in-context and/or object-in-location is formed within the hippocampus and surrounding structures and during a specific testing manipulation or region inactivation; we look for behavioral or neuronal firing changes. Experiments have been carried out using a clear arena that is continuously rotating, where rats must learn to avoid a specific room based location in order to avoid a foot shock. The active place avoidance task presumably causes the rats to have two spatial reference frames, one of the rotating arena and another of the testing room. When neural activity of the dorsal hippocampus is temporarily inactivated, rats spend increased time within the room based avoidance zone (Cimadevilla, Wesierska, Fenton, & Bures, 2001). These results are consistent with experiments assessing hippocampal function in navigational tasks such as the Morris water maze, and further support the vital role of the rodent hippocampus in navigation.

Additional studies have utilized the active place avoidance task and found that an intact hippocampus is required for rodents to coordinate information from the two dissociated spatial reference frames (Kelemen & Fenton, 2010). During this experiment, rats were trained two avoid two locations, one within the clear rotating arena and the other in a fixed room based location. Following a unilateral infusion of the potent neurotoxin Tetrodotoxin (TTX) into the dorsal hippocampus, rats lost the ability to avoid the room based location while in the rotating arena, but their behavior was unimpaired when the arena was stationary. Furthermore, CA1 place cell activity recorded from rats trained in this task showed that the place cell system anchored to either spatial reference frame at a time, rapidly switching between the two. The place cells representing the spatial reference frame that was most behaviorally relevant (arena or room) were observed to be active at any given time (Kelemen & Fenton, 2010). These findings not only support the vital role the dorsal hippocampus pays in spatial processing, but further demonstrate that more than one spatial reference frame can be active during a given task and that bilateral hippocampal function is required for spatial reference frame integration when the environment is moving. A different study tested avoidance of an object instead of location using comparable aversive stimuli (Telensky et al., 2011). Here, two groups of rats were trained to avoid a robot, either while it was moving around the environment or stationary. Bilateral hippocampal inactivation by a TTX infusion significantly impaired task performance. Rats trained to avoid the object while stationary did not show changes in avoidance behavior following the infusion (Telensky et al., 2011). The study concludes that the hippocampus is required for the dynamic process of flexible navigation, but possibility of impaired object recognition or object-in-location memory

disruption is not discussed. As mentioned above, hippocampal activity disruption does not impair the "rules of the game". Rats with their hippocampus temporarily inactivated will still search for a platform in the Morris water maze, just in the wrong location (Riedel et al., 1999). Therefore, it is not surprising that avoidance behavior in the stationary robot group was not affected by the TTX hippocampal infusion. The rats do not need to specifically identify the object, or where it is, they just need to remember the rules of not to get too close to something during the task.

Few studies have investigated the influence moving objects have on hippocampal neuronal activity but recordings from rat hippocampal CA1 place cells in the presence of a moving toy car demonstrate that when the object has salience, its movement can affect the hippocampal place map (Ho et al., 2008). In this study, two groups of rats were trained to walk a in order to receive a rewarding intracranial stimulation. One group received stimulation while following the moving toy car and coming within 20 cm of it whereas the other group had to walk 150 cm, independent of the car location. Although the authors of this study concluded that the hippocampal neurons did not develop a spatial reference frame anchored to the toy car, they found that when the car was paired with reward, place cells were highly influenced by movement and turning of the toy car (Ho et al., 2008). Similarly, a study conducted on place field activity in the presence of a second rat found that it wasn't simply the presence of the rat but the distance between the two that disrupted the place cell firing patterns (Zynyuk, Huxter, Muller, & Fox, 2012). It is probable that these observed effects are a result of attention modulation due to the behavioral task. Fenton et al. reported finding strong attention-like modulation of place cell activity, depending on what task the rats were trained to perform (Fenton et al.,

2010). Taken together, these findings indicate that although the place cell map is not largely affected by moving identities in the environment, hippocampal activity is affected by proximity to non-spatial moving identities, possibly due to exploratory behavior or attention modulation. It is unlikely that a separate spatial reference frame is anchored to a moving object since unstable "landmarks" such as the object used in typical object recognition studies have been shown not to be used to navigate (Chan, Baumann, Bellgrove, & Mattingley, 2012).

1.9 Fear, Anxiety and the Hippocampus

As previously mentioned, the hippocampus does play an important role in contextual memory but its activity is largely influenced by a fearful event. Contextual fear conditioning, where a specific environment is paired with aversive stimuli such as a mild foot shock, is impaired in hippocampal lesioned rats (Maren, Aharonov, & Fanselow, 1997). The lack of fear demonstration during testing is not due to problems with fear expression, but likely a result of inaccessible contextual memory. When emotional input from the amygdala projects to the hippocampus during a fearful event, such as fear conditioning, the consolidated memory becomes much stronger than memories formed during neutral events. Conversely, when LTP in the basolateral amygdala is impaired, using a gene knockout mouse (RasGRF), contextual fear conditioning is impaired, supporting the notion that input from the amygdala is required to associate the context the aversive stimuli (Maren, 1999). Temporarily inactivating the basolateral amygdala, by infusing muscimol prior to fear conditioning, reduced cFos expression by neurons in the dorsal hippocampus (Huff et al., 2006). Furthermore, hippocampal place cells have been shown to be directly affected by contextual fear

memories. Recordings from mouse CA1 neurons following a predator odor presentation within a familiar environment showed that following an exposure to fearful stimuli, the place field system remapped and remained highly stable for a significantly longer period of time compared to when no fearful stimuli was paired with a familiar context (Wang et al., 2012).

The hippocampus does play a role in anxiety. There is a functional subdivide between the dorsal and ventral hippocampus along the septotemporal axis. As previously stated, the dorsal part of the hippocampus is vital for spatial memory whereas there is growing evidence for the role of the ventral hippocampus in anxiety related behaviors (Bannerman et al., 2004). Rodents with impaired ventral hippocampi show reduction in anxiety during tasks such as elevated plus maze, a plus shaped arena with two arms in the open and two enclosed (Kjelstrup et al., 2002). Rodents tend to spend more time in the closed arms, but when given an anxiolytic, such as diazepam, increase time spent exploring the open areas. The anxiety processing within the ventral hippocampus is distinct from fear processing of the amygdala. Rodents with lesioned amygdala will not demonstrate reduced anxiety, although both the ventral hippocampus and amygdala seem to contribute similarly to fear conditioned freezing (Bannerman et al., 2004). Taken together, these data suggest that both the amygdala and the ventral hippocampus affect the dorsal hippocampus during fearful and anxious inducing situations within a specific environment.

1.10 Current Study: Purpose and Hypothesis

The main focus of this dissertation is to explore hippocampal involvement in object memory, and in particular, with respect to object movement and object identity

discrimination. Previous work examining hippocampal role in object memory has focused on object-in-location or object-in-context and have placed a high emphasis on eliminating the spatial aspect of the tasks at hand, either by reducing the contextual information presented, or ignoring the effects a context might have on a task. Here, we introduce movement in order to avoid presenting an object in a specific location while enhancing the spatial features associated to the object.

Chapter 2 examines temporary time duration of diminished neural activity following a local muscimol infusion into the dorsal CA1 region by using a custom made unilateral infusion recording electrode. Furthermore, Chapter 2 demonstrates new evidence for object specific neuronal activity within the CA1 region of the dorsal hippocampus of wild type mice. Chapter 3 examines object avoidance behavior, electrophysiological neuronal activity and immunohistochemical marker expression following multiple exposures to moving objects with a focus on the CA1 region of the dorsal hippocampus and supporting structures. Findings from an elevated plus maze experiment following a dorsal hippocampal inactivation are also presented. The fourth and final chapter will discuss the findings in the light of existing literature with focus on how the results from the current studies provide further support for the involvement of CA1 region in non-spatial object memory.

The main hypothesis of this dissertation states that non-spatial object memory is highly dependent on the dorsal hippocampus in rodents but that acquisition of such memory is dependent upon a stable context memory for some time. Additionally, once consolidated, retrieval such hippocampal dependent object memory is not tied to specific contextual map. Furthermore, hypotheses state that moving objects affect the

hippocampal neural circuit in a different manner compared to stationary objects but these differences cannot simply be explained by alterations in spatial mapping, but rather by a parallel hippocampal non-spatial information processing.

PART II: OBJECT RELATED FIRING IN CA1 OF THE RODENT HIPPOCAMPUS

2.1 Abstract

Research into the function of the rodent hippocampus has demonstrated a clear role for the dorsal hippocampus in spatial memory. Both behavioral impairments following hippocampal inactivation and *in vivo* recordings of place cells provide consistent evidence for the notion that neuronal activity within the dorsal hippocampus supports functions required for successful navigation. The hippocampus does receive two major sources of cortical inputs: projections from the medial entorhinal cortex provide spatial/contextual information, while projections from the lateral entorhinal cortex convey item/object information. More recent work has also demonstrated that this structure is vital for the support of non-spatial object memory, yet singe cell recordings have not yielded clear correlates with non-spatial memory functions. Memory for objects has been shown to be sensitive to a temporary inactivation of the dorsal hippocampus, yet we have little evidence for types of changes to neuronal activity occur following commonly used drug infusions. The current question was regarding what happens to the activity of the neurons within the CA1 region following a local infusion of muscimol? Furthermore, I set out to answer the question whether there are neurons within the CA1 region of the dorsal rodent hippocampus that respond at the location of 3D objects? Here, I determined that all recorded CA1 hippocampal neuronal activity is completely silenced for several hours following a local infusion of muscimol into the dorsal CA1 region of the hippocampus. Furthermore, I recorded CA1 neuronal activity from the rodent dorsal

hippocampus while mice freely explored empty arenas and while exploring objects once placed into a familiar arena. I found a subset of CA1 neurons exhibiting object-related firing when the mice were actively engaging in exploration of novel and familiar 3D objects. Together, these findings support the notion that the rodent hippocampus is highly engaged in processing object-related information and indicate that this activity is disrupted following a temporary inactivation of the region, resulting in observation of behavioral impairments.

2.2 Introduction

The medial temporal lobe, including hippocampus and associated regions, is essential for spatial and nonspatial aspects of episodic memory in mammals (H. Eichenbaum, Yonelinas, & Ranganath, 2007; Morris et al., 1982; Squire, Stark, & Clark, 2004). The hippocampus receives two parallel but interrelated streams of information: spatial orientation/self-location from the medial entorhinal cortex, and external cues, items and objects from the lateral entorhinal cortex (Knierim, Neunuebel, & Deshmukh, 2014; Lisman, 2007; van Strien et al., 2009). The hippocampal cognitive map represents locations where relevant items or objects were encountered, and where specific events occurred within a contextual or spatial reference frame (John O'Keefe & Nadel, 1978). Temporary and permanent lesion studies have established that the hippocampus is required for encoding, consolidating, and retrieving spatial memory (Morris et al., 1982; Riedel et al., 1999). Further, individual principal hippocampal neurons of the CA1 and CA3 regions represent place by firing at distinct rates when a rodent occupies different spatial locations (J. O'Keefe & Dostrovsky, 1971; M. Wilson & McNaughton, 1993). CA1 complex-spiking neurons are thought to also represent configural associations of

odor-in-location (Komorowski et al., 2009) and object-in-location (Burke et al., 2011; S. S. Deshmukh & Knierim, 2013; Manns & Eichenbaum, 2009); such representations are in keeping with predictions of the cognitive map theory. Temporary and permanent lesions of the rodent hippocampus also impair object recognition (OR) memory, under specific testing conditions (R. E. Clark, Zola, & Squire, 2000; Cohen et al., 2013; de Lima, Luft, Roesler, & Schröder, 2006; Hammond, Tull, & Stackman, 2004), for a review see (Cohen & Stackman Jr, 2015). Furthermore, when mice explore novel objects as compared to familiar objects, CA1 neurons fire at higher rates and glutamate efflux is increased in dorsal hippocampus (Cohen et al., 2013). These findings are consistent with reports demonstrating the contribution of the CA1 region of the rodent hippocampus to nonspatial memory (R. E. Clark et al., 2000; Pena, Pereira-Caixeta, Moraes, & Pereira, 2014; Riedel et al., 1999). To the contrary, there is also evidence that OR is spared following hippocampal lesions (Ainge et al., 2006; Mumby, Tremblay, Lecluse, & Lehmann, 2005; Oliveira et al., 2010; Winters & Bussey, 2005; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). Such conflicting results have led to the suggestion that hippocampal contributions to object memory processes depend on particular task requirements imposed (Cohen & Stackman Jr, 2015).

It has been argued that object memory impairments observed following CA1 inactivation result from disturbing recognition of the spatial or contextual attributes of the behavioral task; a view consistent with a hippocampal-dependent *object-in-context* memory (Bussey, Duck, Muir, & Aggleton, 2000; Good, Barnes, Staal, McGregor, & Honey, 2007; Mumby et al., 2002; Save et al., 1992; Winters et al., 2004). In the standard OR task, rodents likely encode an event memory comprising the "object-explored-within-

a-familiar-context", and arguably the CA1-dependence observed by many, may reflect the contextual attributes of the task. If the CA1 contribution to OR comprises the spatial/contextual aspects of the task, then one might consider the potential for context alterations to change the sensitivity of CA1 place cells to OR task-induced remapping, or to change the sensitivity of OR performance to CA1 inactivation. However, whether hippocampal-dependent object memory is guided by object-context configural representations formed by modifications of CA1 place cell activity as the object memory is encoded remains unanswered. Our prior report suggested that CA1 place cell activity established before object memory training was not altered by subsequent OR performance (Cohen et al., 2013), suggesting that object and context information may be dissociable, or that these processes involve functionally distinct hippocampal output streams.

In order to support the notion that a muscimol infusion into the hippocampus disrupts neuronal activity I recorded CA1 activity while simultaneously infusing muscimol into the adjacent region. Our results indicate that when the rodent hippocampus is temporarily inactivated using muscimol infusion neuronal activity is completely silenced and then recoveres 330 minutes following the infusion. I further report evidence of object-related firing of non-bursting CA1 neurons during object exploration recorded from freely moving mice. Our object-related activity may resemble recently reported activity recorded from the rat dorsal hippocampus (S. S. Deshmukh & Knierim, 2013).

2.3 Materials and Methods

2.3.1 Mice and surgery

Male C57BL/6J mice (7-10 wk old; Jackson Labs) were housed 1 per cage following the surgical electrode implant with ad libitum access to food and water. All procedures were conducted in accordance with NIH guidelines and were approved by the Florida Atlantic University's Institutional Animal Care and Use Committee. Surgical implantation of or tetrodes (n = 14) was completed when all mice were 8-11 weeks old, one week after acclimatization to the vivarium.

2.3.2 Electrode construction and implantation

A miniaturized custom-built microelectrode was constructed (adapted from (Stackman et al., 2002)) to carry out extracellular recordings from individual CA1 neurons in the mouse dorsal hippocampus. To determine the time course by which muscimol silences CA1 neuronal activity, the design of the electrode was further modified to accommodate a microinfusion guide cannula (constructed from 18 G metal tubing), thereby facilitating the infusion of drugs while simultaneously recording CA1 neuronal activity. A microdrive carrying four tetrodes (each tetrode comprised four 25-μm diameter Nichrome wires that were twisted together by spinning) was implanted directly above the right dorsal CA1 region, A/P - 2.0 mm, M/L + 1.5 mm, D/V - 1.1 mm from bregma in 1 mouse found to contain neurons demonstrating object-related activity, or A/P - 2.0 mm, M/L + 1.2 or 1.15 mm, D/V - 1.1 mm from bregma for 3 of 4 mice where neurons were shown to demonstrate object-specific firing. The microelectrode/microdrive assembly was anchored to the skull using miniature stainless steel screws (000-120, Antrim) and cold-curing dental acrylic (ColdPac, Chicago, IL).

Each mouse was administered buprenorphine (0.5 mg/kg, IP) after the surgery was completed and triple antibiotic ointment was applied to the wound. Mice were individually housed following surgery to protect the microelectrode implant. Beginning 7 days after surgery, mice were habituated to the recording arena and tetrodes slowly lowered (~ 5-20 μm/day) until neuronal activity from cells within the CA1 of the hippocampus was detected. To confirm the time course of muscimol's silencing effect on CA1 neurons, mice remained in their home cages throughout the experiment. Baseline activity was recorded prior to the drug infusion, during the infusion and in 5-min intervals after the infusion, for the following 5.5 hours.

2.3.3 Recording apparatus and protocol

During daily screening or recording sessions, the mouse was briefly restrained in order to mate the implanted microdrive/tetrodes assembly to the recording cable headstage that contained 16 unity gain operational amplifiers. Unit activity was then monitored while mice moved freely about the floor of the high-walled cylinder or square arenas. The positions of two light-emitting diodes (one red and one green LED) within the headstage and mouse behavior were acquired by a CinePlex video tracking system (Plexon Inc., Dallas, TX). Neuronal activity (amplified 10,000x, filtered at 150-8000 Hz and digitized at 40 kHz) was simultaneously recorded with a 16-channel MAP system (Plexon Inc., Dallas, TX). Spikes were discriminated and analyzed by manual and automatic sorting algorithms based on waveform characteristics using OfflineSorter software (Plexon v3.2.1). The putative CA1 pyramidal neurons were classified using the following criteria: 1) low baseline firing rate (< 15 Hz) and irregular firing pattern; 2) dominant short interspike interval (3-10 ms) by histograms showing a characteristic peak

at 3-5 ms followed by a rapid exponential decay; and 3) a waveform latency of > 300 µsec. The putative interneurons were classified as having relatively narrow waveforms (< 250 µsec) and high firing rates (> 5 Hz), and the interspike interval histograms exhibited a later peak and a much slower decay compared to putative pyramidal neurons. All interneurons were excluded from further analysis. Only units with clear boundaries and less than 0.5% of spike intervals within a 1-ms refractory period were included in the analyses. Neurons with location-specific firing and putative interneurons were excluded from further analysis.

2.3.4 Object-specific activity protocol

Hippocampal activity was recorded throughout multiple sessions in various arenas before, and after the introduction of objects. All recording sessions were 5-10 min long and contained either no objects, one object or two objects of similar size as those used during common OR tasks. Multiple different arenas and objects were used during the CA1 recordings. Once neuronal activity was clearly observed at the location of an object, I performed specific retrograde analyses to determine whether the object-related activity had been present during previous recordings. Then activity was recorded over as many days as possible, as long as object-related activity was detected.

2.3.5 Data analyses

For the simultaneous muscimol infusion while recording CA1 neuronal activity, firing rates for all cells were normalized prior to statistical analysis. Sorted CA1 pyramidal neuron spikes were combined with the position coordinates provided by Plexon CinePlex Editor and place x firing rate maps were constructed using NeuroExplorer (Version 3.266, Nex Technologies, Littleton, MA). Place x firing rate

maps (32 x 32 pixel matrices) were exported to MATLAB R2011b (Version 7.13.0.564), and noise filtered using a conservative smoothing technique. Autocorrelograms, perievent histograms and basic firing properties of the object-location cells were obtained using NeuroExplorer (Version 3.266, Nex Technologies, Littleton, MA) following cluster cutting and the construction of place x firing rate maps, as described above.

2.3.6 Histology

At the conclusion of *in-vivo* recordings, each mouse was deeply anesthetized with 5% isoflurane or ketamine (100 mg/kg, i.p.), and mice were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde before brain dissection. All brains were cryoprotected, then sectioned at 50 μm using a sliding microtome (Leica SM2010) with an automatically controlled freezing stage (Physitemp Instruments, Clifton, NJ). Tetrode placements were confirmed by examination of cresyl violet-stained sections under a light microscope. The data for any mice determined to have an inappropriately placed recording cannula were excluded from the analyses.

2.4 Results

2.4.1 Time course of muscimol-induced inactivation of dorsal CA1

Local neuronal activity was recorded (n = 6) before, and after muscimol, a GABA-A agonist, was infused (1 μg/μl) into the CA1 region of the dorsal hippocampus through a cannula positioned ipsilateral and adjacent to the tetrode array. Activity was recorded in 5-min epochs every 45-105 min (totaling 8 time points). For all of the neurons recorded, intra-CA1 muscimol induced a rapid decline in neuronal firing rates that lasted for hours (Figure 5). A Friedman Repeated Measures ANOVA on Ranks analysis on firing rates yielded a significant main effect of time ($\chi^2(39) = 181.76$, *P* <

0.01). By 320-324 min post-injection, firing rates had recovered to a level equivalent to pre-drug administration ($t(29) = 1.412$, *n.s.*). No attempts were made to determine whether place cell, object-related activity or interneurons were recorded.

2.4.2 Object-related firing

Activity from four dorsal CA1 cells, demonstrating a similar pattern of non-place related characteristics, was analyzed (for representative tetrode placement see Figure 6a). One CA1 neuron was found to fire at peak rates when the mouse was actively exploring objects during OR testing, while simultaneously recorded place cell place cells maintained consisted firing (Figure 6b). Three other object-related firing cells were subsequently identified from distal CA1 under different experimental conditions. The activity of these cells was not influenced by the location of objects, nor did these cells fire at above background rates when objects were not present in the arena (Figure 6c for representative place x firing rate maps). Autocorrelograms from these four cells demonstrate lack of complex spike activity, while indicating a more regular firing pattern (Figure 6d). Contrary to what has been reported for hippocampal place cells (Wang et al., 2012), these data suggest that the average in-field firing frequency of these "object cells" increased over time (i.e. recording days) while maintaining a baseline firing frequency of 0 Hz when no objects were present. A one-factor (time) repeated measures ANOVA was conducted on the average in-field firing frequency recorded over several days for the 4 "object cells" (Figure 6e), yielding a significant main effect of time ($F_{2, 5} = 37.72$, $P < 0.01$). Further, a similar analysis of 4 randomly chosen CA1 place cells (3 were recorded simultaneously with "object cells") (Figure 6f), yielded a non-significant effect of time ($F_{2, 4} = 0.94$, *n.s.*).

2.5 Discussion

The mammalian dorsal hippocampus contributes significantly to non-spatial memory. Non-spatial memory in rodents is likely supported by neurons exhibiting object-specific activity recorded from neurons in the distal and medial sub-regions of CA1, which receive direct input from the perirhinal and lateral entorhinal cortices (Naber, Witter, & Da Silva, 1999), which primarily convey item-related information (Graves et al., 2012; Jacobs & Schenk, 2003; Knierim et al., 2014; van Strien et al., 2009). The specific role that distal CA1 neurons play in object memory is not clear; however, here I report a subset of CA1 neurons with firing modulated by the exploration of objects independent of spatial location. These cells fired at higher rates when the animal was at the location of objects, in a manner unlike hippocampal complex-spiking pyramidal neurons, but were recorded simultaneously with typical place cells within the same region.

Previous work has reported CA1 neurons firing in proximity to items or barriers within specific contexts but these cells also demonstrated complex-spike activity; likely reflecting a place cell subtype (Rivard, Li, Lenck-Santini, Poucet, & Muller, 2004). Interestingly, our unique CA1 cells fired exclusively at objects, regardless of the object identity, possibly indicating that their activity represents a partial and incomplete representation of the object; much like a place field represents a sub-region within a specific context. Future studies could test whether the activity of a given object-specific cell fires concurrently for object-identity or is dependent upon input from the LEC or perirhinal cortex. In addition, the peak firing rates of these object-related cells increased across several object exposures, an experience-dependent effect that I and others have not

observed during repeated recordings of place cells (Table 1). That is, hippocampal place cells tend to maintain a variable, firing rate independent of the presence objects (Burke et al., 2011) (and see Figure 6f). CA1 place cells recorded while rats traversed a circular track with or without objects, demonstrate that place fields close to an object remain stable when an object is exchanged for a different one, yet some of the CA1 neuronal firing patterns are sensitive to a particular object, or to the combination of specific objects placed in a specific location (Burke et al., 2011). This finding suggests that there are cells in the hippocampus that process whole object identity distinct from object location.

A separate study reported that when rats are trained to complete paired context/location-specific item-reward associations, changes in CA1 and CA3 complex-spike activity were observed (Komorowski et al., 2009). Komorowski et al. reported that repeated conditional discrimination training resulted in increased item-location firing frequencies; however, only cells demonstrating complex-spike activity were reported. Notably, throughout training, there were no differences in the firing properties of stable place cells, but the firing location of some cells changed to represent the location that matched the goal digging locations (Komorowski et al., 2009). Therefore, it is plausible that item-location activity is reflected in the firing of object-specific neurons, which modulate the spatial map over time or across training.

Other studies have recorded CA1 activity recorded while rats are in the presence of objects and found neurons that discharge at a specific distance and direction from objects. These landmark-vector cells have been reported to maintain their firing fields after the object has been removed, or relocated between sessions, and can even follow the object to its new location while continuing to fire at locations where it had been found

previously (S. S. Deshmukh & Knierim, 2013). Furthermore, object location does appear to influence how information is processed by the hippocampus. Manns & Eichenbaum (2009) detected no object-specific activity, independent of location, when recorded from the dorsal CA1, but they identified different activity due to different objects within the same location as training progressed (Manns & Eichenbaum, 2009).

Place cell recordings conducted in the presence of objects have demonstrated that objects are only capable of influencing place cell stability when placed near the periphery of the arena and not placed centrally within the arena (Cressant et al., 1997). The hippocampal place cell system must be capable of using object identity to anchor the location of place fields (Cressant et al., 1999). Together, these studies have a few things in common. First, activity of hippocampal CA1 neurons is screened for complex-spike activity prior to further analysis, potentially eliminating signals from other, non-bursting neurons. Second, many of the findings of the above discussed studies report changes to the activity following intense training or multiple exposures to both the recording arena and objects used. Although training is often required in order to test specific behaviors, drawing conclusions from changes to the firing properties of few neurons when the function of the neuronal network within the structure is being altered should be done cautiously.

Interestingly, Graves and colleagues (Graves et al., 2012) characterized two distinct pyramidal cell types, referred to as bursting and regular spiking, within the CA1 and subiculum from acute hippocampal slice recordings. Graves et al. concluded that these distinct cell types process different types of information in parallel, with the bursting cells receiving inputs from the medial entorhinal cortex (spatial information),

46

and the regular spiking cells receiving direct projections from the lateral entorhinal cortex (non-spatial information) (Graves et al., 2012). Accordingly, our object-specific neuronal activity likely reflects regular spiking (non-bursting) pyramidal cell activity since these neurons did not exhibit complex-spike activity, a hallmark characteristic of CA1 place cells. Based on the previous findings stated above, it is not implausible that the variations observed in the firing properties of hippocampal CA1 place cells, following training where non-spatial items are combined with locations; are a result of changes to the firing frequency of the object-firing neurons. The currently reported consistent increase in firing frequency of object-cells recorded over time could provide an explanation to the findings in experiments discussed above. As the CA1 neural network receives input from distinct areas processing either spatial or non-spatial stimuli, we would expect to observe traces of both types of information within the structure. Whether these two types of information streams remain fully separate or get partially integrated remains up for debate.

Taken together, my reported object-specific activity provides electrophysiological evidence for direct CA1 object information processing and lends support to the view that spatial and non-spatial information is processed in parallel within the CA1 region of the dorsal hippocampus.

PART III: AVOIDANCE AND DISCRIMINATION OF MOVING OBJECTS

3.1 Abstract

The contribution of the rodent hippocampus to spatial memory and navigation has been studied extensively. Although there is growing evidence for the hippocampal contribution to non-spatial memory such as object recognition, some suggest that the impaired object memory observed following temporary inactivation of the hippocampus or CA1 specifically, is a direct result of inaccessibility to object-in-location memory. Here, I set out to answer the question of whether the hippocampal dependence of object memory is simply due to hippocampal processing of information about the spatial location of the to-be-remembered object. I address this question while utilizing temporary hippocampal inactivation, cFos immunohistochemistry, and *in vivo* hippocampal place cell recordings. In order to tease apart spatial and non-spatial processing of the hippocampus, I enhanced the spatial aspects of 3D objects by utilizing movement. By demonstrating that memory of objects while moving is equivalently impaired as that of memory of stationary objects, I provide novel evidence for hippocampal dependent object memory. Here, an Enemy Avoidance task, adapted from (Telensky et al., 2009) was used to ask whether avoidance of an object is dependent upon a functioning hippocampus or environmental manipulations. The current study further utilized a novel object discrimination behavioral task, Knowing Your Enemy. The task, adapted from Enemy avoidance, involved training mice to discriminate between two objects, and learn to avoid one of them, both while the objects were moving around the environment, or were

stationary. The current findings first demonstrate that object discrimination as required for the Knowing Your Enemy task, is impaired following hippocampal CA1 inactivation. Additionally, the findings suggest that learning to associate an object with a foot shock is context dependent, but once acquired, object memory can be retrieved independent of where it is presented. I further present evidence demonstrating that CA1 place cell activity is not altered in the presence of a moving object, indicating that the neuronal resources devoted to spatial processing are not transformed to process location of a given object. Lastly, the results of quantification of neurons expressing the immunohistochemical activity marker, cFos, strongly support the vital role that CA1 has in object memory retrieval, regardless of whether the objects presented are moving or not. Additionally, cFos activity counts reveal that information processing by brain regions thought to support object recognition exhibit drastically different cFos expression based on whether objects are moving or not. Together, the current findings provide support for hippocampal dependence of object memory retrieval, emphasizing that once fully consolidated; object memory is not context or location dependent.

3.2 Introduction

Early work examining avoidance learning has demonstrated that rats will avoid a specific location, or a visual cue, within an environment when associated with a mild foot shock. A study using female rats utilized a grid floor to deliver shocks when the animals were in a discrete location with a chamber, or when the rat approached a box placed into the chamber. When avoidance was tested 24-hours later, rats demonstrate comparable fear response to a location compared with a box, but when retested later, differences in avoidance were observed (Blanchard & Blanchard, 1970). The authors conclude that

49

shock objects differing in discriminability elicits difference in behavioral patterns following training. Furthermore, acquisition and the retention of a one-trial avoidance response has been shown to be impaired in hippocampal lesioned rats (Kimura, 1958). Rodents have been suggested to use different strategies to solve various avoidance problems, depending on whether a location or a cue has been associated with a noxious stimulus. When animals have been trained in an avoidance task that requires recognition of a location, hippocampal lesioned animals show a profound deficit, which has been linked to navigational impairments (Black, Nadel, & O'Keefe, 1977).

The function of the rodent hippocampus has been studied extensively with a focus on spatial cognition. Navigational tasks such as the Morris water maze (Morris et al., 1982) and radial arm maze (Olton & Samuelson, 1976) have proven to be valuable tools to study animal behavior along with electrophysiological single cell recordings exploring hippocampal pyramidal neuron activity support the notion that hippocampal function provides a flexible spatial map (J. O'Keefe & Dostrovsky, 1971). The conjunctive activity of hippocampal place cells (J. O'Keefe & Conway, 1978), single neurons that are highly active in distinct locations within an environment, are thought to provide animals with an internal map but when disrupted, cause impaired navigation (Riedel et al., 1999). Previous work has shown that individual place cells can be anchored to more than one spatial reference frame (Zinyuk, Kubik, Kaminsky, Fenton, & Bures, 2000) and can be driven by the behavioral task at hand (Kelemen & Fenton, 2010) as well as attention (Fenton et al., 2010). Studies have suggested that the hippocampal spatial map can anchor to other features such as landmarks and can be affected by objects in motion when behaviorally relevant (Ho et al., 2008).

The rodent hippocampus has also been deemed a vital structure for non-spatial object memory (Cohen et al., 2013; de Lima et al., 2006). However, many argue that the hippocampus does not support consolidation of object recognition memory (Forwood, Winters, & Bussey, 2005; Langston & Wood, 2006; O'Brien, Lehmann, Lecluse, & Mumby, 2006; Winters et al., 2004) but is only required for the spatial memory of the object location and not the object representation (Oliveira et al., 2010). One way of circumnavigating the issue of object-in-location is to attempt to create a memory of an object, independent of a specific location. A way of doing that is to introduce objects that move within the arena. Functional inactivation of the dorsal region of CA1 leads to impairments in avoiding a moving object following successful training (Telensky et al., 2011). Findings from the same study suggested that the hippocampus is not required to avoid a stationary object but this notion could be supported by previous studies showing that a hippocampal inactivation does not disrupt behavior related to the rules of the task. Following hippocampal inactivation, rodents trained in the Morris water maze demonstrate normal searching behavior but in an incorrect location (Riedel et al., 1999). In the case of object avoidance, mice are not required to identify the stationary object in the arena; they simply have learned to follow the rule not to approach it, a process that has not been shown to be hippocampal dependent (Riedel et al., 1999). The current sets of experiments were designed to test hippocampal involvement in discrimination and avoidance of moving objects. Here, I test my main hypothesis that the rodent hippocampus plays a crucial role in object memory, independent of location.

In attempt to address the main hypothesis, I first adapted the Enemy Avoidance (EA) task for mice. While performing the EA task, trained mice will avoid approaching a

fearful stationary object as well as actively move out of the trajectory of it when moving around the training arena. Mice are trained to do so over several days by delivering a mild foot shock when the distance between the mouse and the object drops below a certain measure. I then tested the hypothesis that successful object avoidance is sensitive to hippocampal inactivation; and that avoidance of a moving object is impaired when presented within a novel context. Furthermore, it was of high interest to determine whether acquisition of such avoidance is dependent upon a stable training context. Prior to addressing the hypothesis of hippocampal involvement in object discrimination, I tested the hypothesis that temporary inactivation of the dorsal hippocampus via muscimol infusion into the CA1 region does not result in increased anxiety levels in mice while freely exploring the elevated plus maze.

Next, I developed a behavioral task where mice were trained to discriminate between two objects and actively avoid one to prevent receiving a mild foot shock. Comparable to Enemy Avoidance, the Knowing your Enemy task (KYE) trains mice to acquire the memory of two distinct objects and associate one of them with a foot shock, both when stationary and moving around the environment. Hippocampal inactivation resulted in impaired object discrimination both while moving and stationary. Further, my results indicate that the memory formed for the objects during training was again highly tied to the training context but not dependent upon it. To test the remaining hypotheses, I quantified number of cFos expressing neurons both within the hippocampal sub-regions, basolateral amygdala, and lateral entorhinal cortex, following exposure to stationary or moving familiar objects. Here, the hypothesis was that the CA1 region yielded higher numbers of cFos positive neurons following such exposures to objects, compared to

52

exposures to an empty training context or control mice. Lastly, I tested the hypothesis that the hippocampal place cell system is not affected by the presence of moving objects by utilizing *in vivo* single cell recordings.

The findings demonstrated within this chapter support the notion that the rodent hippocampus is vital in not only processing object-in-context and object-in-location but also provides novel evidence for its role in processing object identity, required for object discrimination. Taken together, I conclude that the rodent hippocampal system not only processes object identity and contextual information simultaneously, but movement of objects affects activity within the hippocampal formation in a drastically different way compared to the way the typical stationary object setup does.

3.3 Materials and methods

3.3.1 Mice and surgery

Male C57BL/6J mice (7-10 wk old; Jackson Labs) were housed 1 (electrode) or 4 (behavior) per cage with ad libitum access to food and water. All procedures were conducted in accordance with NIH guidelines and were approved by the Florida Atlantic University's Institutional Animal Care and Use Committee. Surgical implantation of guide cannulae (n = 50) or tetrodes (n = 4) was completed when all mice were 8-11 weeks old, one week after acclimatization to the vivarium.

3.3.2 Cannula surgical implantation and infusion

Mice were surgically implanted with chronic bilateral guide cannulae (Plastics One, Inc., Roanoke, VA) above the CA1 region of dorsal hippocampus (A/P - 2.0 mm, M/L ± 1.5 mm, D/V - 1.1 mm from bregma; corresponding to intermediate CA1). The guide cannulae were mounted to the skull using skull screws (000-120, Antrim) and cold-

curing dental acrylic (ColdPac, Chicago, IL). Each mouse was administered buprenorphine (0.5 mg/kg, IP) after the surgery was completed and triple antibiotic ointment was applied to the wound. Behavioral testing began 7-10 days later to permit postoperative recovery. Each mouse received a "mock infusion" each day prior to training. The mock infusion procedure involved a brief restraint of the mouse, during which the protective cap and dummy internal cannula were removed, and dummy infusion cannulae inserted into each guide cannula. These dummy infusion cannulae did not project beyond the tip of the implanted guide cannulae. Once the infusion cannulae were inserted, the mouse was released into an empty polycarbonate mouse cage for the 3 min duration of the "infusion". Each mouse was again briefly restrained to remove the infusion cannulae, replace the dummy internal cannulae and protective cap, and then placed in a holding cage for 20 min until training begun. For the actual microinfusions, mice received bilateral (0.35 µl/side, 0.334 µl/min) intra-hippocampal muscimol (1 µg/µl in 0.9% saline, Tocris) or saline 20 min prior to avoidance testing.

3.3.3 Elevated Plus Maze

The elevated plus maze was comprised of two open and two closed arms (29 cm x 6.5 cm x 16 cm, l x w x h) extending from a common central platform (5 cm x 5 cm). The apparatus was positioned 115 cm from the floor. The central platform and the floor of the maze were white, while the side walls of the closed arms were black. Closed and open arms were evenly lit. A camera was mounted directly above permitted video recording of mouse behavior using Ethovision. Each mouse was placed in the maze for 5 min starting from the central platform facing away from the experimenter. Video recording began <1 sec after the mouse was placed into the apparatus and the times spent in the closed arms

and the open arms were measured using Ethovision 11. The plus maze was cleaned after each trial using 10% ethanol to minimize olfactory cues. Mice received a bilateral infusion of either Muscimol or saline directly to the CA1 region of the dorsal CA1 region 20 min prior to anxiety testing on the plus maze.

3.3.4 Histology

At the conclusion of behavioral testing and *in-vivo* recording, each mouse was anesthetized with 5% isoflurane and received 0.1 ml Euthasol IP injection before brains were dissected. Mice used for immunohistochemistry were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde before brain dissection. All brains from behavior and *in-vivo* recording were cryoprotected, then sectioned at 50 µm using a sliding microtome (Leica SM2010) with an automatically controlled freezing stage (Physitemp Instruments, Clifton, NJ). Cannulae and tetrode placements were confirmed by examination of cresyl violet-stained sections under a light microscope. The data for any mice that were determined to have inappropriately placed cannula were excluded from the analyses, see Figure 7a & b for representative intrahippocampal infusion sites within the CA1 region of dorsal hippocampus for all infusion experiments (EA & KYE one context CA1 inactivation, and EA & KYE multiple contexts CA1 inactivation) and representative photomicrograph of cannula placement.

3.3.5 Electrode construction and implantation

A miniaturized custom-built microelectrode was constructed (adapted from (Stackman et al., 2002) to carry out extracellular recordings from individual CA1 neurons in the mouse dorsal hippocampus. A microdrive carrying four tetrodes (each tetrode comprised four 25-µm diameter Nichrome wires that were twisted together by spinning)

was implanted directly above the right dorsal CA1 region, A/P - 2.0 mm, M/L + 1.5 mm, D/V - 1.1 mm from bregma for the place cell recordings. The microelectrode/microdrive assembly was anchored to the skull using miniature stainless-steel screws (000-120, Antrim) and cold-curing dental acrylic (ColdPac, Chicago, IL). Each mouse was administered buprenorphine (0.5 mg/kg, IP) after surgery and triple antibiotic ointment was applied to the wound. Mice were individually housed following surgery to protect the microelectrode. Beginning 7 days after surgery, mice were habituated to the recording arena and tetrodes slowly lowered (~ 5-20 μm/day) until CA1 neuronal activity was detected.

3.3.6 Recording apparatus and protocol

During daily screening or recording sessions, the mouse was briefly restrained in order to mate the implanted microdrive/tetrodes assembly to the recording cable headstage containing 16 unit gain operational amplifiers. Unit activity was then monitored while mice moved freely about the floor of the high-walled cylinder or square arenas. The positions of two light-emitting diodes (one red and one green LED) on the headstage were acquired by a CinePlex video tracking system (Plexon Inc., Dallas, TX). The position of a blue light emitting diode on the object was also tracked during neuronal recording experiments where a stationary or moving object was utilized. Neuronal activity (amplified 10,000x, filtered at 150-8000 Hz and digitized at 40 kHz) was simultaneously recorded with a 16-channel MAP system (Plexon Inc., Dallas, TX). Spikes were discriminated and analyzed by manual and automatic sorting algorithms based on waveform characteristics using OfflineSorter software (Plexon v3.2.1). The putative CA1 pyramidal neurons were classified using the following criteria: 1) low

baseline firing rate (< 15 Hz) and irregular firing pattern; 2) dominant short interspike interval (3-10 ms) by histograms showing a characteristic peak at 3-5 ms followed by a rapid exponential decay; and 3) a waveform latency of > 300 μsec. The putative interneurons were classified as having relatively narrow waveforms (< 250 μsec) and high firing rates (> 5 Hz), and the interspike interval histograms exhibited a later peak and a much slower decay compared to putative pyramidal neurons. All interneurons were excluded from further analysis. Only units with clear boundaries and less than 0.5% of spike intervals within a 1-ms refractory period were included in the analyses. Stability of place x firing rate maps was determined by computing a pixel-by-pixel Pearson cross-correlation analysis between the place x firing rate maps of two recording sessions. The average cell stability for each cell was calculated from the Pearson cross-correlation from the three cue card rotation sessions. Measures of spatial coherence and spatial information content were computed for each unsmoothed place x firing rate map using custom written MATLAB scripts. Correlation stability scores, spatial coherence and information content values were analyzed using parametric statistics in SigmaPlot 11.0 (Systat Software, Inc. SigmaPlot for Windows). Place cell activity in the presence of an object was manually coded from video files for times when mouse for "near" or "far" from the object and isolated for further analysis of spatial coherence and information content. The mouse was considered to be "near" the object when it was facing the object and less than ~5 cm away from it. The mouse was considered to be "far" from the object when in any location in the arena over ~10 cm away from the object, independent of whether its head was oriented towards the object or not.

Mice implanted with hippocampal tetrode arrays were initially screened in a cylindrical arena without objects in order to isolate CA1 units with location-specific firing properties. Once signals were detected the responses of putative place cells were recorded during a sequence of three 5-min cylinder or square arena sessions in which the cue card was rotated 90° counterclockwise from its 3 o'clock standard position, and then when the cue card was rotated back to its standard position (Figure 21a). These sessions were conducted to verify that the cue card exerted the expected stimulus control over the place cell's place field; place fields of cells were expected to rotate positions in the cylindrical arena by an amount approximating that of the rotated cue card (Muller et al., 1987). Within all of the cue card rotation sessions an object was placed into the arena, moving around or placed into a location corresponding to the location of the cue card (stationary). The mouse was removed for 2-4 min between sessions while the arena was cleaned with 10% ethanol and the cue card rotated for the subsequent session. Only place cells that showed complex-spike activity and demonstrated stable place fields during cue card rotations (r > 0.6 for the pixel-by-pixel cross-correlation comparison of the place x firing rate maps using the entire 300 sec recoding file) were included in all subsequent analyses.

3.3.7 Avoidance tasks

Enemy Avoidance (EA) and Knowing Your Enemy (KYE) were developed to train mice (and test) object avoidance/discrimination. EA task utilizes one object which is either stationary within the environment or moving around, alternating states between four 2 min sessions. When the object is moving, the mouse has to actively move out of the trajectory of the object in order to avoid a mild foot shock. For the KYE task, two

distinct objects are used. The mouse must identify the objects as different identities and learn to associate a foot shock with one of them (other remains neutral/no association). The training and testing parameters for both EA and KYE remain similar during these experiments, except the KYE paradigm has two objects that alternate between moving and stationary states (Figures 8 and 9). Further description of training and testing parameters are described below.

Open field white square arena constructed of white acrylonitrile butadiene styrene (ABS), measuring 37.5 cm x 37.5 cm x 50 cm high was used for all experiments. The arena walls were modified during experiments requiring contextual change using clear plastic liners and printed paper with various patterns. The floor was made of a galvanized steel metal sheet which would be kept slightly wet with DI water during training and testing to increase electrical conductance when shock was delivered. An overhead camera tracked the location and movement of the mouse and object/s within the arena using Ethovision 11 color detection. Custom written Python software delivered a mild foot shock (1 Hz) during the time when the distance between the mouse and the shock associated object dropped below 6 cm. A thin insulated electrical cable was attached to a copper hook that clipped onto a subcutaneous ear tag clipped into the scruff between the shoulder blades on each mouse (Kent scientific, Torrington, Connecticut 06790). Ear tags were placed in the shaved and cleaned scruff between shoulder blades while mice were under mild anesthesia (isoflurane). Antibiotic ointment was applied on the skin around the tag. Behavioral training begun 2-20 hours following the scruff implant. One or (Enemy Avoidance) two (Knowing Your Enemy) object/s (Hexbug nano (Innovation First Labs, Inc. Greenville, TX 75402) that were either kept stationary (off)

or randomly moved around the environment (on) were used to train and test object avoidance behavior. The arena floor was cleaned with 10% ethanol between subjects and lightly sprayed with DI water prior to the next mouse.

3.3.7.1 Habituation and training

For both Enemy Avoidance (Figure 8) and Knowing Your Enemy (Figure 9) tasks the procedures are as follows. On day one, mice received a 2-min empty context session where free exploration of the testing arena was allowed with no objects present. For Enemy Avoidance only one object was used for avoidance training and testing. For Knowing Your Enemy, two distinct objects were used for training and testing. Mice were trained to avoid one of the objects (bad) by receiving a mild foot shock when in close proximity whereas the other object remained neutral (good), with no foot shocks delivered upon close proximity. Each mouse remained in the arena while the object/s was/were lowered into the arena for a 2-min session (both objects stationary). Every time the Ethovision video tracking system detected that the distance between the mouse and the bad bug was less than 6 cm, a proximity event was registered, and mild foot shock would be triggered and delivered for the duration of the time the mouse were within 6 cm of the bad object. Proximity events, velocity, and thigmotaxis were recorded throughout all sessions. The bad bug was then turned on for a two-min "moving" session (while good remained stationary (Knowing your enemy only) (Figure 9b). Two more 2-min sessions were then carried out where the bad bug was stationary and then moving again (good moving and stationary (Knowing your enemy only)). On the following days of training, mice were brought into the same room and each placed into the same arena prior to the bug/s for four 2-min sessions, receiving two training bins per day (Figures 8c and 9c).

These bins consisted of two alternating "moving" and two "stationary" sessions for experiments including only one object (Enemy Avoidance, Figure 8). For experiments utilizing two objects (Knowing Your Enemy, Figure 9), the bins consisted of two four 2-min sessions where either object was moving while the other was stationary, alternating every two minutes. Shocks were delivered each time the distance between the "bad" bug and mouse was detected to be below 6 cm, while no shocks were delivered when the mouse was in proximity of the good bug, although proximity events were recorded for both bugs for future object discrimination analysis. Mice later to be tested in an experiment utilizing a familiar unpaired context were exposed to a 4-min session within an empty context prior to training, each day of the protocol, in order to permit familiarity to a neutral context.

3.3.7.2 Avoidance testing

Following training (6 first training bins), avoidance testing was conducted (bin 7). For experiments utilizing hippocampal inactivation, mice received a bilateral infusion/injection of muscimol (GABA-A agonist) directly into the dorsal region of the CA1 20 min prior to avoidance testing. For experiments testing contextual manipulations, arena alterations were conducted. Prior to testing, the shock component was deactivated so no mice received foot shocks during the testing and control bin (bins 7 and 9). This was done in order to test object avoidance behavior specifically, and eliminate the possibility that mice were simply avoiding the foot shock rather than the object associated with the fear memory paired with the foot shock. Aside from the testing manipulation (CA1 infusion, contextual change), both the testing bin (bin 7) and control bin (bin 9) were identical to the training bins except no shocks were delivered. Four 2-

min testing sessions ensued where proximity events, velocity, and % time spent thigmotaxic were recorded. The control bin (bin 9) was carried out 24 hours after testing, several hours after training bin 8. For experiments testing avoidance behavior following hippocampal inactivation, mice received a bilateral infusion/injection of saline directly into the dorsal region of the CA1 20 min prior to avoidance testing (control). For experiments testing contextual manipulations, arena conditions were kept identical to training (no change to training context). Prior to the control session, the shock component was deactivated so no mice received foot shocks. Statistical analysis of the acquired data was conducted using linear mixed-effects models to determine any changes in proximity events encountered following the experimental manipulation, while controlling for the velocity and thigmotaxis of each mouse.

3.3.8 Immunohistochemistry

One day after mice completed KYE training and testing, they were randomly assigned to one of four groups (cage control, empty context, stationary objects, moving objects). The mice in the cage control were not exposed to any additional experimental condition prior to euthanasia. Mice in the empty context group were handled identically to the days prior where they were brought into the testing room and placed into the training context where they had been trained and tested in a non-pharmacological manipulation KYE task. They remained in the empty training context for time duration corresponding one training/testing bin (4 x 2 min). Mice in the stationary object group were treated in the same manner as mice in the empty context group with the addition of the training objects being placed into familiar locations within the training arena. The objects were not touched by the experimenter or moved during the duration of the

session. Mice in the moving object group were treated in the same manner as the stationary objects group except they were exposed to a regular KYE bin where the objects alternated between moving and stationary conditions. No shocks were delivered during context or object exposures. Eighty minutes following the corresponding testing exposure (empty context, moving objects, stationary objects) mice were deeply anesthetized with Euthasol and transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde. Brains were then dissected and fixed in 4% paraformaldehyde for 3-5 days. Brains were then sectioned at either 20 (n=12) or 30 (n=8) μm using a cryostat microtome. Every third section was collected into a set so that each section was 60 or 90 μm apart from the previous, creating three sets of tissue from each brain.

One set of sections were stained using a standard immunohistochemical technique specifically targeting the cFos immediate early gene (Perrin-Terrin et al., 2016). The following standard protocol was used for staining. Day 1- tissue was soaked in 1% hydrogen peroxide. Endogenous peroxidase was then blocked with a solution of 0.3% triton and 5% normal goat serum solution in 0.1 M phosphate buffer. The tissue was then incubated in 0.1 M phosphate buffer containing cFos rabbit polyclonal antibody (1:500), and 3% normal goat serum and left rotating overnight. Day 2- tissue was washed with 0.1 M phosphate buffer and incubated in biotinylated goat anti-rabbit secondary antibody (1:200) and 3% normal goat serum and two hours later the tissue was washed and processed with avidin-biotinylated peroxidase enzyme complex (ABS, 1:40) in 0.1 M phosphate buffer. The tissue was then placed in diaminobenzidine (DAB), allowing for visualization of the chemical reactions before the tissue being placed in a final wash of

0.1 M phosphate buffer. Following the cFos staining procedure, the sections were mounted on gelatin-coated slides, counter stained with cresyl violet and cover slipped.

When slides had fully dried, stereological estimate of total neuron number expressing cFos within subregions of the hippocampus (CA1, CA3, DG), basolateral amygdala and lateral entorhinal cortex were estimated using the Optical Fractionator principle (West, Slomianka, & Gundersen, 1991) with Stereoinvestigator software (MicroBrightField Inc., Colchester, VT) on a Zeiss Axioplan microscope using a 100X oil-immersion lens. Total cell number (N) were calculated by using the formula $N = \Sigma Q- \times (t/h) \times (1/asf) \times 1/ssf$, where $Q-$ = total number of cells counted, t = section thickness, h = height of optical disector, asf = area of sampling fraction = a(frame)/a(x,y step) and ssf = section sampling fraction. Coefficients of error (CE) of the cell estimates were calculated according to Gundersen et al. (Gundersen, Jensen, Kieu, & Nielsen, 1999) and ranged between 1.5-5% within CA1, 1.6-6% in CA3, 1.2-3% in DG, 1.2-3% in amygdala and 1.2-7% in LEC. Using systematic random sampling scheme along with sections selected for volume measurements, neurons were quantified using an optical dissector 7μm deep, centered within the z-axis of the histological preparation to avoid knife errors and other biases. Each section was surveyed at equal sample distances (x,y step) by a motorized stage attached to the microscope that was under computer control. Neurons were counted by the unit counting method (i.e., neuronal nuclei), when they first came into focus, and for each x,y step, counts were derived from a known fraction of the total area by using an unbiased counting frame 40μm × 40μm in dimension and a grid size of 70μm × 70μm. Cell estimates were derived by multiplying the sum of the neurons that were counted by the reciprocal of the fraction of the layer that was sampled (derived from

the section sampling interval; x,y step size and section thickness). Using these sampling parameters, estimates were calculated in 169–1311 dissectors per animal. Mean estimated neuron numbers (±SEM) were calculated for each experimental group (Cage control, Empty context, stationary objects, moving objects) and compared by two-way ANOVA followed by post-hoc t-test comparisons.

3.4 Results

3.4.1 Enemy Avoidance

3.4.1.1 Experiments and rationale

To determine whether avoidance of an object, both moving and stationary is dependent upon a fully intact hippocampus I first replicated the approach of Telensky et. al (Telensky et al., 2011); using a modified Enemy Avoidance task with mice in place of rats. Instead of training two groups of mice to avoid a moving or stationary object, I trained all mice to avoid an object under both conditions. Following training, an infusion of muscimol into the dorsal CA1 region of the hippocampus impaired avoidance of an object both while stationary and while moving. After determining that avoidance of the object under both conditions is significantly impaired following a dorsal CA1 inactivation (Exp. 1a) I examined whether comparable results would be obtained following testing in a novel context (Exp. 1b). I conducted these manipulations with the prediction that if the behavioral impairments observed following hippocampal inactivation were simply due to the contextual memory being unavailable, a similar pattern of results should be obtained under both experimental conditions.

To further examine contextual dependence on object avoidance, while simultaneously eliminating the effects of novelty, I trained and tested mice in an unpaired

familiar context (Exp. 1c). For this experiment, mice were exposed to two arenas; a neutral empty context which they were exposed to each day prior to training, and the actual training context, where they learned to associate the object with a foot shock. After determining that moving object avoidance in a novel context is impaired but avoidance is fully spared within a familiar unpaired context, I was interested in testing object avoidance acquisition without a stable training context. I trained mice using the Enemy Avoidance protocol within multiple novel contexts in attempts to reduce the novelty effect (Exp. 1d). If mice are repeatedly encountering the same objects across different training contexts, then the avoidance rule might be acquired or generalized across contexts. Following training, mice avoided the object while stationary but were unable to do so when the object was in motion. I then inactivated the dorsal CA1 region via muscimol infusion prior to testing without any observed changes in avoidance behavior.

3.4.1.2 Training

In order to determine if mice in each experiment were successful at learning to avoid the object during training, I conducted linear mixed-effects regression models predicting the total number of proximity events during a training sessions 1-5, as a function of the total number of training bins prior to testing (i.e., did proximity events decrease with more training?). For each of the four Enemy Avoidance experiments mice experienced significantly fewer proximity events over the course of training under both the moving and stationary object conditions (see inserts in graphs in Figure 10) (for table with corresponding p-values see table 2). This result demonstrates that mice improved their behavioral performance between training bins 1-5 by learning to avoid the object both while moving and stationary.

To determine if performance of the mice in all experiments was equivalent following training and prior to testing, I conducted a linear mixed-effects regressions predicting total number of proximity events during training bin 6, controlling for animal velocity and % time thigmotaxic. In doing so, I first estimated a null model containing only velocity and thigmotaxis as fixed-effects predictors. I then compared this model to an alternative model that included dummy codes indicating the experimental conditions. In the moving object conditions, there was a significant difference between proximity events at training bin 6 as a function of experimental conditions ($\chi^2\Delta$ (3) = 16.99, p < .001). Post-hoc analyses indicated that mice in experiment 1d (Multiple contexts Muscimol) experienced more proximity events than mice in the other three experiments when the object was moving. This suggests that when mice were trained to avoid an object in multiple novel contexts, as in experiment 1d, they did not successfully learn to avoid the object while moving. In the stationary object conditions, there were no statistically significant differences in number of proximity events between the four experiments at training session six ($\chi^2\Delta$ (3) = 5.15, p = 0.16).

To determine if mice in all experiments maintained equivalent levels of training between testing, I conducted a linear mixed-effects regressions predicting total number of proximity events training bin 8, controlling for animal velocity and thigmotaxis. In doing so, I first estimated a null model containing only velocity and thigmotaxis as fixed-effects predictors. I then compared this model to an alternative model that included dummy codes indicating the experimental conditions. In the moving object conditions, there was a difference between proximity events at training bin eight as a function of experimental conditions ($\chi^2\Delta$ (3) = 40.48, p < .001). Post-hoc analyses indicated that mice in

experiment 1d experienced more proximity events than in the other three experiments. Post-hoc analyses also showed that mice in experiment 1b had more proximity events than those in experiment 1c, although this effect was relatively small (b = -1.94, p = .027). In the stationary object conditions, there were no statistically significant differences in proximity events at training session eight ($\chi^2\Delta$ (3) = 6.58, p = .09). Together, these data indicate that mice in all four experiments performed the Enemy Avoidance task equivalently prior to and after testing when the object was stationary. Furthermore, mice trained to avoid a moving object in multiple novel contexts cannot acquire the task but mice in the other three experimental conditions successfully acquired and performed the task before and after testing equivalently.

3.4.1.3 Testing

In order to determine if the behavioral performance of each Enemy Avoidance experiment changed during testing, I used linear mixed-effects regression models to compare proximity events at testing bin 7 vs. control bin 9 after controlling for mouse velocity and thigmotaxis. To do so, I used contrast codes (.5 for test and -.5 for control) as indicators for each session. These codes allow us to directly compare the average number of proximity events at the two sessions with the regression coefficient (b) reflecting the difference between the two conditions. I allowed this effect to vary across mice (random effect).

For experiment 1a (One context Muscimol) I found a statistically significant difference in proximity events between bins 7 and 9 when the object was moving (b = 3.20, p = .042), with more proximity events during testing bin 7. I also found a statistically significant difference in proximity events between bins 7 and 9 when the

object was stationary (b = 1.52, p = .024), with mice experiencing more proximity events during bin 7 (Figure 10a). These results indicate that CA1 neuronal activity is required to avoid a familiar object within a familiar context, not only when the object is in motion.

For experiment 1b (Novel context) I found a statistically significant difference in proximity events between bins 7 and 9 when the object was moving (b = 2.33, p = .029), with more proximity events during testing bin 7. There was no statistically significant difference in proximity events between bins 7 and 9 when the object was stationary (b = .28, p = .58) (Figure 10b). These results indicate that mice are unable to avoid a familiar moving object when encountered in a novel context but the environmental change has no effect on avoiding the object while stationary. Since the average amount of proximity events received during training bin 7 appeared considerably higher compared to what was seen during bin 9, I conducted an additional MANCOVA comparing proximity events with only velocity as a covariate within experiment 1b. A visible increase in velocity was observed during the first stationary session of the test during bin 7 during this experiment. The test did not yield a significant difference in proximity events between bin 7 and 9 after controlling for velocity ($F_{1, 21}$= 0.1, p=0.76). This result further supports the findings from the original analysis that when the increase in velocity is accounted for, mice do not experience more proximity events within the novel context, compared to the training context.

For experiment 1c (Familiar context) there was no statistically significant difference in proximity events between bins 7 and 9 when the object was moving (b = -.90, p = .32), or stationary (b = -.48, p = .31). These results indicate that successful object avoidance performance can be retained between two familiar contexts, even if mice have

never encountered or been trained to avoid the object in the familiar unpaired context (Figure 10c). Together with the results from experiment 1b (Novel context change), these findings indicate that a familiar spatial map is required to avoid a familiar moving object, yet stationary object avoidance is not as sensitive to environmental manipulation.

For experiment 1d (Multiple contexts Muscimol) I found no statistically significant differences in proximity events between testing bin 7 and 9 when the object was moving (b = -.43, p = .62) or stationary (b = .57, p = .18) (Figure 10d). These results suggest that a local inactivation of CA1 region does not alter the behavioral performance during the task, even after the successful avoidance acquisition of the object when stationary and unsuccessful avoidance acquisition of the object in motion.

Lastly, since velocity of mice during the testing manipulation (context/infusion) was occasionally observed to increase when the object was stationary, I ran a two-way ANOVA comparing velocity measures between bins 7 and 9 across the four experiments. The ANOVA yielded a non-significant main effect of experiment ($F_{3, 200}$= 2.06, p=0.11) but a significant main effect of testing bin ($F_{1, 200}$= 34.95, p<0.001) where Holm-Sidak post hoc tests indicated that bin 7 had higher velocity compared to bin 9. Furthermore, the ANOVA yielded a significant interaction between the variables ($F_{3, 200}$= 3.03, p=0.03) where corresponding post hoc analysis indicated that within bin 7, mice in experiments 1b (Novel context) and 1d (Multiple contexts Muscimol) had significantly higher velocity compared to experiment 1c (Familiar context). These results indicate that when a novel identity (context/state) is introduced, locomotion behavior can be altered without directly affecting avoidance behavior. Under the moving object condition, mice were observed to move significantly faster and more consistently throughout the

70

experiments compared to when the object was stationary. This observation is likely due to the notion that mice move around more when they are required to actively avoid a moving object. As a result of this ceiling effect and since velocity change has been accounted for as a covariate in the proximity event analysis above, additional comparisons of velocity with a moving object would be unnecessary.

3.4.2 Elevated plus maze and hippocampal inactivation

To ensure that the impaired object avoidance observed following CA1 inactivation was not a result of reduced expression of anxiety I utilized the elevated plus maze to measure levels of anxiety following such an inactivation. In order to determine if muscimol infused into the dorsal region of CA1 affected anxiety levels I conducted an elevated plus maze test 20 min following the CA1 inactivation. I examined whether there was a difference in time spent within the closed and open arms based on which treatment the mice received by conducting a t-test on the square root values of the % time spent in open arms (Time in open arms/Time in all arms) (Figure 11). The test yielded a non-significant effect on time spent following a muscimol (M= 0.64, SD= 0.15) or saline (M= 0.56, SD= 0.05) infusion into the dorsal CA1 of the hippocampus t(16)=1.56, p= 0.14. Furthermore, I conducted a t-test on the square root value transformation of % of entries into the open arms. The t-test yielded a non-significant difference in % number of entries into open arms based on what treatment (muscimol (M= 0.50, SD= 0.14), saline (M= 0.40, SD= 0.08) was infused into the dorsal CA1 region of the hippocampus t(16)=1.92, p= 0.07. Lastly, to evaluate whether the intra-CA1 infusion of muscimol or saline affected locomotion of the mice during the test I conducted t-tests on the distance traveled and velocity measured during the test. The t-test comparing distance traveled

between the two treatment groups (muscimol M= 1782.7, SD= 422.4) (saline M= 1664.3, SD= 107.1) found no differences in distance traveled following the infusion t(16)= -0.66, *p*= 0.51. The second t-test yielded a non-significant effect on velocity measures following a muscimol (M= 6.33, SD= 0.88) or saline (M= 5.92, SD= 0.68) infusion into the dorsal CA1 of the hippocampus t(16)= 1.11, *p*= 0.28. These results indicate that there is no difference in levels of locomotion or anxiety measures of mice after receiving a microinfusion of muscimol or saline into the CA1 region of dorsal hippocampus.

3.4.3 Knowing Your Enemy

3.4.3.1 Experiments and rationale

To test the hippocampal dependence of object avoidance and discrimination, I infused muscimol into the CA1 region of the dorsal hippocampus in mice trained in the KYE task. In order to determine whether discrimination of moving or stationary objects is sensitive to hippocampal inactivation I infused muscimol into dorsal CA1 following successful avoidance training (Exp. 3a). Discrimination of the objects, both while moving and stationary was impaired following CA1 inactivation. Since the results of experiment 3a where CA1 inactivation impaired discrimination of objects both while moving and stationary, I tested discrimination within a novel context (Exp. 3b) with the prediction that if the behavioral impairments observed following the inactivation were simply due to inaccessibility to the contextual memory, comparable results should be observed in a novel arena. For experiment 3b, no pharmacological manipulations were made, yet the novel context had a drastic effect on object discrimination. When the objects were stationary, enhanced object discrimination was observed but while moving, discrimination of the objects was abolished.

To further examine contextual dependence on object discrimination and reduce the effects of novelty, I tested trained mice in an unpaired familiar context (Exp. 3c). For this experiment, mice were familiar with two arenas; a neutral empty context which they were exposed to each day prior to KYE discrimination training, and the actual training context, where they learned to associate one of the objects with a foot shock. Findings from experiment 3c suggest that when mice are tested within a familiar unpaired arena, discrimination of objects is preserved both under moving and stationary conditions. Next, I was interested in exploring object association acquisition in the absence of a consistent context, and later testing the hippocampal dependence of such object memory. I trained mice using the KYE protocol within multiple novel contexts in attempts to reduce the novelty effect (Exp. 3d). If mice are constantly encountering novel environment under these conditions the expectation is that the experience of repeatedly encountering novel environments would no longer be novel. Although mice significantly improved at avoiding both objects throughout training, they were unable to discriminate between them during/after training under both moving and stationary conditions. I then inactivated the CA1 region of the dorsal hippocampus via bilateral muscimol infusion, which did not have an effect on avoidance behavior or object discrimination. Lastly, in order to further support the notion that anxiety does not play a large role in successful discrimination of moving or stationary objects, I tested trained mice in the KYE paradigm following an IP injection of the anxiolytic drug diazepam (Exp. 3e). For this experiment, mice were trained within one context and tested in the same context following an injection of diazepam. No changes in discrimination or avoidance were observed while the objects

were moving, in mice following treatment with diazepam compared to following treatment with saline.

3.4.3.2 Training

The statistical analysis of the training data for the five Knowing Your Enemy experiments will be described below. To determine changes in behavior during training I conducted linear mixed-effects regression models predicting the total number of proximity events during training bins 1-5, as a function of the total number of training sessions prior to testing (i.e., did proximity events decrease with more training?). For all five KYE experiments, I found a significant decrease in proximity events for both objects under both moving and stationary conditions during training (See table 3 for p-values), likely indicating that mice are both learning to avoid and/or are becoming habituate to the objects throughout training. In order to determine any differences in task performance between mice in the five experiments prior to testing I conducted a linear mixed-effects regression predicting total number of proximity events during training bin 6, controlling for animal velocity and thigmotaxis. In doing so, I first estimated a null model containing only Velocity and Thigmotaxis as fixed-effects predictors. I then compared this model to an alternative model that included dummy codes indicating the experimental conditions. I found no significant differences in proximity events to the good object while stationary ($\chi^2\Delta$ (4) = 3.28, p = .51) or moving ($\chi^2\Delta$ (4) = 1.31, p = .86) but detected significant differences in proximity events to the bad object while stationary ($\chi^2\Delta$ (4) = 31.11, p < .001) and moving ($\chi^2\Delta$ (4) = 30.82, p < .001) with post-hoc tests demonstrating higher values in experiment 4 (multiple context CA1 muscimol). These results indicate that mice trained in multiple novel contexts do not perform task equivalently to mice trained in a

single context. Mice trained within one context demonstrated equivalent performance during training, experiencing comparable amounts of proximity events to the bad object. Furthermore, to determine if mice were affected following testing and prior to the control bin, I conducted a linear mixed-effects regression predicting total number of proximity events during training bin 8 controlling for animal velocity and thigmotaxis. In doing so, I first estimated a null model containing only Velocity and Thigmotaxis as fixed-effects predictors. I then compared this model to an alternative model that included dummy codes indicating the experimental conditions. These findings were consistent with results from bin 6 where I found no significant differences in proximity events to the good object while stationary ($\chi^2\Delta$ (4) = 0.38 p = .984) or moving ($\chi^2\Delta$ (4) = 0.94, p = .918) but I did detect significant differences in proximity events to the bad object while stationary ($\chi^2\Delta$ (4) = 24.33, p < .001) and moving ($\chi^2\Delta$ (4) = 24.60, p < .001) with post-hoc tests showing higher values in experiment 4 (multiple context CA1 muscimol) compared to the other four KYE experiments.

3.4.3.3 Testing

The statistical results from each of the five different Knowing Your Enemy (KYE) experiments will be described below. To determine the extent to which mice learned to discriminate between the two objects prior to and during testing I conducted linear mixed-effects regression models using the number of proximity events mice encountered from each object (controlling for velocity and thigmotaxis) while moving and stationary during bins 6-9. Furthermore, in order to determine the effect the testing manipulation had on discrimination and avoidance behavior compared to the control session during each respective experiment I used linear mixed-effects regression models

to compare proximity events to either object at bin 7 vs. bin 9, both while moving and stationary. To do so, I used contrast codes (.5 for test and -.5 for control) as indicators for each session. These codes allowed me to directly compare the average number of proximity events at the two sessions with the regression coefficient (b) reflecting the difference between the two conditions allowing this effect to vary across mice (random effect).

Experiment 3a (One context CA1 muscimol)

A cohort of mice (n=12) previously cannulated and tested within the EA (one context, CA1 inactivation) experiment was trained in the KYE task. Prior to testing, muscimol was infused into the CA1 region of the dorsal hippocampus, and the shock generator was inactivated. Avoidance behavior of mice within a novel context was measured 20 minutes following the infusion. Avoidance behavior was then measured again 24 hours later within the training context where shocking generator was again inactivated, 20 minutes following an infusion of saline into the CA1 region of the dorsal hippocampus (control) (Figure 12).

-*stationary discrimination*: During bin 6 (pre-test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -1.92, p = .001) but during bin 7 (test) mice encountered comparable amount of proximity events to the bad object and to the good object (b = -1.49, p = .116). Mice encountered significantly less proximity events due to the bad object compared to the good object (b = -2.56, p < .001) during bin 8 (inter-test), and during bin 9 (control) mice encountered significantly less proximity events due to the bad object compared to the good (b = -2.28, p < .001). These results demonstrate that during the two training bins, before and after testing, as well as

during the control session, discrimination of the stationary objects was observed. Mice did not discriminate between the two stationary objects after the infusion of muscimol into the CA1.

-*moving discrimination*: During bin 6 (pre-test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -3.25, p < .001) but during bin 7 (test) mice exhibited comparable number of proximity events to bad object compared to the good object (b = -1.11, p = .167). Mice encountered significantly less proximity events due to the bad object compared to the good object (b = -3.79, p < .001) during bin 8 (inter-test), and during bin 9 (control) mice encountered significantly less proximity events due to the bad object compared to the good (b = -3.11, p < .001). These results demonstrate that during the two training bins, before and after testing, as well as during the control session, discrimination of the moving objects was observed. Mice did not discriminate between the two moving objects following the muscimol infusion into the CA1 region.

-*stationary Muscimol vs. Saline*: The analysis yielded no statistically significant difference in the number of proximity events to the good object when it was stationary during bin 7 vs. 9 (b = 1.33, p = .343) and a marginally statistically significant difference in of proximity events to the bad object when it was stationary during bin 7 vs. 9 (b = 2.12, p = .047) (Figure 12a). This result demonstrates that when the objects were stationary, the lack of discrimination following the muscimol infusion can be explained by the increase in proximity events experienced in the presence of the bad object.

-*moving Muscimol vs. Saline*: The analysis yielded no statistically significant increase in the amount of proximity events to the good object when it was moving during bin 7 vs.

bin 9 (b = 1.81, p = .057) and a significant increase in number of proximity events to the bad object when it was moving during bin 7 vs. 9 (b = 2.27, p = .025) (Figure 12b). This result demonstrates that while the objects were moving, the lack of discrimination following the muscimol infusion can be explained by the increase in proximity events experienced in the presence of the bad object.

Experiment 3b (Novel context change)

A novel cohort of mice (n=17) was trained in the KYE task. Following training, the shock mechanism was inactivated and avoidance behavior of mice placed into a novel context was measured. Avoidance behavior was then measured again 24 hours later within the training context and shocking mechanism inactivated (control) (Figure 13).

 -*stationary discrimination*: During bin 6 (pre-test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -1.88, p = .002) and during bin 7 (test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -3.16, p < .001). Mice committed significantly less proximity events to the bad object compared to the good object (b = -1.78, p = .003) during bin 8 (inter-test), but during bin 9 (control) no significant differences in proximity events due to either stationary object were detected (b = -0.88, p = .139). These results demonstrate that during the two training bins, before and after testing, as well as during the test session, following the novel context change, discrimination of the stationary objects was observed. Unexpectedly, mice did not discriminate between the two objects when stationary during the control session (bin 9). This finding is interpreted not as a lack of discrimination as a result of avoidance to both objects, but rather an effect of habituation to the highly familiar good object.

-moving discrimination: During bin 6 (pre-test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -3.76, p < .001) and during bin 7 (test) mice encountered significantly less proximity events to bad object compared to the good object (b = -1.79, p = .013). Mice encountered significantly less proximity events due to the bad object compared to the good object (b = -3.10, p < .001) during bin 8 (inter-test), and during bin 9 (control) mice encountered significantly less proximity events due to the bad object compared to the good (b = -2.43, p < .001). These results demonstrate that during the two training bins, before and after testing, as well as during the control session, discrimination of the moving objects was observed. However, when encountered in a novel context, the mice did not discriminate between the two moving objects.

-stationary Novel context vs. Training context: There was a statistically significant increase in the number of proximity events to the good object when it was stationary during bin 7 vs. bin 9 (b = 2.33, p = .014) but no difference in number of proximity events to the bad object when it was stationary during bin 7 vs. 9 (b = 0.60, p = .251) (Figure 13a). This result demonstrates that when the objects were stationary, the successful (and enhanced) discrimination following the novel context change can be explained by the increase in proximity events experienced in the presence of the good object.

 -moving Novel context vs. Training context: There was no statistically significant difference in the number of proximity events to the good object when it was moving during bin 7 vs. 9 (b = 0.26, p = .766) and no statistically significant difference in of proximity events to the bad object when it was moving during bin 7 vs. 9 (b = 1.64, p =

.072) (Figure 13b). This result demonstrates that while the objects were moving, the lack of discrimination following the novel context change cannot be explained by a drastic change in proximity events experienced in the presence of either object.

Experiment 3c (Familiar context change)

A novel cohort of mice (n=12) was trained in the KYE paradigm. Following training, the shock generator was inactivated and avoidance behavior was measured in a familiar context, in which the mice had never been exposed to the testing objects. Avoidance behavior was then measured again 24 hours later within the original training context with the shocking generator inactivated (control) (Figure 14).

-*stationary discrimination*: During bin 6 (pre-test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -1.57, p < .001) and during bin 7 (test) mice encountered significantly less proximity events to bad object compared to the good object (b = -1.75, p = .026). Mice encountered significantly less proximity events due to the bad object compared to the good object (b = -2.05, p < .001) during bin 8 (inter-test), but during bin 9 (control) no significant differences in proximity events due to either stationary object were detected (b = -0.59, p = .312). These results demonstrate that successful discrimination of the two stationary objects pas preserved during the two training bins, before and after testing, as well as during the test session, following the familiar context change. Unexpectedly, mice did not discriminate between the two objects when stationary during the control session (bin 9).

-*moving discrimination*: During bin 6 (pre-test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -3.73, p < .001) but during bin 7 (test) mice encountered comparable number of proximity events to the bad

80

object compared to the good object (b = -1.18, p = .20). Mice encountered significantly less proximity events to the bad object compared to the good object (b = -2.70, p < .001) during bin 8 (inter-test), and during bin 9 (control) mice encountered significantly less proximity events due to the bad object compared to the good (b = -1.93, p = .021). These results demonstrate that during the two training bins, before and after testing, as well as during both familiar context change and the control bin, discrimination of the moving objects was observed. This finding indicates that when familiar objects are presented in an unpaired familiar context, discrimination of the objects whether stationary or moving, is unaffected.

-stationary Familiar context vs. Training context: There was no statistically significant difference in the amount of proximity events to the good object when it was stationary during bin 7 vs. 9 (b = 1.13, p = .298) and no significant difference in proximity events to the bad object when it was stationary during bin 7 vs. 9 (b = 0.38, p = .377) (Figure 14a). This result demonstrates that when the objects were stationary within an unpaired familiar context, there was no change in number of proximity events experienced in the presence of either object supporting the observation of the successful discrimination.

-moving Familiar context vs. Training context: There was no statistically significant difference in the amount of proximity events to the good object when it was moving during bin 7 vs. 9 (b = 0.51, p = .645) and a marginally significant difference in proximity events to the bad object when it was moving during bin 7 vs. 9 (b = 2.12, p = .053) (Figure 14b). This result demonstrates that while the objects were moving within the unpaired familiar context, the successful discrimination following the familiar context

change was not a result of changes in number of proximity events experienced to either object.

Experiment 3d (Multiple contexts CA1 muscimol)

A cohort of mice (n=14) previously cannulated and tested within the EA (multiple context, CA1 inactivation) task was trained in the KYE task. Following unsuccessful training within multiple novel contexts, muscimol was infused into the CA1 region of the dorsal hippocampus, and the shock generator inactivated during the testing bin. Discrimination and avoidance behavior of mice within a novel context was measured 20 minutes following the infusion. Object discrimination and avoidance behavior was then measured again 24 hours later within a novel context where the shock generator was again inactivated, 20 minutes following an infusion of saline into the CA1 of the dorsal hippocampus (control) (Figure 15).

-*stationary discrimination*: During bin 6 (pre-test) mice did not encounter different amounts of proximity events to either object (b = 0.86, p = .311). Also, during bin 7 (test), mice encountered comparable number of proximity events to bad object compared to the good object (b = -1.41, p = .14). Mice did not encounter different number of proximity events to either object (b = -0.02, p = .977) during bin 8 (inter-test), and during bin 9 (control), no significant differences in number of proximity events to either stationary object were detected (b = -0.77, p = .22). These results demonstrate that during the two training bins, before and after testing, as well as following the muscimol (test) and saline (control) infusions, discrimination of the stationary objects was never observed.

82

-moving discrimination: During bin 6 (pre-test) mice did not encounter different number of proximity events to either object (b = -0.07, p = .933). Also, during bin 7 (test), mice made a comparable number of proximity events to the bad object compared to the good object (b = 0.21, p = .794). Mice did not encounter different number of proximity events to either object (b = 0.01, p = .994) during bin 8 (inter-test), and during bin 9 (control), no significant differences in proximity events due to either stationary object were detected (b = -0.68, p = .30). These results demonstrate that during the two training bins, before and after testing, as well as following the muscimol (test) and saline (control) infusions, discrimination of the moving objects was never observed. Despite the mice never successfully learning to discriminate between the good and the bad objects, their behavior was measured following the muscimol and saline infusions into the CA1 region of the dorsal hippocampus. This was done to ensure that no changes in avoidance or discrimination were observed while the CA1 region was inactive.

-stationary Muscimol vs. Saline: There was no statistically significant difference in the number of proximity events to the good object when it was stationary during bin 7 vs. 9 (b = 0.35, p = .750) and no significant difference in number of proximity events to the bad object when it was stationary during bin 7 vs. 9 (b = 0.85, p = .231) (Figure 15a). This result demonstrates that while the objects were stationary, the continuous unsuccessful discrimination following the muscimol infusion is a result of changes in proximity events experienced in the presence of either object.

-moving Muscimol vs. Saline: There was no statistically significant difference in the number of proximity events to the good object when it was moving during bin 7 vs. bin 9 (b = 0.62, p = .608) and no significant change in number of proximity events to the bad

object when it was moving during bin 7 vs. 9 (b = 1.33, p = .152) (Figure 15b). This result demonstrates that while the objects were moving, the continuous unsuccessful discrimination following the muscimol infusion is not a result of changes in number of proximity events experienced in the presence of either object.

Experiment 3e (Diazepam IP injection)

A subset of mice (n=9) previously trained in the KYE task and used in the novel context exposure experiment were tested following an IP injection of Diazepam. Following training, the shock generator was inactivated and object discrimination and avoidance behavior of mice placed in the familiar training context was measured 20 min following an IP injection of Diazepam. Object discrimination and avoidance behavior was then measured again 24 hours later following an IP injection of saline (control), where the shocking generator was again inactivated (Figure 16).

-*stationary discrimination*: During bin 6 (pre-test) mice did not encounter different number of proximity events to either object (b = -1.42, p = .161). Also, during bin 7 (test), mice encountered comparable number of proximity events to the bad object compared to the good object (b = -1.32, p = .153). Mice did not encounter different numbert of proximity events to either object (b = -1.09, p = .232) during bin 8 (inter-test), and during bin 9 (control), no significant differences in number of proximity events to either stationary object were detected (b = -0.52, p = .574). These results demonstrate that during the two training bins, before and after testing, discrimination of the stationary objects was observed. Following the Diazepam (test) and saline (control) IP injections; unexpectedly, successful object discrimination was not observed.

-moving discrimination: During bin 6 (pre-test) mice encountered significantly less proximity events to the bad object compared to the good object (b = -2.96, p = .007) and during bin 7 (test) mice encountered significantly less proximity events to bad object compared to the good object (b = -2.83, p = .006). Mice encountered significantly less proximity events due to the bad object compared to the good object (b = -2.51, p = .002) during bin 8 (inter-test), and during bin 9 (control) mice encountered significantly less proximity events due to the bad object compared to the good (b = -3.61, p < .001). These results demonstrate that during the two training bins, before and after testing, as well as following the Diazepam (test) and saline (control) IP injections, successful discrimination of the moving objects was observed. These findings indicate that the anxiolytic drug treatment did not affect object discrimination.

-stationary diazepam vs. saline: There was no statistically significant difference in the number of proximity events to the good object when it was stationary during bin 7 vs. 9 (b = 1.78, p = .252) and no significant difference in of proximity events to the bad object when it was stationary during bin 7 vs. 9 (b = 0.67, p = .411) (Figure 16a). This result suggests that while the objects were stationary, the lack of clear object discrimination following the diazepam injection was not a result of anxiolytic effects on behavior, but rather a result of the mice not demonstrating object discrimination during the last training bins and following the saline injection (bin9). Together, these findings indicate that object discrimination behavior does not change following a drug injection of an anxiolytic compound.

-moving diazepam vs. saline: There was no statistically significant difference in the number of proximity events to the good object when it was moving during bin 7 vs. bin 9

(b = 1.61, p = .21) and a non-significant increase in number of proximity events to the bad object when it was moving during bin 7 vs. 9 (b = 1.53, p = .096) (Figure 16b). This result demonstrates that while the objects were moving, the successful discrimination following the diazepam injection was not a result of change in proximity events experienced in the presence of either object.

3.4.4 Immunohistochemical findings

3.4.4.1 cFos positive neurons in dorsal hippocampus, BLA and LEC

In order to determine what neurons and brain regions were most active during the KYE task, I stained for cFos IEG following exposures to variations of the KYE task (cage control, empty context, stationary objects, moving objects). Estimated total number of cFos-expressing neurons within dorsal hippocampal regions, basolateral amygdala and lateral entorhinal cortex were quantified from mice (n=20) exposed to one of four experimental groups (Figure 17) (cage control, empty context, stationary objects, moving objects) (Table 4). I conducted a one-way ANOVA on the square root values of total cFos positive cells within the CA1 region of the dorsal hippocampus for the four exposure groups. The ANOVA yielded a significant effect of group ($F_{3, 16}$ = 26.62, p<0.001). The multiple comparisons procedures (Holm-Sidak) indicated that both stationary and moving objects groups had significantly higher numbers of cFos expressing neurons in CA1 compared to the control and context exposure groups, which did not differ in amount of cFos expressing neurons.

I then conducted a one-way ANOVA on square root values of estimated cFos positive cells within the CA3 region for the four groups. The ANOVA yielded a significant difference in cell counts between groups ($F_{3, 16}$ = 33.99, p<0.001) and the

multiple comparisons procedures (Holm-Sidak) indicated that all four groups differed in number of cFos-expressing cells. The Moving objects group had the highest number of cFos-positive neurons, followed by those of the stationary objects group, context exposure and the control group had the lowest number of cFos expressing neurons within the CA3 region of dorsal hippocampus.

Lastly, I ran a one-way ANOVA on the square root values of cFos positive cells within the dentate gyrus of the dorsal hippocampus for the four groups. The ANOVA yielded a significant effect of group ($F_{3, 16} = 9.55$, $p<0.001$). The multiple comparisons procedures (Holm-Sidak) indicated that the moving objects group had significantly higher numbers of cFos expressing cells compared to the other three groups. Mice exposed to stationary objects, empty context and cage controls did not significantly differ in number of cFos expressing neurons within the dentate gyrus (Figure 18a).

In order to examine the differences between neuronal activities within non-hippocampal regions following the experimental exposures I conducted a one-way ANOVA on the square root values of total cFos positive cells within the basolateral amygdala for the four groups (Figure 18b). The one-way ANOVA yielded a significant main effect between the exposure groups ($F_{3, 16} = 65.14$, $p<0.001$) and multiple comparisons procedures (Holm-Sidak) indicated that the cage control group had significantly fewer neurons expressing cFos within the basolateral amygdala compared to the other three groups. The notion that there is no difference in amygdala cFos expressing neurons between the mice exposed to the empty context and mice exposed to the context with objects (moving or stationary) suggests that mice developed a fear memory not just to the objects but primarily to the training context.

I conducted a one-way ANOVA on the raw counts of cFos-positive neurons within the LEC for the four groups (Figure 18c). The one-way ANOVA yielded a significant main effect of group ($F_{3,\ 16}$ = 99.74, $p<0.001$) and multiple comparisons procedures (Holm-Sidak) indicated that the LEC cFos expression within the stationary objects group was highly significantly more compared to the other three groups ($p<0.001$). Furthermore, the moving objects group showed highly significantly more cFos expression within the LEC compared to the cage control group ($p<0.001$), and a significant difference ($p=0.04$) in cFos expression within the LEC was detected between Moving objects and context groups as well as between the context and control group.

3.4.4.2 cFos expression correlates of mouse behavior

In order to determine whether the mice used for immunohistochemistry staining performed the task successfully following training and prior to tissue collection I quantified the proximity events associated with each object during the test, prior to tissue collection. I conducted a Mann-Whitney Rank sum test on the proximity events measured from mice exposed to the stationary objects condition prior to tissue collection (Figure 19a). The test yielded a significantly higher number of proximity events associated with the good bug (Mdn= 7) compared to the bad (Mdn=5), U= 88.0, $p=$ 0.002 indicating that mice successfully discriminated between the two stationary objects. I then examined object discrimination of mice exposed to the moving objects condition (KYE) using t-tests. A Mann-Whitney Rank sum test on the proximity events measured from mice in the presence of the stationary object within the arena yielded significantly higher values associated with the good bug (Mdn= 11) compared to the bad (Mdn=4), U= 18.5, $p=$ 0.018 indicating successful discrimination. Lastly, discrimination of the moving objects

was assessed with a t-test that again demonstrated that mice experienced significantly higher number of proximity events ($t(18)= -2.58$, $p=0.02$) associated with the good bug (M=11.3, SD= 2.95) compared to the bad bug (M= 8.6, SD= 1.5). These results indicate that in both object exposure groups, mice successfully discriminated between the objects like they had been trained to (Figure 19b).

I conducted a one-way ANOVA on the distance traveled during each exposure conditions for the three experimental groups (Moving objects, Stationary objects, Empty context). The ANOVA yielded a significant main effect based on exposure condition ($F_{2, 59}$ = 10.544, $p<0.001$) with corresponding multiple comparison Holm-Sidak post-hoc measures demonstrating differences between all three groups. The mice exposed to the moving objects traveled the longest distance and the mice exposed to the objects only while stationary traveled the shortest (Figure 20a). In order to determine if there was an association between distance traveled and number of cFos expressing neurons within distinct regions of the dorsal hippocampus (CA1, CA3, DG) following the exposure (empty context, stationary objects, moving objects) I began by conducting a one-way MANOVA on the raw counts with distance traveled as a covariate. I found no significant effect on neuron counts within each region based on distance traveled, ($F 3, 11$ = 1.54, p = .25; Wilk's Λ = 0.70, partial $\eta 2$ = .29). This result suggests that distance traveled during the exposure was not the main contributor to the significant differences detected between cFos expressing neurons within sub regions of the hippocampus.

I then examined whether the numbers of cFos expressing neurons within each hippocampal region in mice exposed to moving or stationary objects (not empty context) differed after controlling for distance traveled. I conducted a one-way ANCOVA with

distance traveled as a covariate on cFos neuron counts from each of the three hippocampal regions (CA1, CA3, DG). The ANCOVA conducted on counts from CA1 region yielded a non-significant effect (F2, 9 = 0.42, p=0.54) and the ANCOVA conducted on counts from region CA3 also did not yield a significant effect (F2, 9 = 0.36, p=0.57) (raw values demonstrated a difference between moving and stationary object groups. See above). The ANCOVA conducted on DG counts did however yield a significant effect (F2, 9 = 8.034, p=0.025), indicating that after controlling for the significant differences in distance traveled during the exposure, cFos expressing neurons within the DG were still significantly greater following an exposure to moving objects compared to stationary ones. These findings indicate that factors other than the distance traveled during the exposure were contributing to the increased cFos expression within DG in the moving objects group compared to the stationary.

Additionally, in order to examine the relationship between distance traveled and number of cFos expressing neurons within the hippocampus of mice exposed to either moving or stationary objects I conducted a linear regression analysis using the two measures (cFos expressing neurons, distance traveled) for each region. A non-significant regression equation was found for cFos expressing neurons within CA1 ($F_{1, 9}$= 1.47, p=0.26) with an R^2 of 0.16. The predicted distance traveled was equal to 2496.09 + (0.49 *(CA1)) when cFos expressing neurons were quantified within CA1 region of the hippocampus. I found a significant regression equation for cFos expressing neurons within CA3 ($F_{1, 9}$= 7.28, p=0.03) with an R^2 of 0.48. The predicted distance traveled was equal to 1858.15 + (0.99 *(CA3)) when cFos expressing neurons were quantified within CA3 region of the hippocampus. Finally, a non-significant regression equation was found

for cFos expressing neurons within DG ($F_{1, 9}$= 1.5, p=0.26) with an R^2 of 0.16. The predicted distance traveled was equal to 2393.23 + (0.65 *(DG)) when cFos expressing neurons were quantified within the dentate gyrus region of the hippocampus (Figure 20b). These findings indicate that CA3 neuronal activity is significantly influenced by the distance traveled during either moving or stationary object exposures.

3.4.5 Hippocampal CA1 place cells and moving objects

Hippocampal function has been strongly linked to spatial navigation and its neuronal activity is affected by changes the environment. As movement is very spatial in nature it is of high interest to further determine how moving items affect the spatial navigation system. In order to examine the effects moving objects have on hippocampal neuronal activity I conducted *in vivo* electrophysiological recordings of CA1 neurons and isolated activity from 35 stable place cells. Place cell activity from mice (n=3) was recorded during a cue card rotation protocol (Figure 21a). Unilateral tetrode placements within the medial region of dorsal CA1 were histologically verified following the experiment (Figure 21b). In order to determine the effect moving and stationary objects have on the firing fields of CA1 place cells during cue card rotations I quantified field stability between sessions, spatial coherence and spatial information content of each cells place x firing map as well as maximum firing frequency and spike count from each place cell (moving n=18 stationary n=17) (Figure 21c). I conducted a two-way ANOVA on the correlation measures between the "heat" maps created from the entire 300 sec recording file for each session. The ANOVA yielded a non-significant effect of movement ($F_{1, 99}$ = 0.01, p=0.90), session type ($F_{2, 99}$ = 0.15, p=0.86), and a non-significant interaction between the two ($F_{2, 99}$ = 1.66, p=0.19). Next, I conducted a two-way ANOVA on the

91

calculated spatial coherence obtained from each session. The ANOVA yielded a non-significant effect of movement ($F_{1, 99}$ = 1.77, p=0.18), session type ($F_{2, 99}$ = 0.28, p=0.75), and a non-significant interaction between the two ($F_{2, 99}$ = 0.26, p=0.77). Furthermore, I conducted a two-way ANOVA on the log10 transformation of the calculated spatial information content obtained from the same recordings. The ANOVA yielded a non-significant effect of movement ($F_{1, 99}$ = 1.65, p=0.20), session type ($F_{2, 99}$ = 0.06, p=0.94), and a non-significant interaction between the two ($F_{2, 99}$ = 1.51, p=0.22). Furthermore, I examined the maximum firing frequency and the average spike count recorded from each place cell during the three cue card rotation sessions. I conducted a two-way ANOVA comparing maximum Hz between place cells recorded in the presence of stationary or a moving object and found no significant main effect of movement ($F_{1, 99}$ = 1.82, p=0.18), recording session ($F_{2, 99}$ = 0.02, p=0.98) or and interaction between the variables ($F_{1, 99}$ = 0.64, p=0.53). I then conducted a two-way ANOVA on the spike counts observed from those same sessions and found no significant main effect of movement ($F_{1, 99}$ = 0.16, p=0.69), recording session ($F_{2, 99}$ = 0.05, p=0.95) or and interaction between the variables ($F_{1, 99}$ = 0.28, p=0.76). These findings support the notion that simply the presence of a stationary or a moving object within the arena does not drastically affect the overall firing properties of CA1 place cells.

In order to explore the relationship between spatial coherence and information content obtained from each 300-sec recording session under either the moving or stationary object conditions, I ran simple linear regression analysis for both conditions (Figure 21d). A linear regression predicting spatial coherence based on information content with a stationary object present yielded a significant regression equation (F1,

52=8.65, p=0.005), with an R^2 of 0.143. Each cells' predicted spatial coherence was equal to 0.36 + 0.05 when information content was obtained. A linear regression predicting spatial coherence based on information content with a moving object yielded a non-significant regression equation (F1, 52=1.76, p=0.19), with an R^2 of 0.03. Each cells' predicted spatial coherence was equal to 0.36 + 0.03 when information content was obtained. These results indicate that there is a significant relationship between spatial coherence and information content when a stationary object is present within the recording arena, but that relationship is non-existent when the object is moving.

In an attempt to exclude influence of object exploration on hippocampal place cell activity during cue card rotations I manually coded video files according to whether the mouse was in close proximity to the object, exploring it (Near), or was walking around the arena away from the object (Far). I quantified time spent (sec) "near" or "far" from the object during each 300-sec session (East, North, East2) with an object moving or stationary, and conducted a three-way ANOVA on the values (Figure 21e). The ANOVA yielded a significant main effect of proximity ($F_{1, 83} = 328.22$, $p<0.001$), with post-hoc tests demonstrating that mice spent more time far from the object compared to near it. The ANOVA also yielded a significant main effect of movement ($F_{1, 83 =} 26.95$, $p<0.001$), with post-hoc tests indicating that more time was quantified as available for overall analysis when the object is stationary (mice spend more time "near" the object when it is stationary compared to moving). The ANOVA did not yield a significant main effect of session ($F_{2, 83} = 0.47$, $p=0.63$) or an interaction between any of the variables. Due to restricted location data sampling when the mouse was near the object, analysis of the neuronal data would be inappropriate, and was excluded from the data set. In order to

93

examine whether the simple presence of a moving or stationary object; while excluding direct object exploration affects hippocampal place cell activity, I quantified the correlation stability, spatial coherence, information content, maximum Hz and spike count using heat maps created from the manually coded "far" correlation maps (Figure 21f). In order to account for differences in sampling time between the stationary and moving objects conditions, I controlled for sampling time in our analysis using time as a covariate. The multivariate test yielded significant difference in the five place cell measurement values based on sampling time prior to heat map plotting ($F_{5, 99}$= 5.56, p<0.001; Wilk's Λ = 0.78, partial $\eta2$ = .22). Controlling for sampling time resulted in no statistically significant effect on place field correlation measure ($F_{1, 99}$= 2.82, p=0.09; partial $\eta2$ = .03), Spatial coherence of place fields ($F_{1, 99}$= 3.14, p=0.08; partial $\eta2$ = .03), and maximum Hz of the cells ($F_{1, 99}$= 5.56, p=0.82; partial $\eta2$ = .04) but differences in both information content ($F_{1, 99}$= 6.06, p=0.01; partial $\eta2$ = .06) and Spike count ($F_{1, 99}$= 4.56, p=0.03; partial $\eta2$ = .04) were not explained by differences in sampling time (Values plotted based on comparable sample time- "not raw" Figure 21g). For both information content and spike count, having a moving object within the recording arena yielded higher values. These results indicate that the place field system is not largely affected by moving objects within the testing arena although we cannot exclude that spatial coherence would be observed to decrease when the mouse is approaching or in close proximity to a moving object like others have suggested (Zynyuk et al., 2012). Although differences in information content were detected, it is likely that these findings are due to sensitivity of the measure to limited sampling, and therefore an inflated calculation was obtained from the "moving far" sampling heat maps. Furthermore, the

increase in spike count measured in the presence of a moving object was not accounted for by the sampling time. Although this effect was not observed independent of exploratory behavior, this finding could indicate that the other place field measures are not sensitive enough to the manipulation and differences in place field properties would be detected under different experimental conditions. Taken together, the notion that place cells are not directly affected by moving objects or identities is not yet conclusive and further testing is required to determine the effects movement has on the spatial mapping system.

3.5 Discussion

The findings from the present sets of experiments provide strong support for the overall hypothesis that the CA1 region of the rodent hippocampus plays a crucial role in object memory processing, independent of location. Temporary hippocampal inactivation impaired successful object discrimination under both moving and stationary object conditions when mice were tested in the KYE task. In addition, I found supporting evidence for the notion that object association acquisition is highly context dependent given that mice were not able to acquire the KYE task after days of training within multiple novel contexts. Further, I found new evidence demonstrating that retrieval of such association learning is not, as seen by successful object discrimination and avoidance when trained mice were tested within an unpaired familiar context. When familiar objects were presented in a novel context, discrimination of stationary objects was enhanced whereas moving object discrimination was highly impaired. This finding is indicative of the notion that movement of the objects has an inhibiting effect on object memory retrieval. Furthermore, measurements of cFos-expressing neurons within

temporal lobe structures quantified after mice were exposed to moving objects demonstrated that while the CA1 region of the dorsal hippocampus is just as active in mice exposed to stationary objects, differences in numbers of cFos-expressing neurons within the LEC are drastically lower. As the LEC projects non-spatial information to the CA1 region and other regions of the hippocampal system (Andersen, 2007), it is an interesting finding that equivalent number of cFos expressing neurons were not detected within the region following exposures to the objects while either moving or stationary. One way of interpreting result is that when objects are moving, they may be harder to either identify or perceive, compared to when stationary; providing an additional explanation to why mice take longer to acquire the avoidance tasks when objects are moving. A different possibility is that the hippocampal system provides inhibitory feedback to neuronal activity within the LEC when objects are moving around the environment. Further studies are required to specifically address the significant difference in number of cFos expressing neurons within the LEC following exposures to moving objects compared to stationary.

Spatial and non-spatial information are not integrated into a single representation within the CA1 region of the dorsal hippocampus. My findings that moving objects do not affect firing properties of established CA1place cells is indirectly supportive of hippocampal processing of object memory, and suggests that spatial and non-spatial information remains separate within the CA1 region. The parallel map theory states that the hippocampus processes the spatial bearing map and the object/local cue sketch map separately is supportive of the notion that activity within the spatial mapping system should remain largely unaffected by the presence of objects (Jacobs & Schenk, 2003).

The absence in change of place cell firing properties in the presence of moving or stationary objects indicates that the CA1 place cell system does not directly process the location or the increased spatial information gained when objects are moving around the environment.

Together, these findings support my overall hypotheses that object information retrieval is dependent on the dorsal hippocampus, not simply because of spatial properties such as object-in-location or movement. My findings do, however, also provide support for the notion that when rodents are learning about a given object, the consolidation process is highly dependent on an accessible spatial memory. Future research aimed at determining when the newly acquired object memory becomes non-context dependent during multiple encounters within an environment is vital in order to fully answer questions pertaining to true hippocampal dependent object memory.

3.5.1 Elevated plus maze and anxiety

Impaired object discrimination and avoidance following hippocampal inactivation is not a result of reduced anxiety or changes in locomotion. As changes in anxiety can largely affect animal behavior I first wanted to determine that the local muscimol infusion used for hippocampal inactivation did not have anxiolytic effects or influence locomotion. It is well established that compounds that reduce anxiety will alter the behavior of rodents during the elevated plus maze test, when given systemically (Pellow, Chopin, File, & Briley, 1985). I detected no significant differences in time spent in open arms or number of entries into open arms of the elevated plus maze between mice treated with intracranial infusion of muscimol compared to those treated with saline. This result indicates that a local infusion of the GABA-A agonist muscimol into the CA1 region of

the dorsal hippocampus does not decrease anxiety in male C57BL/6J mice to the level where significant differences are measured on the elevated plus maze. I also detected no differences in velocity or distance traveled between the two treatment groups, indicating that a local CA1 infusion of muscimol does not affect locomotion of the mice. Together, these findings indicate that a local infusion of muscimol into dorsal CA1 does not significantly alter the observed behavior of mice.

Reduced anxiety does not alter object discrimination or avoidance in the KYE task. In order to assess the effects an anxiolytic drug has on object discrimination and avoidance behavior I tested mice in the KYE task following a systemic diazepam injection. I detected no significant difference in discrimination of the objects while moving and although avoidance appeared to decrease, this effect was non-significant. Although successful object discrimination was not observed under the stationary conditions it is important to point out that discrimination of the objects was not detected during the three adjacent training sessions and following the saline injection (control). This finding is likely a result of lower animal numbers and an inexplicable poor performance of this cohort of mice, but is not deemed detrimental as the stationary condition is considered the easier version of the task. The notion that I didn't observe behavioral changes associated with reduced anxiety on the elevated plus maze following muscimol into dorsal CA1 of the hippocampus, and that an injection of anxiolytic compound did not affect KYE performance, I conclude that the discrimination and avoidance impairments detected following the hippocampal inactivation are not due to reduced anxiety, but rather demonstrate the hippocampal dependence of the task.

3.5.2 Object identification and discrimination

It is very important to ensure that the impairments that follow experimental manipulations, such as the hippocampal inactivation in the current study, is in fact a result of the true function of the brain region of interest. Because the Enemy Avoidance (EA) task only utilizes one object it would be incorrect to make the claim that true object memory has been impaired following a hippocampal inactivation. It is impossible to state that the representation of the specific object mice were trained to avoid is inaccessible following the muscimol infusion, when previous work has shown clear impairments in spatial memory following such inactivation in rats (Riedel et al., 1999). Following the CA1 inactivation, presumably both the contextual and object memory are inaccessible, and although we observed impairments under both moving and stationary object conditions, we cannot exclude the possibility that the impaired avoidance is due to inaccessibility of the contextual memory.

To eliminate these possibilities, I inactivated the CA1 region of the dorsal hippocampus in mice trained in the Knowing Your Enemy (KYE) task and observed significantly impaired object discrimination both while moving and stationary. It is difficult to attribute the impaired object discrimination following the hippocampal inactivation simply to impairments in spatial processing. If neuronal activity within cortical temporal lobe regions were adequate to support true object identity memory, we would expect to observe no impairments in object discrimination under both stationary and moving conditions following the temporary inactivation of the hippocampus. The findings that proximity events encountered with the bad object increased significantly, to an equivalent level to the good bug, even when the extremely familiar objects were

stationary strongly supports the notion that the CA1 region of the dorsal hippocampus is vital for object memory retrieval as others have reported (Cohen et al., 2013).

3.5.3 Avoidance of moving objects requires a familiar contextual map

Having previously encountered a location does appear to have a positive effect on avoidance of a moving object. The current findings demonstrate that object avoidance (EA) and discrimination (KYE) remains unaffected when mice are tested within an unpaired familiar context, but the respective behaviors are impaired when the object, or objects, are encountered in a novel context. If the object discrimination impairments observed following hippocampal inactivation were simply due to impaired spatial processing, we would expect to observe comparable results when trained mice are tested within a novel context; yet the results from these types of experiments are unique. When object avoidance (EA) was tested within novel context, the association between context and object identity is not accessible (due to spatial memory encoding and consolidation), resulting in impairments in avoidance of the object while moving. Stationary object avoidance was spared, as object recognition remained intact (hippocampal dependent or not). In comparison, when trained mice were tested in the KYE task within a novel context, object discrimination impairments were observed only while the objects were in motion. I hypothesize that this finding is due to the fact that the locations within the novel context were unfamiliar, leading to impairments in continuous updating of location of the objects within the environment. The observed impaired object discrimination was likely not due to inaccessibility to the object memory per se, but rather to the inability to "navigate" around the unfamiliar environment.

Interestingly, discrimination of the objects while stationary was significantly enhanced, a finding that has rarely been reported without hormonal or pharmacological manipulations (Oliveira et al., 2010). Although there is little clear supportive evidence for this finding, I hypothesize that when the neutral familiar object (good object) is removed from the fearful training context, fear responses are reduced and the animal is less anxious to explore the good object. This hypothesis is supported by the cFos immunohistochemistry results which showed that even when mice are exposed to the empty training context, comparable numbers of cFos expressing neurons are quantified within the basolateral amygdala. This result indicates that mice are not only trained to fear and avoid a specific object, they develop a contextual fear memory throughout training. When mice were removed from the training context and presented with the two familiar objects (familiar unpaired context), avoidance remained intact for the object paired with the aversive stimuli but exploration of the neutral object was significantly increased. Although my current results are consistent with previous work (Oliveira et al., 2010), it is difficult to relate the findings due to differences in experimental protocols. The conclusions of Oliveira et al. that hippocampal activity can interfere with object memory consolidation when an object is encountered in an unfamiliar environment is difficult to apply to the current results. The authors do not address object memory retrieval specifically, but their conclusions could be supportive of the current findings obtained when object avoidance and discrimination was tested in an unpaired familiar context.

Since we know that novelty highly affects the hippocampus (Arias, Méndez, & Arias, 2015); I wanted to exclude any influence a novel context might have on object

avoidance, yet examine the contribution context may have on object memory. When I tested mice in an unpaired familiar context, avoidance, and discrimination behavior, remained fully intact in EA and KYE respectively, indicating that object memory retrieval is not dependent on a specific paired context. Encountering a familiar object in an unpaired familiar context, a location where the object has never been seen before does not affect successful object memory retrieval. This finding provides further evidence for dissociation of object and context memory, as observed previously (Cohen et al., 2013), and provides unexpected support for a flexible cognitive ability rarely studied in mice.

3.5.4 Object memory acquisition is context dependent

Learning about an object appears to be dependent upon where it is encountered. Based on the current findings that EA testing of mice in a novel context impairs avoidance of a moving object, yet avoidance remains unaffected in a familiar context; I was interested in testing object avoidance acquisition across multiple novel contexts with the goal of reducing the novelty of the experience. The goal was to train mice to avoid the object, both while moving and stationary while never presenting the task in a familiar environment. As others have suggested, learning to associate an item with reward is highly context dependent (Biegler & Morris, 1993) and even under these highly motivating conditions our results support that notion. Although our findings demonstrate that mice trained to avoid one object (EA) in multiple novel contexts cannot learn to avoid the object while moving, they are learning something about the task, as seen by decreased number of proximity events when the object is stationary. Under these circumstances, mice are likely unable to retrieve object identity information due to the lack of familiar contextual information, and since they are not required to identify the

object they likely associate "it" with a "do not approach" rule when presented with novel context conditions. Because of the learned rule not to avoid an object when presented in a novel context, temporary hippocampal inactivation does not have an effect on avoidance of the stationary object. Furthermore, because the task was never acquired, no differences in behavior are observed when the object is moving. This finding indicates that avoidance acquisition of a moving object is more dependent upon a stable contextual memory compared to a stationary object.

Although mice successfully avoid one object while stationary within multiple novel contexts; stationary object discrimination is never acquired under the same training conditions in KYE. This finding further supports the notion that object memory acquisition is context dependent. The result that mice cannot learn to discriminate between two objects, moving or stationary, when trained in multiple novel contexts supports the notion that learning about object associations highly depends on the training context. Mice can recognize a stationary familiar object in a novel context after encoding and consolidating the memory within a consistent context. However, mice cannot avoid a familiar object while moving, likely because a familiar spatial map is required, or hippocampal processing of spatial information interferes with object memory retrieval, as suggested by Oliveira et al, (Oliveira et al., 2010). Although the current findings do not explain how information about moving objects get processed within the hippocampal system, they do suggest that once the information about the moving object is learned, avoidance of the object while moving is highly dependent on hippocampal processing.

3.5.5 cFos marker activation

Hippocampal neurons are strongly activated when mice are exposed to object, which supports the notion that this structure plays a role in object memory. Its activity is further altered when those objects are moving around the environment where they are presented. Measurements of cFos expressing neurons within the CA1 and CA3 regions of the dorsal hippocampus were increased following exposures to either the empty training context, or the context containing moving or stationary objects. Both regions showed a considerable increase in cFos positive neurons compared to the cage control mice although the moving objects group stands out with significantly higher numbers of cFos expressing neurons within the DG. Furthermore, differences in cFos activity within LEC were considerable between all groups and especially under the stationary object condition, where the expression was exceptionally high. Expression of cFos within neurons of the BLA demonstrates that the region acts as a good control area since all 3 exposure groups demonstrate similar but significantly higher neuronal marker expression compared to the cage control group. These findings indicate that not only have the mice been conditioned to avoid an object, amygdala cFos expression is consistent with the view that they are also conditioned to fear the training context. The differences in cFos expression counts between all four groups used to test influences of moving vs. stationary objects suggest that when movement is introduced, information flow through the brain changes drastically.

My findings that numbers of cFos expressing neurons within the CA1 region is comparable between both object exposure groups, yet significantly higher compared to the empty context and cage control is interesting, given the expression within the other

two hippocampal sub-regions. This result indicates that the CA1 region of the dorsal hippocampus becomes highly active during the presence of 3D objects within a familiar environment, independent of whether they are moving or not. Only focusing on stationary objects, the current finding is consistent with others who report increased cFos expression within CA1 in animals shown objects within a familiar context, compared to the empty familiar context alone (VanElzakker, Fevurly, Breindel, & Spencer, 2008). The same study reports detecting no differences in numbers of cFos expressing neurons within the DG following those experimental conditions, which is consistent with the current findings when the empty context and stationary object exposure groups are compared.

The current findings do indicate that the DG is highly active when trained mice are exposed to familiar moving objects, but is not significantly influenced by the presence of familiar stationary objects. This finding could be explained by the notion that the DG functions both as a gate and a filter of the information projected from entorhinal and perirhinal cortices. The DG gate opens when persistent and repetitive stimulation is received but it also filters consistent patterns and only the most highly potentiated patterns are passed through (Hsu, 2007). The information available during the moving objects condition is considerably higher compared to the stationary object condition as the objects have an increased spatial aspect due to the continued movement as well as the animal is forced to pay more attention to the location of the objects in order to avoid the expected foot shock (shock was inactive during the exposures). Previous work examining cFos expression in the DG has found a correlation between number of cFos expressing neurons and distance traveled on a running wheel but no relationship was found in relation to the distance traveled passively within the home cage (P. J. Clark,

Bhattacharya, Miller, & Rhodes, 2011). It's unclear whether the exercise affected the increased DG cFos expression or the notion that wheel running is an engaging task, but it is difficult to exclude the possibility that the movement of the wheels during running has an effect on DG neuronal activity. Further studies are required to determine the specific function of DG neurons and the information flow through the structure during various tasks.

The lateral entorhinal cortex processes object-relate information. Previous work has found strong evidence for the vital role LEC plays in object information processing using behavioral analysis and immunohistochemistry (D. I. Wilson et al., 2013) or single cell recordings (Tsao, Moser, & Moser, 2013). The current results provide support for these earlier studies as demonstrated by the highly significant increase in number of cFos expressing neurons within the LEC following exposures to stationary objects compared to the empty context. Although the cFos expression within LEC following an exposure to moving objects was also found to be significantly higher compared to the expression following exposure to the empty training context, the effect was not as extreme as the LEC expression following exposure to the stationary objects. Somewhat unexpected, this finding was not surprising when taken into account that mice are likely still paying high attention to the stationary objects as demonstrated by the successful discrimination during the exposure. During the object exposure, it is likely that mice are able to obtain maximal object identity information as the objects are not moving around the environment. The finding that numbers of cFos expressing neurons within the LEC were still found to be significantly higher following exposures to the moving objects compared to the empty context not only suggests that although the objects move around, adequate object

information was obtained and successful object discrimination is observed but also that the presence of objects highly influences neuronal activity within the LEC.

The rodent hippocampus has been studied extensively with regards to spatial memory and navigation. Place cells are thought to be the building blocks of a cognitive spatial representation of the external world and their activity is strongly correlated with the animals' location at a given time (J. O'Keefe & Conway, 1978). Previous work exploring activity markers within the dorsal hippocampus has found that when rats receive two sequential exposures to different environments, just less than 40 % CA1 neurons are active within each environment. Furthermore, immunohistochemical markers detected after an environmental exposure matched evidence from electrophysiological recordings of hippocampal neuronal ensembles (J. F. Guzowski, McNaughton, Barnes, & Worley, 1999). Although not well established, expression of immunohistochemical markers for activity within the hippocampus, such as cFos, would be expected to correlate with the amount of area covered, or the distance traveled prior to tissue collection. With the assumption that place cells would express IEG markers following recent activity, I explored the relationship between the distances traveled during the exposures and numbers of cFos expressing neurons within sub-regions of the hippocampus. Although the experience that the mice are exposed to during the stationary and moving object conditions may vary drastically, I found no significant differences in number of cFos expressing neurons within the CA1 and CA3 regions of the dorsal hippocampus, after controlling for distance traveled during the exposure. However, I did detect differences within the DG, indicating that the high activation of neurons within this structure is likely not due to spatial processing, but the exposure to moving objects.

Although it is unlikely that the main variable contributing to neuronal activity within the CA1 and CA3 regions is the distance traveled during the exposure, I found evidence for the notion that it is a significant contributor. These findings do support the well-established spatial processing function of the dorsal hippocampus. Furthermore, the significant relationship between cFos neuronal activity marker within the CA3 region and distance traveled during the object exposures further supports the significant spatial processing within that region. Additionally, the absence of such a relationship within the CA1 region does indicate that factors other than distance traveled contribute to the enhanced number of cFos expressing neurons following exposures to the objects.

Taken together, my findings indicate that the presence of objects within a familiar arena highly engages the hippocampus, but when those objects are in motion, the information flow through the structure changes in a drastic way. I hypothesize that when stationary objects are presented to the mice, object information flows to the CA1 directly from the LEC and less information is projected through the hippocampus via the trisynaptic loop. In contrast, under the moving object condition, the DG is highly engaged and the information flow is altered in a manner that enhances activity through the hippocampal trisynaptic loop while maintaining or reducing the information projected directly to CA1 from the LEC. This hypothesis could be tested by recording individual neurons within the sub-regions of the dorsal hippocampus in the freely moving animal during exposures to stationary or moving objects. The results from the current cFos marker study would suggest that individual neurons within the CA3 and DG regions of the dorsal hippocampus would be observed to have different firing properties when mice are presented with stationary objects compared to when moving.

3.5.6 Place cells are not influenced by a moving object.

The hippocampus is well known for being the home of the location specific firing place cells yet it remains unlikely that disruption in hippocampal place cell activity results in impairments in avoidance of a moving object. These neurons are thought to process the animals' location within the environment but their activity does not appear to be affected by smaller items or objects, whether they are moving or not. Previous behavioral work has examined the extent to which items or objects are used as reliable navigational landmarks. Findings from such studies indicate that unstable landmarks, such as small objects, found in various locations are rarely used to navigate (Chan et al., 2012) and would therefore not be expected to influence the activity of the hippocampal place cell system. The notion that place cell activity has been observed to anchor to more than one contextual spatial reference frame, even with one of them continuously rotating (Zinyuk et al., 2000), but never to moving objects, is highly supportive of such navigational studies. These previous findings would indicate that the rodent hippocampal system easily distinguishes between stimuli within the environment and is capable of determining what features belong to the context and what features belong to unique objects.

The current finding that CA1 place fields remain largely unaffected in the presence of a moving object when compared to a stationary one, supports the notion that the despite receiving additional spatial information from objects, spatial processing within the CA1 is not altered. Previous work exploring differences in hippocampal place cells in the presence of stationary 3D objects suggest that the hippocampal spatial mapping system remains mostly unaffected by the presence of objects, yet individual

fields tend to be smaller and more scattered when multiple objects are added to the arena (Burke et al., 2011). Interestingly, the significant relationship currently found between spatial coherence and information content when place cells were recorded in the presence of a stationary object could indicate that the stability of the hippocampal place cell system is reduced in the presence of moving objects. Considering that, it is likely that the other quantifications of CA1 place fields are not sensitive enough to detect influences of moving objects.

The hippocampal place cell system has not been shown to be highly influenced by non-spatial items moving within the environment. Hippocampal recordings carried out in the presence of a toy car found that although CA1 neurons are modulated by changes in its turning angle of the car when paired with reward, place cell firing properties remained relatively stable and consistently represented the location of the rat (Ho et al., 2008). This finding indicates that even when the animal is rewarded for paying attention to a location or movement of an object, place cells do not anchor to the item or form a spatial reference frame based on the items location. Further, studies of rat CA1 neurons, recorded in the presence of a second rat, have found that as the distance between the subject and the other rat decreases, spatial coherence of CA1 place fields diminishes as well (Zynyuk et al., 2012). These findings are hard to compare to the current findings due to differences in experimental protocols, but it is plausible that similar results would be obtained with increased sampling. The continuously moving object in the current experiment does not allow for abundant exploration by mice within the recording window; the mouse must follow the object around in order to get a close view or somatosensory information, which is more difficult than while exploring the object while

110

stationary. Future experiments should aim towards replicating the findings from Zynyuk et al. using inanimate items where the distance between the mouse and the object can be continuously and reliably tracked.

Taken together with previous work, the current findings suggest that spatial processing by CA1 region of the rodent hippocampus is not directly affected by the presence of objects, whether they are moving or not. Although it is currently unknown how neurons within other hippocampal regions and supporting structures are affected by movement of objects, findings from the current cFos imaging studies would support the hypothesis that DG neurons increase their firing under conditions where moving objects are presented. Interestingly, studies examining LEC neurons, recorded in the presence of 3D objects yield higher spatial information content compared to when the rat was exposed to an empty recording arena (Sachin S. Deshmukh & Knierim, 2011). Further, neurons within the perirhinal cortex show consistent increased firing at the location of 3D objects, independent of whether they are novel or familiar (Burke et al., 2012), although the contrary has been reported (Brown & Aggleton, 2001). These findings support the notion that neurons within cortical areas that have been associated with processing object related information are likely responsible for projecting the information to the hippocampus, independent of the spatial information received from other cortical regions.

The current results suggest that established place cells are not influenced by moving objects, supporting the notion that avoidance impairments observed following hippocampal inactivation cannot simply be due to the place cells being offline.

3.5.7 Conclusions

The rodent hippocampus plays a vital role in object memory acquisition and retrieval although there is evidence that these distinct processes are dependent upon the same temporal lobe structures. The current study demonstrates for the first time compelling evidence that movement of objects affect the hippocampal formation in a different manner compared to the commonly studied stationary objects. The experiments used in this study provide new insight into examining information flow throughout this well studied structure and how a small but important variable, like movement, can alter information flow throughout the rodent brain.

PART IV: GENERAL DISCUSSION

The present sets of experiments were designed to produce answers to questions raised regarding object memory processing within the rodent dorsal hippocampus. The current results support the general hypothesis that object memory retrieval in rodents is reliant upon the dorsal hippocampus. However, the findings indicate that the different processes involved in encoding, consolidating and retrieving hippocampal dependent object memory are not easily elucidated and require further research spanning across rodent species. Although it is common to generalize experimental findings across rodent species, it is imperative to keep in mind that differences in behavioral performances have been reported between the commonly used rats and mice. Previous work, comparing the species, has suggested that rats may have spatial skills superior to mice. During a one day water maze task, rats were shown to rely more on spatial cues compared to mice, when locating the hidden escape platform (Frick, Stillner, & Berger-Sweeney, 2000). In contrast, a study utilizing navigational tasks, either involving locomotion in water or on land, concluded that mice are only inferior to rats in navigational tasks involving swimming (Whishaw & Tomie, 1996). Differences in species performance in studies examining non-spatial memory has not been clearly defined so although it is possible; it is unlikely that the findings from both previous studies and the current findings discussed within this dissertation are inconsistent across rodent species.

The EA and KYE tasks vary drastically from the more common ways of studying object memory. These avoidance tasks do not rely upon the predominant exploratory

behavior; much like novel object recognition does, but rather examines learned behaviors. The intense KYE training likely ensures that the mice have learned about the objects based on their identity, given that we observed clear discrimination between the objects following the training. Because of the clear discrimination and appropriate avoidance of objects following training, we can draw clearer conclusions of behavioral performance following the experimental manipulations of a contextual change or a drug administration. Furthermore, the object memory learned during training in the EA and KYE tasks is expected to be much stronger compared to the object memory obtained during tasks such as novel object recognition. Training in the EA and KYE tasks requires that the mice have multiple long duration exposures to the objects, and acquire fear associations to at least one of the objects. Utilizing the foot shock during training likely engages the amygdala, which is known to affect hippocampal activity and enhance consolidation following an experience (Huff et al., 2006; Maren, 1999). The emphasis on ensuring that a strong object memory is formed during training in the EA and KYE tasks augments the reliability of other inactivation studies which have shown that non-spatial object memory is hippocampal dependent. Furthermore, the current findings raise additional questions regarding how movement affects processing of object memory within the mammalian brain and.

4.1 The hippocampus and object memory correlates

Despite a debate in the literature, there is increasing evidence for the notion that the CA1 region of the dorsal hippocampus in rodents plays a vital role in object memory processing, along with cortical regions such as LEC and the perirhinal cortex (Cohen &

Stackman Jr, 2015). Despite abundant findings from behavioral studies, *in vivo* recordings studies have yielded little direct indication of object memory correlates.

Several studies have examined whether hippocampal neuronal ensemble firing properties change as rodents explore objects but none have found direct neuronal correlates that respond to an object independent of its location (Burke et al., 2011; S. S. Deshmukh & Knierim, 2013; Manns & Eichenbaum, 2009). The present study provides compelling evidence for firing correlates during *in vivo* recordings of CA1 neurons of the mouse hippocampus. Although different from conventional place cells (and other spatial firing neurons), it is impossible for us to fully determine whether the observed firing properties of these "object cells" convey a different variant of spatial firing. Despite that, the finding that these neurons increase their in-field firing frequency over time does suggest that they do not simply process the spatial information about the object. Since the perceptive input (i.e. the features of the objects) over days of recording does not drastically change over time, we would expect the neuronal firing properties to remain consistent, much like has been reported from CA1 place cell recordings (Burke et al., 2011). The finding that the firing frequency consistently increased with experience, may reflect acquisition of object memory, object salience, or object familiarity, rather than changes due to alterations to perceptive input. The dorsal CA1 region receives information from several cortical regions but the perirhinal cortex, which processes object related information specifically, projects information to the CA1 region through two distinct pathways. The perirhinal cortex projects indirectly to CA1 through a distinct pathway via the trisynaptic loop, where information gets modulated by the dentate gyrus and CA3 regions before reaching CA1. The direct pathway projects the same information

directly onto CA1 neurons from the perirhinal cortex, without being processed by other hippocampal regions (Naber et al., 1999). The perirhinal cortex is within close anatomical and functional proximity to the LEC, also projects information directly to the CA1 region (Andersen, 2007; Brown & Aggleton, 2001). Interestingly, immunohistochemical mapping studies have found that exposures to familiar objects are associated with decreased number of LEC neurons expressing neuronal activity markers, likely as objects become more familiar to the rats (Arias et al., 2015; J. F. Guzowski et al., 1999). These findings are consistent with electrophysiological recordings from the perirhinal cortex, where reduced firing rate of neurons is observed as an object becomes increasingly familiar (Brown & Aggleton, 2001). These findings appear to be in contrast to the results from the current object-related activity of neurons recorded within CA1. This negative relationship perhaps demonstrates that information processing within these brain regions change as the animal becomes more accustomed to exploring objects. Combined with previous work, the current report of "object cells" within the CA1 region of the dorsal hippocampus suggest that the brain regions responsible for processing object information function in harmony, in a manner where the previous experiences affect retrieval of object memory.

Previous theories have been proposed regarding how cortical process object information, throughout the hippocampal formation. The bucket theory, based on the model of strong vs. weak memories (Squire et al., 2007), states that as an animal learns about an object, perirhinal cortical neurons begin to encode this information. Once the information within the preirhinal region reaches a critical level, neuronal activity is conveyed to the LEC and the hippocampus, where the memory of the object becomes

dependent upon intact function of the hippocampal formation (Cohen & Stackman Jr, 2015). Although the bucket theory is largely based on how rodents create novel object memories, and not aimed towards explaining strong object memory function, the theory can also be extended to such. Despite the strong object memory being dependent upon the hippocampus, both previous work and the current data suggest that the object memory becomes less dependent upon the perirhinal and lateral entorhinal cortices as experience increases. It is not implausible that when the rodent is no longer learning about the features of an object, the memory becomes mostly dependent upon the hippocampus. Following along with the previously proposed bucket theory (Cohen & Stackman Jr, 2015), one could hypothesize that as the object memory continues to increase in strength, it gets handed from the perirhinal cortex to the CA1 region of the hippocampus. A novel addition to the bucket theory is that the process of information transfer from perirhinal cortex to CA1 does not discontinue once the memory "reaches" the hippocampus.

Once information from these cortical regions reaches the CA1 region, two distinct types of pyramidal neurons process the input in a bursting or non-bursting manner. The bursting type of pyramidal neuron is thought to receive spatial information via the indirect pathway, whereas the non-bursting or regular spiking neurons receive the information directly from the perirhinal cortex (Graves et al., 2012). It is well established that one of the hallmarks of the place cell firing property is the complex-spiking or bursting characteristic. Because of this, hippocampal activity recorded during behavioral studies is commonly screened for such bursting properties and neurons that do not show these characteristics are excluded from further analysis. The current finding that the CA1 neurons discharging at the location of 3D objects do not demonstrate this complex spike

activity suggests that the object-responsive neurons are the pyramidal cells receiving non-spatial information directly from lateral entorhinal or perirhinal cortices. Taken together, it is likely that the current report of CA1 neurons found to discharge at the locations of 3D objects are actively processing or receiving non-spatial information regarding the object in a way that it fully, or partially, represents the object as a whole.

4.2 The hippocampus and moving objects

The KYE paradigm was adapted from an enemy avoidance protocol designed to study spatial avoidance behavior of objects (Telensky et al., 2009). The current study first replicated the findings from Telensky et al. (2011) where rats were found to demonstrate impaired avoidance of a moving object following bilateral micro-infusion of TTX into the CA1 region of the dorsal hippocampus. During several days of training, the enemy avoidance task is utilized to train rats to avoid a moving or a stationary object by delivering a mild foot shock each time the rat was in close proximity to the object. Rats quickly acquire the task, requiring only several sessions of training to demonstrate successful object-shock associations.

Although the current findings did not fully replicate the findings from Telensky et al. (2001), where rats did not show impaired avoidance to a stationary object following a hippocampal inactivation, the differences in the two experimental protocols provide a potential explanation for the varying results. First of all, the current study utilized mice instead of rats for the behavioral studies, which although unlikely, could explain some variances in the findings. Second, instead of training two separate groups of mice to either avoid a stationary object or a moving one, all of the mice in the current study were trained to avoid the object under both conditions. These differences in training protocols

likely explain why a more similar pattern of results was not obtained. Previous work has demonstrated that following a hippocampal inactivation, the rules of the learnt task are not impaired (Riedel et al., 1999). This notion could explain why both groups of rats in Telensky et al. (2011) did not demonstrate impaired behavioral performance following the hippocampal inactivation. Presumably, learning to avoid a stationary object within the environment while the rat walks around requires different brain regions and attention compared to actively avoiding a moving target. If we assume that the rules learned by these two groups of rats are different, the lack of impaired avoidance of the stationary object following hippocampal inactivation can be understood. The findings from the current EA task experiments suggest that a functioning hippocampus is required to avoid an object, both while stationary and moving. This result indicates that during training, mice are learning to associate the object identity with the foot shock, regardless of whether it is moving or not, and the retrieval of the memory for that association becomes impaired following a muscimol infusion into the dorsal hippocampus.

Learning new object associations depends upon the context they are presented in. Previous studies have reported that rodents cannot learn to associate a landmark with a reward location when presented within an unstable reference frame. These studies conclude that a stable contextual memory is required to form the associations between a non-spatial item/landmark and the goal (Biegler & Morris, 1993; Collett et al., 1986). In support of these studies, the current finding that mice trained (EA) in multiple novel contexts they successfully avoid the object while stationary, but not moving, indicates that they are not using the object identity information to create the fear association. Since mice "learn" to associate the foot shock with the object while stationary (within multiple

119

novel contexts), implies that they are not learning the "object-shock" association, but rather learning a distinct task. The finding that mice trained within multiple novel contexts do not avoid the object while it is moving is more difficult to interpret. It is unlikely that this is the result of impairments in object identification while moving, but rather impairments in consolidating the association between object and shock because the contextual information is inconsistent. Further, mice fail to acquire the KYE task when training is conducted in a novel context each day of training. Although mice cannot learn to avoid the bad object (moving and stationary) when trained in multiple novel contexts, the lack of discrimination between the good and the bad objects is the most interesting finding. This pattern of results indicates that although the mice are learning something about the task, as seen by reduced overall proximity events throughout training, mice are not learning about the identity of the object. This finding of impaired discrimination is likely due to the inconsistency of the training context. As stated above, rodents cannot acquire associations to objects without a stable environmental representation (Biegler & Morris, 1993; Collett et al., 1986). The finding that when mice are trained within multiple novel contexts they are capable of avoiding a stationary object (not moving) but cannot discriminate between two objects further supports the notion that object identity memory acquisition depends on the training context. Such conclusions would have been impossible to reach without utilizing movement within the current experimental paradigm.

Despite differences between the EA and KYE tasks, the behavioral results from these tasks are comparable and are supportive of the overall conclusions of this dissertation. The biggest difference in behavioral observations between mice performing

EA and KYE, although the data was not presented, was the average velocity of the mice. During the stationary object sessions in the EA task, velocity of the mice was observed to be significantly lower compared to when the object is moving. All statistical analysis of the behavioral data controlled for both velocity of the mouse and thigmotaxic behavior. No difference in mouse velocity was observed between any of the four sessions within a training bin in the KYE task. This difference in velocity is likely due to the notion that while the object is stationary, the mouse is not forced to actively move out of the trajectory of the object in order to avoid a foot shock, as when the object is moving, but can instead sit and groom from a distance. These differences in behavior and required attention to the object to avoid a foot shock are perhaps not detrimental to interpreting the results but rather indicate that when a moving object is within the environment, more demand is placed on the spatial cognitive map, as the location of the object is continuously changing. Previous work has indicated that rodents don't just recognize the location of an object when it gets there; they learn to anticipate the location throughout training. One study trained rats to press a lever for food reward when an object on a rotating table reached a specific location on the trajectory (Pastalkova, Kelemen, & Bureš, 2003). Although not directly related, their conclusion receives support from the current finding that mice cannot avoid a moving object in a novel context but do so successfully in a familiar unpaired environment. I theorize that when a spatial cognitive map is accessible, like when mice are placed into the familiar unpaired context, independently retrieved object memory can be integrated and successful object avoidance (EA) and discrimination (KYE) is observed. Although the familiar object has never been encountered within that context, the locations within the arena are familiar, so spatial

121

memory encoding is not required. When a memory of the spatial map is inaccessible; like when mice are placed into a novel context, spatial memory retrieval is impossible. While the context memory is encoded and/or consolidated, continuous updating of object location is impossible. The enhanced spatial information associated with the objects while moving, causes anticipation of the object location based on its expected trajectory within a novel context to be too demanding. Although retrieval of contextual information is impossible, retrieval of the object memory is possible as it likely requires a different parallel processing by the hippocampus. As the location of the stationary object does not need to be continuously updated, successful avoidance is observed. Interestingly, discrimination of the objects while stationary (KYE) when tested in a novel context, was observed to be enhanced compared to the control session. This finding indicates that despite the objects being presented within a novel context; retrieval of the object memory is unaffected, further supporting the hypothesized parallel processing of object and contextual processing within the hippocampus. One possible explanation for the increase in time spent with the good object compared to the bad one is that throughout training, mice do not only develop a fear memory to the bad object, but to the context as well, and when the contextual fear memory is not a prominent factor, as when mice are placed into a novel environment, the predominant exploratory behavior is increased. The finding that avoidance of the bad object is unaffected further supports the notion that the memory of the object identity remains unaffected by the object being presented in a novel context.

While the above discussed experiments have demonstrated that acquisition of object memory associations are context dependent, and retrieval of such memories is not; the most central finding is that retrieval of object memory associations are hippocampal

dependent. After demonstrating that stationary object discrimination and avoidance is fully intact within both familiar and novel contexts, the object discrimination impairments observed following a hippocampal inactivation cannot be explained by spatial mapping failures. If the memory of the object/s were dependent on regions other than the hippocampus, successful object discrimination would be expected following the local infusion of muscimol into the CA1 region of the dorsal hippocampus. In addition, if the spatial mapping system of the hippocampus were responsible for processing the object memory, or played a crucial role of integrating the objects location, we would expect to observe such representations by CA1 place cell firing. I detected no overall differences in the firing properties of stable CA1 hippocampal place cells when mice were in the presence of either stationary or moving objects. My finding that place cells maintain stable and consistent firing properties in the presence of a moving object suggests that the spatial mapping system of the hippocampus does not directly engage in processing of the enhanced spatial feature (movement) of the object. I interpret this result as explanatory evidence for the impaired object avoidance (EA or KYE) observed in a novel context. Together, these findings suggest that it is not the movement of objects that has an effect on the spatial mapping processing of the hippocampus, but rather hippocampal encoding of contextual information that impairs the avoidance of a moving object (not stationary). In light of the fact that one cannot have a memory of a location that has never been encountered, this is a result of impaired ability to continuously update the location of the moving object/s.

4.3 Activity marker for object memory

The overall findings within this dissertation are in support of the notion that the dorsal hippocampus plays an important role in non-spatial object memory. In addition to the results from the behavioral studies, the quantification of cFos-expressing neurons provide evidence for changes in information flow throughout the hippocampal formation, and surrounding structures when objects are moving around the environment compared to when presented stationary within the arena. The similarities in number of cFos expressing neurons within CA1 region following exposures to either moving or stationary objects, combined with the differences in expression within DG and LEC, indicate that movement has a profound effect on this memory circuit; yet the CA1 region appears to be influenced mainly by the presence of objects. Given the current results, it is difficult to conclude how the information flow is affected by the presence of moving objects. Future work could aim to determine the differences in information flow by quantifying cFos expressing neurons within additional regions thought to support spatial and object memory functions, such as the medial entorhinal cortex and the subiculum. Furthermore, if we quantified all the neurons within the regions counted, we would know the percentage of activated cells and could estimate with more precision how the KYE task affects activity within various brain regions, compared to the empty context or the presence of stationary objects.

In addition to processing features of spatial and non-spatial episodic memory, the hippocampus has been implemented in detecting the novelty or familiarity of presented stimuli. Electrophysiological recordings of rat CA1 and CA3 hippocampal regions have found that the CA1 region specifically works as a novelty detector (Larkin, Lykken, Tye,

Wickelgren, & Frank, 2014). This study found that place cells within the CA1 region increase their firing rate when the rat is presented with a familiar or a novel object in a novel location. It is important to point out that these increases in firing rates were observed independent of location specific firing and therefore, do not signal changes in the spatial mapping properties, but rather a more generalized indication that something requires further exploration. In support of the finding that neuronal activity within the CA1 is influenced by novelty, a different study examined cFos activity marker expression following multiple exposures to either very familiar objects or novel ones while utilizing a Bow tie maze (Albasser, Poirier, & Aggleton, 2010). This study reported that when rats were shown familiar objects, a higher number of neurons expressing activity marker in the direct pathway (LEC to CA1) were detected. Furthermore, when novel objects were presented, activity was shifted from the PER to the trisynaptic loop, activating the CA1 and CA3 more prominently. More importantly, only the DG of the hippocampus demonstrated increased activity when rats were presented with familiar objects compared to novel ones. One explanation for this difference in activity could be that the DG functions both as a filter and a gate of the incoming information. The DG excludes inconsistent patterns of inputs and only passes on consistent, expected or predicted stimuli (Hsu, 2007). During the Bow tie maze task, rats were trained to respond a certain way when they were presented with an object not previously encountered during recent trials, or a completely novel one. Presumably, it is easier to recognize an object as completely novel, compared to an object you've encountered a few days ago, when making the choice between two presented objects. The information needed to make the attention demanding decisions during the Bow tie maze task is likely effective enough to

125

cause the increased activation. Presumably, when rats are presented with novelty it is easier to detect it as the correct choice, compared to having to recall the temporal order of multiple previously detected familiar stimuli. This enhanced activation within DG during the Bow tie maze running is likely a result of excessive training of the rats. The notion that object identity is behaviorally relevant in order to receive a reward, likely causes the attention of the animals to shift, demonstrating that not just novelty, but object identity engages the hippocampus during performance of this repetitive matching task (Albasser et al., 2010). The result from the currents study, showing increased number of cFos expressing neurons within the DG after exposure to moving objects, compared to stationary objects or an empty context, are not very distinct from the findings reported in Albasser et al. (2010) and are in support of the notion that DG activity may be influenced by attention during a behavioral task. Taken together, hippocampal activity is likely influenced by the novelty or familiarity of the presented stimuli as well as by the difficulty, or attention required for successful performance of the task.

When I examined the behavior of the mice exposed to moving compared to stationary objects, I observed that although both groups successfully discriminate between the objects, mice exposed to moving objects move around more, and experience additional proximity events compared to the mice exposed to the stationary objects. These results suggest that it is more difficult to avoid the objects while moving, which likely requires more attention and more complex behaviors of the animals. Because of this, and what we know about DG function, it is plausible that the DG activity observed following these exposures is highly affected by the differences in tasks, not just the movement of the objects. We cannot make the claim that it is the increased spatial

information provided by the moving objects that is causing the DG activation, but more likely it is the behaviorally relevant avoidance decisions that cause prominent dependence on this hippocampal structure. Future research could aim at determining what type of stimuli or attention is required for such high DG activity during a task. In order to determine whether the increased number of cFos expressing neurons within the DG following exposures to moving objects compared to stationary ones could simply be due to movement, single cell recordings of DG neurons are required. Conducting such recordings in the presence of moving and a stationary object, without requiring the animal to pay increased attention to the object (i.e. no foot shock pairing) would further elucidate whether DG activity is affected by movement of objects, unlike neurons within the CA1 region appear to be.

For the current study, I explored the relationship between distance traveled and number of cFos expressing neurons within the sub-regions of the dorsal hippocampus. Despite finding a positive relationship between the total distance the mouse traveled during the test exposure, and numbers of cFos positive neurons within CA3 region of the hippocampus, other measures indicative of spatial locomotion behavior did not yield comparable results (Average # of pixels visited, total # of pixels visited, average distance traveled- data not shown). In attempts of obtaining a positive relationship between numbers of cFos expressing neurons and other measures of context exploration, I compared both the average number of areas visited (arena divided into 64 even pixels) and total areas visited but found no relationship as strong as the total distance traveled during the task. The lack of corroborative evidence from using other measures indicative of context exploration does not provide strong support the argument that neuronal cFos

expression following the various exposures could partially be explained by spatial processing of hippocampal neurons. Despite of that, there is abundant evidence supporting the role of the hippocampus in spatial memory and navigation which is not up for much debate, nor is it the main focus of this dissertation. It remains likely that the contextual exploration during the exposures partially contributed to the number of cFos expressing neurons quantified within the subregions of the dorsal hippocampus (i.e. place cell activity within CA1 and CA3) but I found no evidence that it was the main contributor, supporting the notion that other stimuli presented during the task contributed activity dependent cFos expression within the hippocampus.

Although immunohistochemical markers of neuronal activity are commonly and widely used within the field of neuroscience, this technique, like many others, has not been shown to be without limitations. Despite the current results demonstrating compelling differences in number of cFos expressing neurons within the hippocampus following the different exposure conditions, previous studies have indicated that immunohistochemical markers are not always good predictors of activity during certain hippocampal dependent tasks. Following a performance of a hippocampal dependent task, comparable numbers of activity marker expressing neurons within the hippocampus were reported as after a different group of rats completed a procedural memory tasks (Shires & Aggleton, 2008). Although staining for cFos and other immediate early gene markers, such as *Arc* are aimed to determine what neurons or regions within the brain are contributing to specific behaviors or activity dependent information processing, it is important to interpret such findings with caution. Previous studies have sought to specifically detect a consistent relationship between neuronal activity and immediate

128

early gene expression following learning, but some findings indicate that such correlations are not always reliable. One such study recorded activity from rat CA1 place cells during various numbers of context exposures, and although they detected consistent and stable place cell activity, immediate early gene expression of *Arc* was not found to represent the recorded activity when rats were repeatedly exposed to the same environment multiple times throughout one day. *Arc* expression within the CA1 region of rats exposed one time to either a familiar or a novel environment was significantly higher compared to the expression in rats that experienced the massed exposures (John F. Guzowski et al., 2006). A different study examined *Arc* expression in primary auditory cortex during associative learning of a nose poke task found comparable results. Examining both firing rates of single cells and *Arc* expression immediately after sensory training, the authors concluded that the two measures were inversely related, yet days following the training, a positive relationship between firing rates and *Arc* expression was detected (Carpenter-Hyland & Plummer, 2010). Together, these findings indicate that expression of immediate early genes shortly following repeated exposures to a task or an environment should be interpreted carefully. For the current study, cFos positive neurons were stained for following a 24-hour break from a 5-day training in the KYE task. Combined with the observed successful performance of the KYE task, the extensive duration of the training would suggest that the temporary abolished relationship between neural activity and cFos expression following training should not have been observed. Although it is difficult to demonstrate that the current observed number of cFos expressing neurons is an accurate representation of the activity prior to staining; one

would expect that if tissue staining occurred following repeated KYE training on day 1, fewer numbers of cFos expressing neurons would be quantified.

4.4 Conclusions

The current findings provide novel support for the notion that the CA1 region of dorsal hippocampus processes object information, independent of location or movement within an environment. Findings from behavioral testing support the notion that object-associations acquisition is highly dependent upon a specific context. In addition, retrieval of such object-associations is not tied to the context where the information is consolidated, yet they remain highly dependent upon the CA1 region of the dorsal hippocampus.

Expression of immediate early gene markers within the CA1 region of the dorsal hippocampus following exposures to familiar objects is in support of the notion that the rodent hippocampus plays an important role in non-spatial object memory. The numbers of cFos expressing neurons within the CA1 are found to be of high similarity following exposures to highly familiar objects, independent of whether they are moving or not. Other sub-regions of the hippocampus appear to be influenced by movement of objects but further research is necessary to determine the role of CA3 and DG play in processing information about moving objects.

Spatial processing by CA1 place cells is not affected by movement of objects. The finding that CA1 place cell firing properties are not significantly affected by the presence of moving objects indicates that movement alone is not likely the main contributor to increased activity within the region. This result supports the notion that spatial and non-spatial information is not well integrated within the CA1 region, but are rather processed

independently and simultaneously. The hippocampal CA1 neurons that were observed to discharge at the locations of 3D stationary objects could provide an explanation for the equivalent levels of CA1 cFos expressing neurons detected between the moving and stationary object groups after mice were either exposed to moving or stationary objects.

With different types of spatial and non-spatial information projected to the hippocampus, it is likely that when mice are exposed to familiar stationary objects, object related information projects to the CA1 region directly from lateral entorhinal and perirhinal regions. As less spatial information is available, compared to when mice are exposed to moving objects, significantly less spatial information projects towards the trisynaptic circuit through CA3 and DG. For mice exposed to moving objects, the CA1 receives less direct information from cortical areas involved with processing object-related information (LEC, Perirhinal), and more information projects through the DG and CA3 regions of the hippocampus. The well-established anatomy of the rodent hippocampal memory circuit receives strong support for the theorized function of the system from the current findings demonstrated within this dissertation.

4.5 Interpretations and alternative approach

The role of the hippocampal formation in episodic memory has been studied extensively, yet some specific functions of this neuronal circuit remain unresolved. Questions regarding how the brain, and specifically how neurons within the hippocampal system, represent the various features of episodic memory in a manner where individual characteristics are isolated, or whether they are kept together in a single representation have proven difficult to address. The current set of experiments was designed in an attempt to separate features of an episodic memory, where the non-spatial characteristics

were presented in a manner independent of its spatial attributes. In the sections below, additional experimental and methodological approaches to acquire and further interpret results related to the project will be discussed.

Behavioral Procedures

The results of the behavioral experiments within this dissertation demonstrate a consistent performance by mice in the Enemy Avoidance and Knowing Your Enemy tasks, despite the fact that individual mice were not exposed to identical experiences during each training bin. Although all mice were trained with an identical protocol, and for the same amount of time, the number of proximity events to the object/s each mouse experienced were not controlled or matched across subjects during training of both behavioral tasks. That is, across a cohort of mice, each mouse experienced a different number of foot shocks, as well as a distinct inter-shock interval during training. In spite of these concerns, the consistent performance and relatively low behavioral variance between mice, after encountering the uncontrolled amount of foot shocks during training, may not be a significant factor in how well each mouse acquires the task. Based on classical studies of Pavlovian conditioning, it has been shown that the stability of conditioned responses and the associative strength acquired to a given stimulus is influenced by the number of pairings of the conditioned stimulus and the unconditioned stimulus, and varying the inter-stimulus interval can dramatically affect conditioning (Vallence, Schneider, Pitcher, & Ridding, 2014). If one considers the typical procedures used for delay or trace cued fear conditioning, for example, one finds that the rigorous adherence to a standard protocol in which the CS and US are presented in a consistent time relative to one another, and the interval between presentations of each CS is

standardized, remains essential for the CS to gain rapid associative strength with the US, in order for subsequent exposure to the CS alone to elicit a strong and consistent conditioned response (Singer, Wei, Chen, Boison, & Yee, 2013). Considering the current findings, the strict adherence to a standardized behavioral protocol during fear association training may not be as vital as previously assumed. Regardless, the Enemy avoidance and the Knowing Your Enemy tasks would likely benefit from improved standardization where the number of shocks delivered to each animal, and the timing of those events is consistent across individual subjects. In order to further standardize the enemy avoidance or KYE task, one might imagine the need to develop a computer-controlled, robotic object so that each mouse of a cohort experiences a consistent number and the same pattern of proximity events during training. Despite the very consistent behavioral performance of mice trained under the current behavioral conditions, given the previous work in fear association learning, one could imagine that variance in performance would be further narrowed with increased standardization of behavioral training.

The influence of novelty and familiarity

Behavioral paradigms involving novelty require a more careful interpretation compared to when familiarity is utilized. Recognizing that an item is novel is presumably easier to do compared to retrieving memories regarding familiar items when presented together. Consider the novel object recognition task. When mice are placed in the arena along with a familiar and a novel object, the mouse does not need to specifically recognize, or retrieve the memory of the familiar object, but it naturally preferentially explores what is novel. For experiments testing object memory retrieval following hippocampal (CA1) inactivation and utilizing novel objects, it can be farfetched to claim

133

that the specific memory of the familiar object is inaccessible. Rather, it is more appropriate to claim that the animal cannot recognize what is novel. For the KYE task, it was of importance to avoid novelty and train the mice to specifically associate a fearful stimulus to one of the objects and not to the other. In order to train mice in that manner, both objects become be very familiar, completely eliminating the novelty feature. In order to train mice to make such behaviorally relevant distinction between the objects, one must associate a fearful stimulus to one, and not the other. Fear memory, like mentioned above, involves structures outside of the hippocampus, such as the amygdala (Maren, 1999). Memory involving emotional or stressful stimuli, such as foot shocks, tends to be stronger in nature, compared to memory for neutral experiences such as object memory. In order to test object memory in the absence of such stressful features, one might assume that the Knowing Your Enemy task could be improved by eliminating the foot shock association. Such a task would of course not involve an "enemy" object, but rather two identical neutral objects that would be experienced both while moving around the environment and stationary, comparable to commonly used object recognition tasks. During training, one would expect not to observe object discrimination, and the number of encounters or time spent in proximity to either object should be comparable. Prior to testing, one of the familiar neutral objects would be exchanged for a novel one and the time spent close to both objects monitored. This version of the task shares similarities to the commonly used Novel Object Recognition task so one would expect to observe that the intact animal spends more time in proximity to the novel object compared to the familiar one. It is worth noting that "training" would be expected to require fewer sessions compared to the KYE task, as mice are not required to form a long-lasting

association of one object to fearful stimuli and actively avoid it. In comparison to the current experiment, when the CA1 region of the dorsal hippocampus of mice is inactivated prior to testing, one would expect to see impaired performance of preferential exploration of the novel object, under both moving and stationary conditions. One reason for why such a task was not utilized for the current behavioral experiments has to do, again, with novelty of the objects used. Previous research has shown that novel stimuli have an effect on hippocampal activity (Albasser et al., 2010; Larkin et al., 2014), yet it is unclear how novelty affects retrieval of established memory. If we were to test object memory under conditions as described above, we could not exclude that the preferential exploration of the novel object was not simply due to the presence of novelty, and the function of the hippocampus as a novelty detector. Testing object memory performance while using two highly familiar objects enhances the probability that the true object representation is being recalled or inhibited by a muscimol infusion, and reduces the odds that the animal is altering its behavior in the presence of novel stimuli.

The current finding of the non-bursting CA1 object cells, observed to be active during exploration of 3D objects provide a potential explanation for neuronal processing of non-spatial features of episodic memory, yet their firing properties do not fully support such simple function. If we assume that these non-bursting neurons are responsible for carrying the functions of object memory retrieval, then the impaired object discrimination following a CA1 inactivation via muscimol infusion into the dorsal hippocampus could be explained as a result of the silenced firing of object cells. However, the unexpected finding that these object-responding neurons were observed to increase in-field firing frequency over time, or with the experience of exploring objects, further dissociates the

135

activity from that of the well-studied place cells (Burke et al., 2011) and indicates that these changes in neuronal firing frequency is not simply coding for a feature of the object. An alternative view of the function of the observed increase in firing rates over time could be representing the familiarity to the task at hand, or potentially functioning as an inhibition of a novelty signal, likely projecting directly from the LEC or PER. Previous research has reported activity representing familiarity within the LEC and PER, whereas such activity was not found within sub-regions CA1 and CA3 of the hippocampus (Atucha, Karew, Kitsukawa, & Sauvage, 2017). Whether the currently reported increased firing rate of object responding neurons within the CA1 region are responding specifically to the non-spatial features of object memory, or a familiarity signal directly from cortical regions currently remains unclear. Further studies utilizing more specialized approach or more advanced techniques would be required to determine what these neurons are representing, and if their change in firing frequency is indicative of any of the above-mentioned features.

One way of assessing whether the object cells could be coding for a familiarity signal would be to utilize the KYE task and the cFos marker, but stain from its presence at a different time point during behavioral training. Based on the findings from the recordings of the object responding cells within the CA1 of the dorsal hippocampus, one would expect to observe higher numbers of cFos expressing neurons when the objects or the experience of exploring objects is more familiar; regardless of whether the object cells are representing object memory, or familiarity of the task. Because of the observed increased firing frequency when mice are familiar with exploring objects, one might expect that as the neurons increase their firing along with experience, the number of

neurons passing the cFos activity expressing threshold following exposures to the familiar good and bad objects would be increased. Given this assumption, it would be of interest to compare number of cFos expressing neurons from mice within the current experiment to that of mice only exposed to one or two sessions of KYE training (i.e. when objects are relatively novel/unfamiliar). Based on the observed increased firing rate of CA1 object-responding neurons as exploration becomes more familiar, one would expect to observe higher numbers of cFos expressing neurons within the CA1 region in mice exposed to full KYE training, compared to that of mice that have not been fully trained. Such pattern of results would indicate that the recorded CA1 object cells were processing a familiarity signal rather than coding for features of the objects.

Circuitry supporting discrimination of moving objects

Information processing by the hippocampal formation changes dramatically when objects are presented while moving around compared to stationary. During behavioral tasks involving moving objects, the assumption is that increased spatial information is available to the animal compared to when objects are presented under stationary conditions. With such differential amount of spatial and non-spatial information projected towards the hippocampus from entorhinal and other cortical regions, it is likely that different pathways are recruited to process the information. The current cFos marker data would indicate that when mice are exposed to stationary familiar objects, increased non-spatial, or object related information projects to the CA1 region directly from LEC and PER. As less spatial information is available, compared to when mice are exposed to familiar moving objects, significantly less spatial information projects towards the trisynaptic circuit through CA3 and DG, as indicated by the lower number of cFos

137

expressing neurons within those sub-regions when mice were exposed to stationary familiar objects. On the contrary, when mice are exposed to moving objects, the CA1 region receives less information directly from the cortical areas involved with processing object-related information (LEC, PER), and more information projects through the DG and CA3 regions of the hippocampus, likely from cortical areas processing spatial information such as MEC. Previous work has been conducted to examine the expression of immunohistochemical markers within the hippocampus following exposures to stationary objects, where findings are relatively consistent to the current results (VanElzakker et al., 2008). However, no known previous work has been conducted to examine immunohistochemical markers following exposures to moving objects. Given the consistencies between previous and the current work regarding stationary objects, it is not unlikely that the expression following exposures to moving objects accurately represents activity within the region under such conditions. The apparent difference in information flow through the hippocampus as non-spatial stimuli become increasingly spatial in nature as movement is introduced could provide insight into how the structure processes various features of episodic memory.

Sub-regions of the dorsal hippocampus, perhaps other than the CA1 may play a larger role in processing object location or movement. The current findings that CA1 inactivation did impair object discrimination, when both of the objects were in motion, as well as stationary, indicates that the CA1 region is not highly involved in processing object location, but rather the object memory specifically. Combined with previous work and the current cFos marker data, it is plausible that the CA3 region of the dorsal hippocampus may play a larger role in processing object location or object movement,

138

compared to the CA1 region. Although functions of the CA3 region have been linked to non-spatial aspects of episodic memory, an inactivation study where both contextual memory and object memory were assessed, found that spatial memory consolidation depended on a functioning CA3 region, yet the same temporary inactivation did not affect object memory (Stupien, Florian, & Roullet, 2003). In addition, a different study conducted simultaneous recordings of CA1 and CA3 place cells following temporary inactivation of PER in rats trained in an object-place recognition task, found differential effects on firing within the hippocampal sub-regions following the muscimol infusion (Lee & Park, 2013). The authors conclude that CA1 neuronal firing is significantly disrupted when PER neuronal activity is compromised, possibly representing the object related information projected from PER to CA1. Furthermore, although the behavioural performance of the rats was impaired, no change in CA3 place cell activity was observed following the PER inactivation, demonstrating the dissociation of object information projected to the hippocampal areas (Lee & Park, 2013). These reports are consistent with the current cFos results where object information does not appear to be highly represented within the CA3 region of the hippocampus, yet the spatial location, or more specifically the movement associated with the object, has an effect on neuronal activity. Due to these findings, it would be of high interest to temporarily silence neuronal firing within the CA3 region in mice trained in the KYE task and then measure object avoidance and discrimination. If the CA3 region actively processes these spatial features of object memory, one might expect that when CA3 activity is silenced, object discrimination would only become impaired while the objects were moving, while stationary object discrimination would presumably remain intact.

The sub-regions of the dorsal hippocampus have been discussed extensively within this dissertation, with the exclusion of the CA2 region. This area has not been studied as broadly as its neighboring CA3 and CA1, which is both represented within the literature as well as this dissertation. The functions of this relatively small region have recently been linked to sociocognitive memory processing, where genetic inactivation of the area results in impaired social memory (Hitti & Siegelbaum, 2014) and stimulation enhances the retrieval of social memories (Leroy, Brann, Meira, & Siegelbaum, 2017). For the current study, activity within the CA2 following exposures to moving objects was not assessed, as visualization of cFos neuronal expression between exposure groups did not appear distinct. Future research examining activity within the CA2 under conditions utilizing moving objects will be of interest as it is not improbable that the mice view moving identities in a different manner compared to stationary objects, and perhaps associate a social aspect to a moving object. As it is very probable that the CA2 region plays a role in processing both spatial and non-spatial aspects of episodic memory, it is exciting to consider future work where the contribution of this area to KYE performance is assessed.

The dentate gyrus further remains an area of high interest when it comes to processing information regarding moving objects. Although no other known studies have explored neuronal activity within the DG following exposures to moving objects, performance of spatial navigation tasks and hippocampal dependent object recognition tasks remain dependent on neurogenesis within the DG (Jessberger et al., 2009). Despite few recording studies exploring DG place firing compared to CA1 and CA3, location specific activity of DG granule neurons has been shown to yield smaller and more

discontiguous fields compared to pyramidal cells (Jung & McNaughton, 1993). These findings perhaps indicate that the DG has further distinct functionality to the remaining hippocampal sub-regions. The DG region remains distinct from the pyramidal layers of the hippocampus both because of the difference in neuron types, as well having an upper and a lower blade. The upper and lower blades of the DG have further been shown to contain neurons demonstrating distinct morphology. The difference in neuronal dendritic branching between the blades provides a potential explanation for the difference in activity seen between the upper and lower blades following spatial or non-spatial tasks (Gallitano, Satvat, Gil, & Marrone, 2016). Studies have shown immediate early gene expression within the upper blade of the DG shortly following performances of tasks involving spatial processing, whereas expression within neurons of the lower blade remains disperse (Chawla et al., 2005; Marrone, Satvat, Shaner, Worley, & Barnes, 2012). However, following longer delays, increased immediate early gene expression within the lower blade of the DG has been reported (Ramírez-Amaya et al., 2005). This dissociation, both between morphology and delay dependent function of the upper and lower blades of the DG potentially provides hints for the notion that the structure plays a role in memory consolidation over time. For the current experiment, no measures were taken to distinctly quantify the number of cFos expressing neurons within the upper and lower blades of the DG, instead the total number of cFos expressing neurons were quantified. As the main focus was initially aimed at examining cFos expression within the CA1 region of the hippocampus, it is with regret that the dissociation of the upper and lower blades of the DG was not made during cFos quantification. Fortunately, findings of cFos expression within the DG is of high interest and raise even further questions

141

regarding the role that granule cells may play during performance of avoidance tasks involving moving objects. It is difficult to make predictions of what the results may have unveiled if the quantification methods used for the current study had made the distinction between the upper and lower blades of the DG, but perhaps such analysis would aid in further dissociation between the functions of the blades. It is not difficult to imagine that perhaps the expression of cFos would be highest in the upper blade when mice were exposed to moving objects, and that little difference would be observed in the lower blades between the four test groups. It is likely that little difference would be observed within the lower blades given the euthanasia time; so that the delayed expression of the lower blades would not have been observed given the time frame between exposure and brain extraction.

Expression of cFos marker within cortical regions associated with processing non-spatial information, provide support for previous work, yet raise questions regarding how the task at hand affects such activity. The dramatically enhanced number of cFos expressing neurons within the LEC following exposures to familiar stationary objects is likely a direct result of the seemingly enhanced object information available to the mice. One can assume that when a moving identity is within the arena, which might need to be avoided, the mice will focus on what and where that item is, compared to observing the detailed features of the object. With this assumption, when mice that have mostly experienced the objects where one of them is continuously moving, are placed into the arena for the stationary object exposure (prior to tissue collection), it is not unlikely that when not required to actively perform the task, mice will increasingly focus on visual details of the objects. Such increase in object detail information would presumably result

in increased activity within areas processing object related information and although dramatic, the significantly higher expression of cFos within LEC under the stationary object condition is not unreasonable. If tissue collection would have allowed, staining for cFos expressing neurons within the MEC would have been expected to be highest within the moving objects group, comparable to that of the DG cFos expression. Previous work demonstrating the role of LEC in non-spatial object memory (Brown & Aggleton, 2001) receive strong support from the current result but further indicate that the task at hand has an effect on cFos expression within the region.

Neurons and neuronal networks

How information is processed within the brain to represent the outside world has not yet been fully established. It is a common assumption that neuronal activity processes information related to a specific event or a specific feature of stimuli and that action potentials neatly code for a given feature. Although in some areas, like the visual cortex, such reliable simplicity beautifully aligns, the reality is that the limited number of neurons within complex brain regions does not permit anything but plastic and detailed network functionality. Presumably, neuronal activity can result in changes within a single cell or the neuronal network that will slightly alter the functionality of the brain structure and as a result, the behavior of the organism can change, like has been shown to occur within the hippocampus (Silva-Gómez et al., 2003, Santarelli et al., 2003). Visualizing the activity or changes within the neurons and network should aid in understanding if, and how information is conveyed within a given brain region and can shed light on whether individual neurons can represent a specific feature in isolation, or if they only

143

function as a part of a complex and plastic network to represent multitude of various features.

Immunohistochemistry is a great tool used to shed light on how experience based information is represented within complex structures such as the hippocampus but it must be used cautiously and the results must be interpreted carefully. The method of staining for such physical changes or newly expressed markers within neurons is a helpful method to determine which brain regions or neurons may play a role in processing the presented stimuli which can aid in our understanding in stimuli representation. The currently utilized neuronal marker, cFos, has been demonstrated to be increasingly expressed within cells following high activity (Bullitt, 1990). One of the main disadvantages of experiments utilizing cFos markers is that it is not only expressed within neurons, but also other cell types within the brain such as glia. Because of this non-specific expression of cFos, the number of cFos expressing neurons can be inflated due to counter error (Tian & Bishop, 2002). Expression of the cFos marker has also been shown not to be exclusively found in excitatory neurons (Torterolo, Yamuy, Sampogna, Morales, & Chase, 2001). In studies utilizing cFos quantification, it is difficult to dissociate whether it is excitatory neurons or inhibitory interneurons preferentially expressing the activity marker without staining for additional markers (Gaykema et al., 2014). For the current experiment, the immunohistochemistry maker results may have been different following the various exposures to objects and contexts if a different neuronal maker were used, or an additional labeling mechanism were used in conjunction to cFos labelling. Perhaps, if *Arc* labeling would have been used, a plasticity marker only expressed within neurons (J. F. Guzowski et al., 1999) and not glia, a more reliable quantification measures would've

144

been observed. On the contrary, as the *Arc* immunohistochemistry marker is expressed following plasticity changes, and not simple activity, much like the cFos marker, additional differences could be observed if future experiments were to utilize *Arc* staining instead of cFos for a comparable behavioral experiment. The mice in the current experiments were trained in KYE task over multiple days and the lack of continuous improvement once the avoidance behavior had reached a plateau, would indicate that plasticity associated learning would not be as high as during initial training. Presumably, once the avoidance behavior has been fully acquired, plasticity within the network is reduced and one might expect not to observe high expression of a plasticity marker (*Arc*), so the expression of an activity marker (cFos) might be a more appropriate measure. Although cFos and *Arc*, as well as other immediate early gene markers, have been shown to yield comparable and somewhat simultaneous expression within the hippocampus, it remains unknown how the ensemble of such gene markers is expressed following an experience, or if various experiences cause differential expression within neurons (Minatohara, Akiyoshi, & Okuno, 2015). Furthermore, few studies have explored the difference in plasticity and activity markers following such extensive training as within the current experiments. Regardless, it remains of high interest to stain for neuronal expression of *Arc* in a different set of brain slices from the mice used for cFos staining and compare counts obtained from each mouse, under the various exposures to further elucidate what neurons may be contributing to the ongoing behavioral performance of trained mice. In addition, previous work has suggested that the counting method used to acquire the number of cFos expressing neurons does not always yield consistent and reliable results (Mura, Murphy, Feldon, & Jongen-Relo, 2004). The notion that the

145

stereological parameters and profile counting methods used to quantify cFos expressing neurons in the same sections do not always produce comparable results does not provide support for the notion that immunohistochemical marker quantification is a reliable tool. The clear difference between experimental groups and low variance within the current cFos results reduces the probability that many of the above discussed flaws of immunohistochemistry had a large effect on the current findings; however, there is room for improvement from standardization in order to isolate the true effects neuronal activity or plasticity following experiences within the hippocampus.

Like stated above, the simple function of neuron representing a single stimulus is perhaps too farfetched given brain regions like the hippocampus. An alternative view is that memory feature representations are not simply coded by neuronal firing or spikes. Perhaps we are interpreting neuronal spike functions in an incorrect way and assigning too much meaning to the action potential. Possibly the information relayed in each action potential or a spike train does not directly represent the information processing of the brain, but rather it is the joint firing of the network that conveys the information. Without such network activity, each neurons firing properties cannot not carry much meaning alone. Recent work has suggested that it would be virtually impossible for a single neuron to have a highly specialized function and perhaps only represent one feature or stimuli. Such specialized response properties would greatly limit the amount of representations the brain or a given region could represent (Fusi, Miller, & Rigotti, 2016). Higher dimensionality of neural representations provides a more appropriate explanation of how the brain can continuously represent increasing amount of information throughout an individuals' life. If neurons or the neuronal network demonstrates such mixed

146

selectivity, while maintaining the ability to represent multiple features or stimuli, perhaps the increased firing of object-cells does not fully represent the explored object, in a complete or a partial manner. Perhaps the firing observed while mice explore 3D objects is not representing the exploration or the object at all, but rather partially conveying information of different feature of the behavior or the experience. Future work is required to elucidate the mutual firing of multiple or all the neurons within the hippocampus to elucidate how information is represented. New technology, such as a head mounted camera, simultaneously visualizing Ca^{2+} expression in numerous cells (Ghosh et al., 2011) will aid in understanding of how such network activity processes information regarding features of episodic memory during encoding and retrieval. With other developments in neuroscience technology we will be able to unveil the specific functions of the hippocampal circuit to truly answer the questions of how the brain represents both spatial and non-spatial episodic memory.

Table 1. Firing properties of the four CA1 neurons that exhibited increased object-specific firing over time.

Mouse # / Session	Cell Type	Average In Field Hz	Mean Overall Hz	Max Hz	# of Bursts	Recording Session Length (sec)	Mean ISI	Context	Object
93-4 Day 1 without object	Ch 7 Place cell	1.5	0.35	17.76	21	607.93	2.84	Novel	
	Ch 14 Object cell	0.8	0.40	3.32	1	607.93	2.49		Novel
93-4 Day 1 with object	Ch 7 Place cell	3	0.47	25.53	44	601.77	2.11	Familiar	
	Ch 14 Object cell	1	0.41	3.23	0	601.77	2.41		Novel
93-4 Day 21 without object	Ch 8 Place cell	3	0.60	10.02	25	601.76	1.15	Novel	
	Ch 14 Object cell	1	0.39	4.29	1	601.76	2.56		Novel
93-4 Day 21 with object	Ch 8 Place cell	2	0.26	4.91	8	623.59	1.47	Familiar	
	Ch 14 Object cell	3.5	0.65	5.41	1	623.59	1.51		Novel
61-4 Day 1 without object	Ch 10 Place cell	3.5	1.43	42.23	58	301.06	0.69	Familiar	
	Ch 13 Object cell	0.8	0.68	3.94	0	301.06	1.37		
61-4 Day 1 with object	Ch 10 Place cell	1.5	0.74	26.09	27	310.43	1.27	Familiar	Familiar
	Ch 13 Object cell	2	1.36	3.32	3	310.43	0.73		
61-4 Day 10 without object	Ch 10 Place cell	3	1.13	18.02	36	304.90	0.88	Familiar	
	Ch 13 Object cell	1	0.82	5.62	2	304.90	1.17		
61-4 Day 10 with object	Ch 10 Place cell	8	1.49	28.90	57	413.26	0.66	Familiar	Novel
	Ch 13 Object cell	4	1.63	5.96	8	413.26	0.61		
72-1 Day 1 without object	*No place cell well isolated*								
	Ch 7 Object cell	0.8	0.62	9.68	0	303.50	1.61	Novel	
72-1 Day 1 with object	*No place cell well isolated*								
	Ch 7 Object cell	1	0.44	7.95	3	345.60	2.22	Novel	Familiar
72-1 Day 6 without object	*No place cell well isolated*								
	Ch 7 Object cell	1.5	0.94	7.69	1	303.96	1.06	Familiar	
72-1 Day 6 with object	Ch 7 Object cell	3	1.15	6.92	0	301.96	0.85	Familiar	Familiar
197-1 Day 1 without object	Ch 5 Place cell	1.5	0.81	10.74	20	301.36	1.23	Familiar	
	Ch 2 Object cell	1	0.83	4.03	0	301.36	1.21		
197-1 Day 1 with object	Ch 5 Place cell	1.5	0.51	5.23	7	301.60	1.94	Familiar	
	Ch 2 Object cell	1.5	0.86	6.95	0	301.60	1.14		Familiar
197-1 Day 11 without object	Ch 5 Place cell	1.5	0.74	9.87	16	300.83	1.33	Familiar	
	Ch 2 Object cell	1.5	1.38	4.84	0	300.83	0.72	Novel	
197-1 Day 11 with object	Ch 5 Place cell	2	0.81	7.20	14	308.26	1.22		
	Ch 2 Object cell	3	2.30	5.66	2	308.26	0.43	Familiar	Familiar

Table 1. Object-specific neuronal firing properties

148

Table 2. *P*-values obtained from analysis of behavioral performance during training in each of four Enemy Avoidance experiment. Significant values indicate that performance of the mice to avoid the object improves after 5 training bins.

Table 2

Enemy Avoidance Experiment	Stationary	Moving
One context CA1 muscimol	b = -1.15, t = -6.02, p < .001	b = -1.93, t = -8.20, p < .001
Novel context change	b = -0.92, t = -5.86, p < .001	b = -0.88, t = -4.60, p < .001
Familiar context change	b = -1.16, t = -6.71, p < .001	b = -1.68, t = -7.43, p < .001
Multiple contexts CA1 muscimol	b = -0.95, t = -6.89, p < .001	b = -1.02, t = -3.72, p < .001

Table 2. Enemy avoidance training p-values listed by experiment

Table 3

Table 3. *P*-values obtained from analysis of behavioral performance during training in each of four Knowing Your Enemy experiment. Significant values indicate that performance of the mice to avoid the object improves after 5 training bins.

Knowing your Enemy Experiment	Good stationary	Good moving	Bad stationary	Bad moving
One context CA1 muscimol	b = -.64, p = .006	b = -.74, p = .004	b = -0.49, p < .001	b = -.89, p < .001
Novel context change	b = -.93, p < .001	b = -1.12, p < .001	b = -0.47, p < .001	b = -1.50, p < .001
Familiar context change	b = -.87, p < .001	b = -1.26, p < .001	b = -0.48, p < .001	b = -1.73, p < .001
Multiple contexts CA1 muscimol	b = -.80, p < .001	b = -.54, p = .03	b = -.41, p = .014	b = -0.68, p = .002
Diazepam IP injection	b = -1.09, p = .001	b = -0.94, p < .001	b = -0.30, p = .036	b = -1.31, p < .001

Table 3. Knowing your Enemy training p-values listed by experiment

Table 4. Quantifications of cFos expressing neurons within sub-regions of the hippocampus, BLA and LEC following exposure to one of four experimental variations.

Range of point counts obtained within region	CA1	CA3	DG	BLA	LEC
	4-48	4-47	9-74	11-71	3-69
Experimental groups	*Range of total cells estimated per region*				
Cage control	140-493	175-516	338-1141	432-947	96-836
Empty context	330-448	487-756	655-1140	2198-2749	557-1329
Stationary objects	1041-1801	856-1409	831-1388	2704-3340	3390-4944
Moving objects	1069-2624	1239-2111	1312-1889	2079-2707	1189-1784

7 sections were probed with dissector samples with a(frame)= 40 x 40 μm and a height (h) of 7 μm at an average of 486 sampling sites per region

Table 4. Range of cFos expression per region

Figure 1. Schematic of memory organization. a) Declarative memory dissociation within the medial temporal lobe. (Adapted from, Squire, 2004) b) Nondeclarative memory organization, thought to depend on brain regions outside of the medial temporal lobe
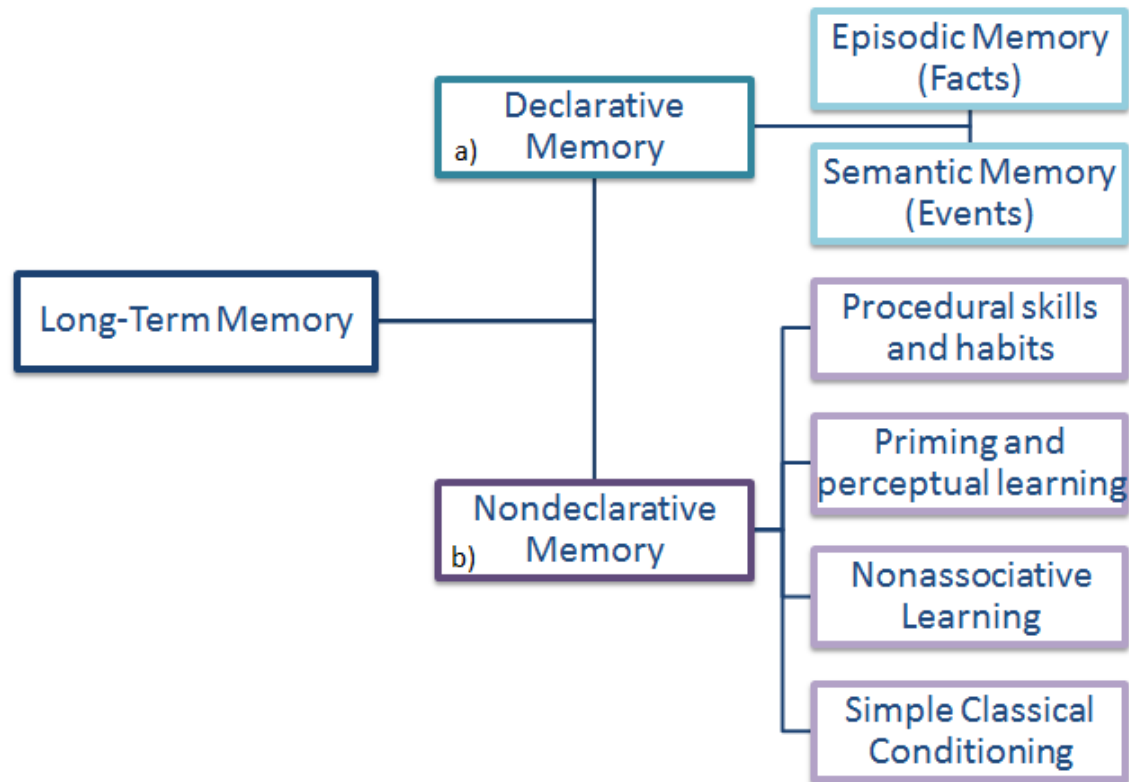
Figure 1. Long-term memory organization

Figure 2. The hippocampal circuit within the medial temporal lobe demonstrating the information network (Hartley, Lever, Burgess, & O'Keefe, 2014). Postrhinal cortex projects spatial information directly or indirectly to the hippocampus through the medial entorhinal cortex. The perirhinal cortex projects non-spatial information to the hippocampus via the lateral entorhinal cortex. The entorhinal cortex projects to the hippocampus directly to subiculum and CA1 or through the perforant pathway, via the dentate gyrus.
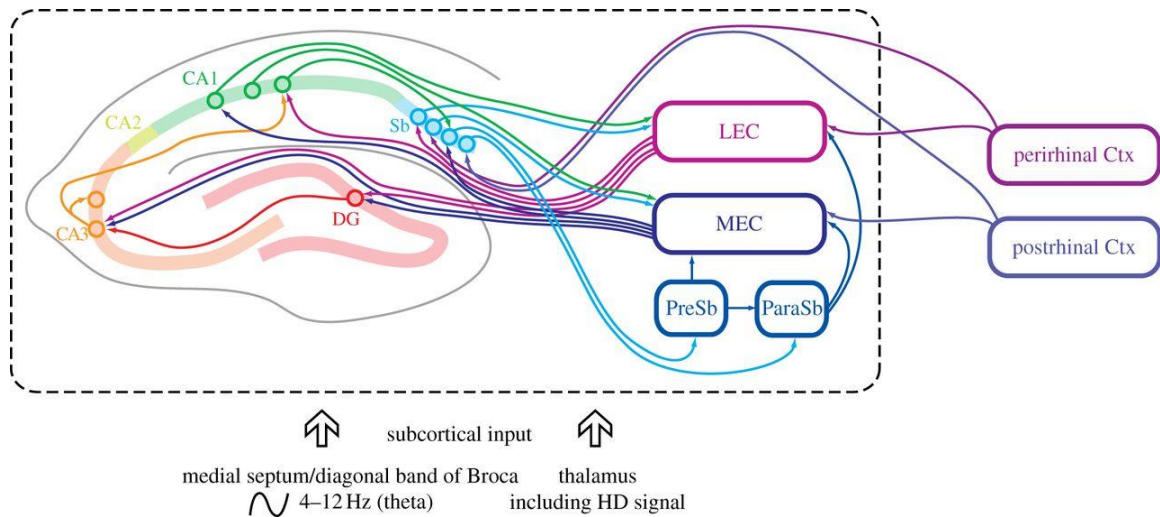
Figure 2. The information network of the hippocampus

Figure 3. a) Radial arm maze used for assessing spatial working memory. Two of the arms are baited with food reward b) Morris water maze task. Left shows typical swimming path of an untrained rodent during first training sessions, and right shows typical path following successful training. c) In vivo hippocampal place cell recording setup. Lights mounted on rats head track position of the animal within the arena. Heat map on right shows color-coded firing frequency of a recorded neuron. Blue colors represent low Hz and red represents maximum average in field Hz with the average in field firing rate shown above the heat map (Bottom figure is modified from: (Muller et al., 1987).
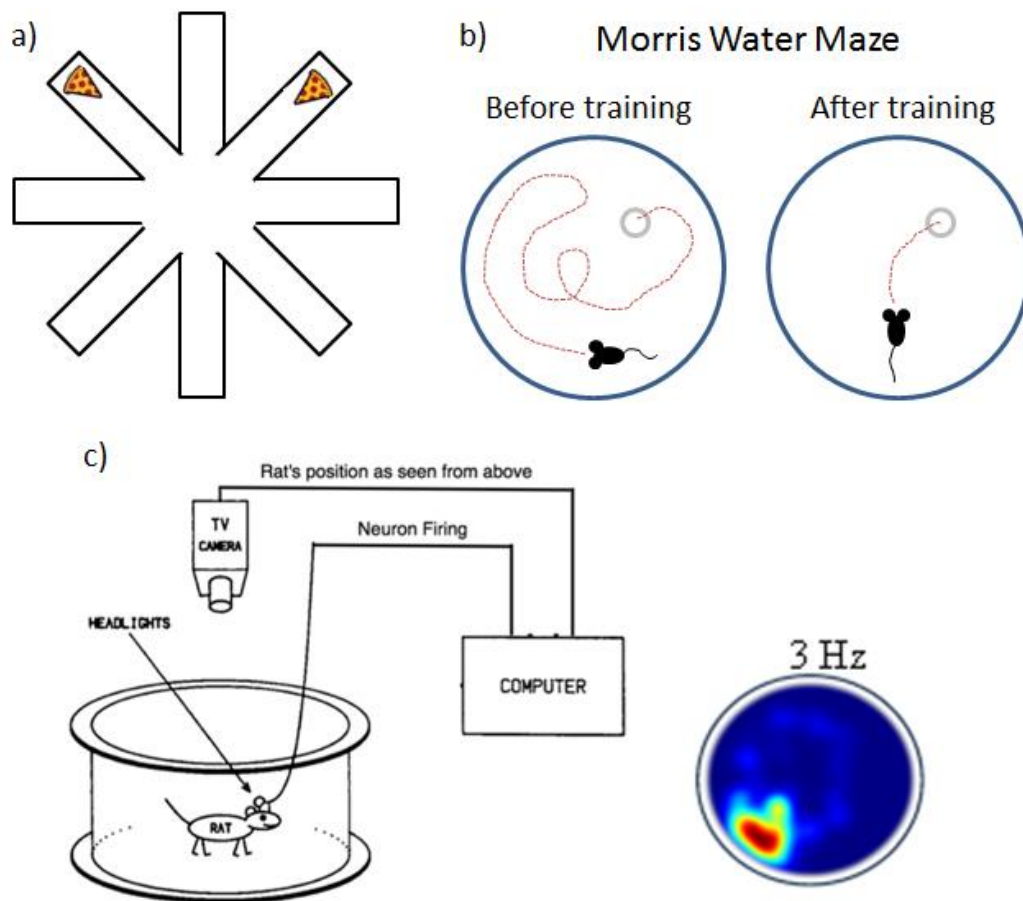
Figure 3. Tools for assessing spatial memory

Figure 4. Novel Object Recognition protocol commonly used for behavioral testing. During normal hippocampal function, the rodent should have the ability to encode, consolidate and retrieve the object memory; resulting in preference for novel object during the Test session. When the hippocampus is inactivated immediately following the Sample session or before the Test session, the rodent is unable to consolidate or retrieve the object memory, respectively. These impairments result in the mouse failing to exhibit an exploratory preference for the novel object during the Test session but rather spending equal time with both objects during test session. Image from: (Asgeirsdottir, 2013).
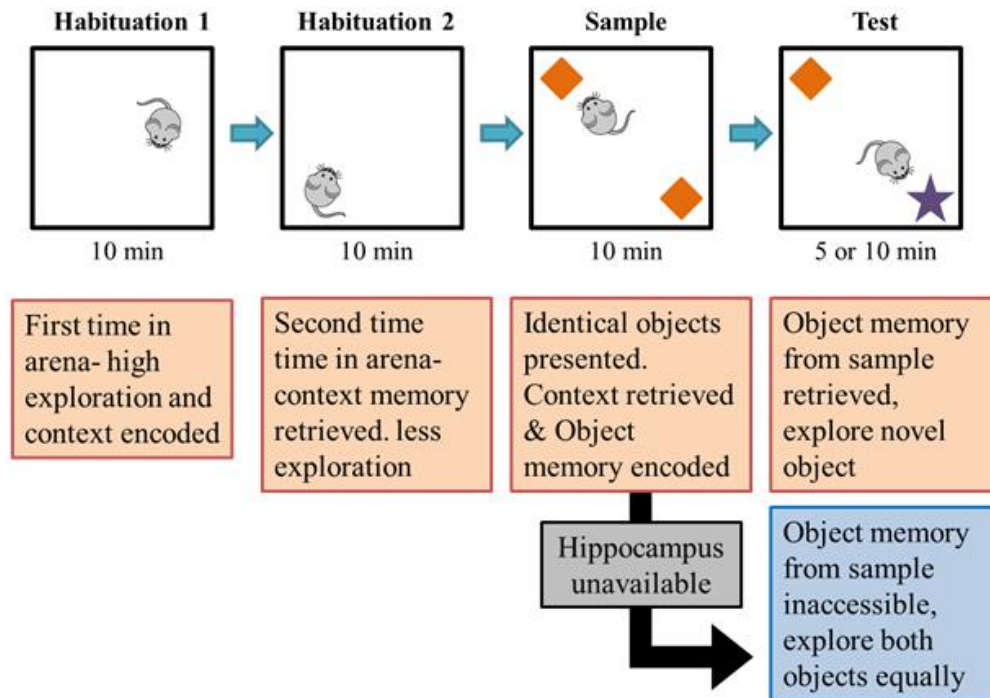
Figure 4. Novel object recognition protocol

Figure 5. Normalized firing frequency of 6 dorsal hippocampal CA1 neurons prior to, during and at multiple time points following a muscimol infusion into the recording region. Grayed block represents time points where muscimol was in effect.
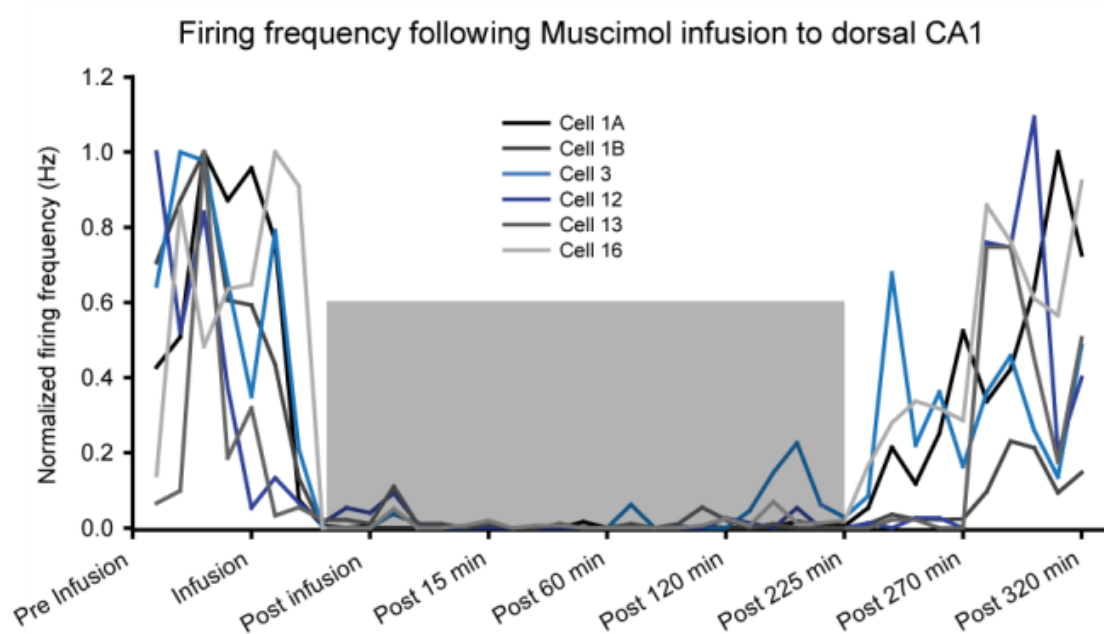
Figure 5. Firing rates of hippocampal neurons following a local muscimol infusion into CA1 region of the dorsal hippocampus

Figure 6. Hippocampal CA1 object-related firing. a) Representative placement of the unilateral tetrode arrays implanted over the CA1 region of the right and medial dorsal hippocampus for four mice (blue-filled circles) that object cell activity was recorded from. b) Representative heat maps from a CA1 place cell and a simultaneously recorded CA1 object cell during habituation 2 and test of the OR task (note difference in Hz scale). The perievent histograms (four graphs on right) depict activity from the place cell (top) and the object cell (bottom) during test of the *Cue Card OR* protocol while the mouse was engaged in object exploration (right) and while not exploring (left). The graphs show averaged spike counts per bin from twelve separate events of object exploration (green dots) and non-exploration (red dots) from manual codes. Time 0 marks the onset of exploration of either object within the arena (Exploring object) or a time when the mouse was away from the object, either walking or looking around (Not exploring). Lower dotted line represents the expected mean firing rate for that cell and the upper dotted line represents the 99% confidence limit.
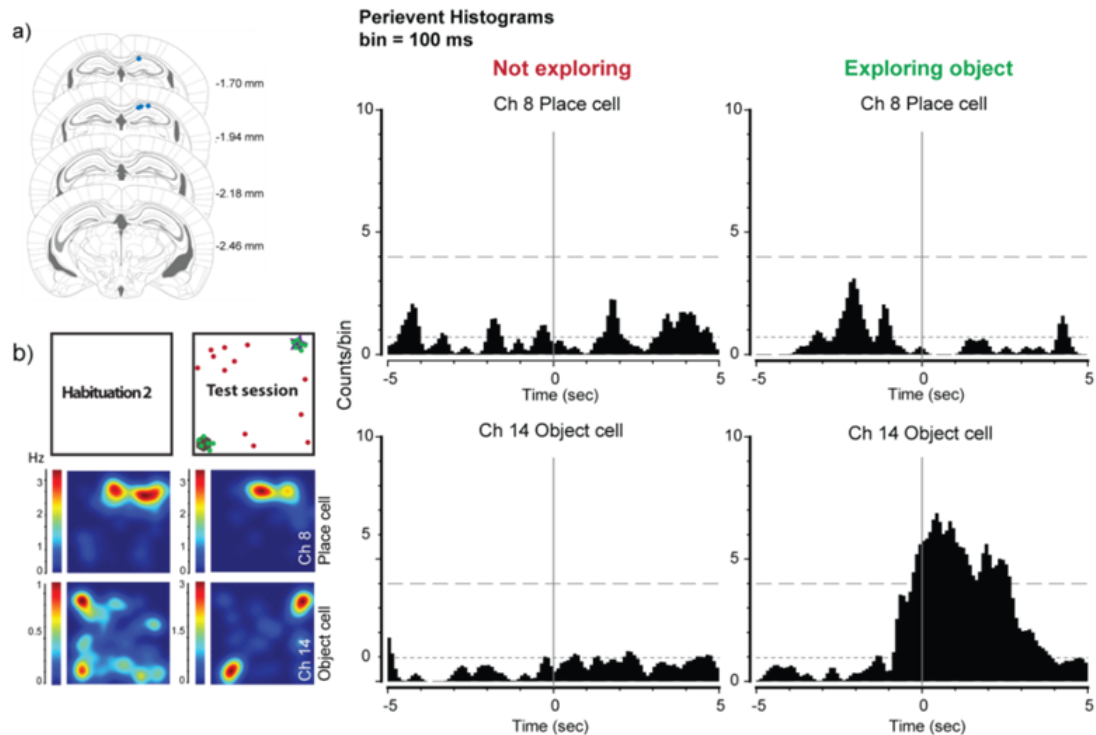
Figure 6. Hippocampal CA1 object-related firing properties

Figure 6 continued. Hippocampal CA1 object-related firing. c) Representative schematic of the protocol, arena, and object locations (top) with place x firing map of a neuron demonstrating maximal firing at locations of 3D objects recorded throughout 11 session across 20 days (bottom) in 4 different contexts. d) Representative autocorrelograms of five CA1 neurons recorded over a 300 sec period. Top: complex spike firing properties of a CA1 place cell. Below: four regular spiking neurons discharging where objects were present (absence of complex spike patterns). e) Firing frequency increased continuously over time (days), discharging specifically at object location (n = 4 CA1 neurons). f) Firing frequency of CA1 place cells recorded simultaneously to object cells exhibit no consistent changes over time (days).
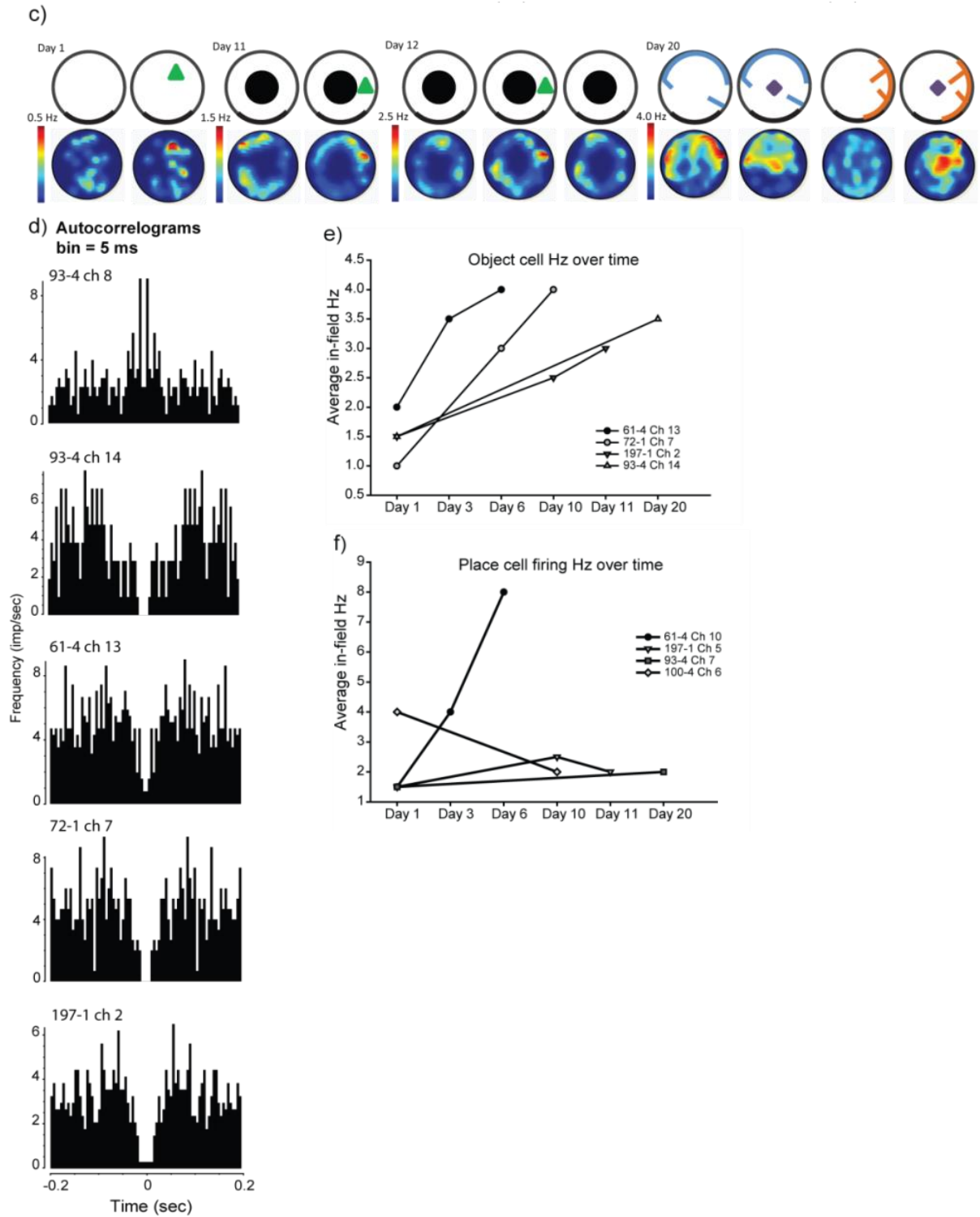
Figure 6 Continued. Hippocampal CA1 object-related firing properties

Figure 7. Representative intrahippocampal infusion sites within the CA1 region of the dorsal hippocampus of mice used for behavioral experiments. Bottom images show a representative photomicrograph of cannula placement. a) Infusion sites within the hippocampus of mice tested in both EA and KYE trained within one context. b) Infusion sites within the hippocampus of mice tested in both EA and KYE trained multiple novel contexts.
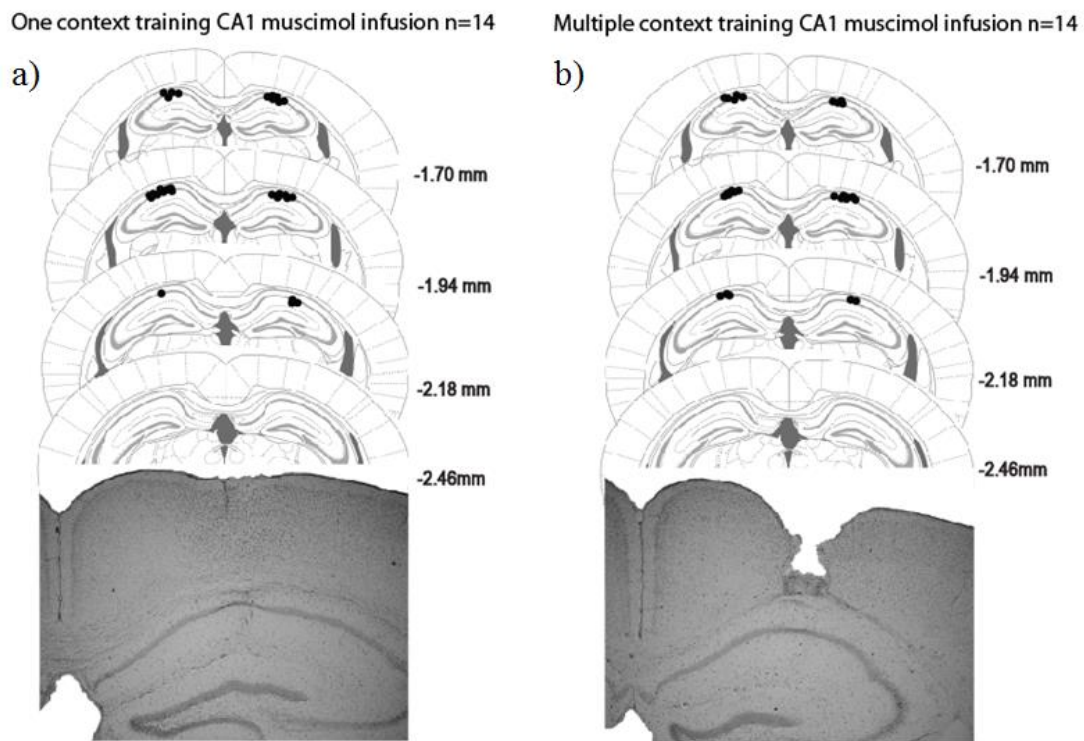
Figure 7. Histological verification of infusion sites for mice used in behavioral studies

Figure 8. The Enemy Avoidance task (EA). a) Experimental setup for training mice in the EA paradigm. Overhead cameral tracks the location of the mouse and bad object within the arena. Computer program detects distance between subjects and delivers shock when the distance between mouse and bad object drops below 6 cm. b) Behavioral protocol for training mice within EA. One training bin is shown, consisting of four 2-min sessions where the object alternates between a moving or stationary state. c) Training protocol over multiple days of the EA paradigm. Time between bars indicates time between training/testing bins. Shock is only delivered during training and is inactive during testing bins 7 & 9.

Figure 8. The Enemy Avoidance task

161
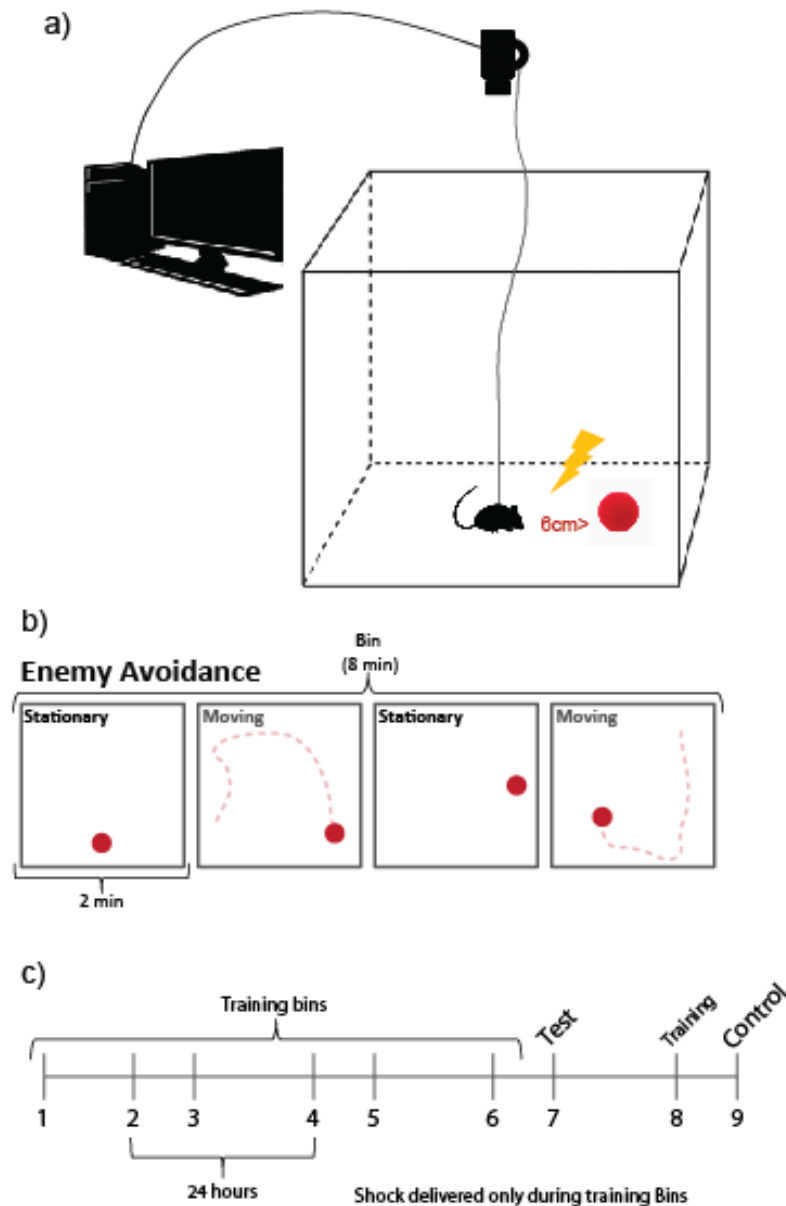
Figure 9. The Knowing Your Enemy task (KYE). a) Experimental setup for training mice in the KYE paradigm. Overhead cameral tracks the location of the mouse and objects within the arena. Computer program detects distance between subjects and delivers shock when the distance between mouse and bad object drops below 6 cm. Shock is never delivered when mouse is in close proximity to the good object . b) Behavioral protocol for training mice within KYE. One training bin is shown, consisting of four 2-min sessions where the good and bad objects alternates between moving and stationary states. c) Training protocol over multiple days of the EA paradigm. Time between bars indicates time between training/testing bins. Shock is only delivered during training and is inactive during testing bins 7 & 9.
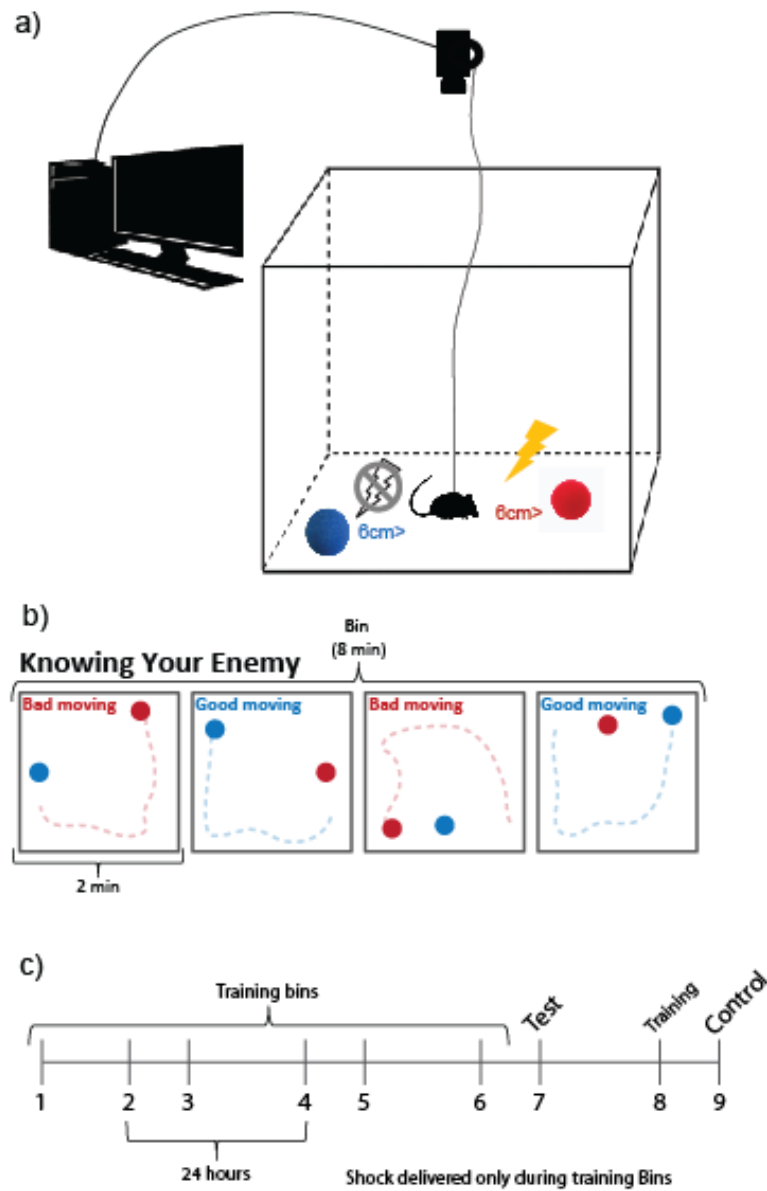
Figure 9. The Knowing Your Enemy task

Figure 10. Behavioral performance in four Enemy Avoidance experiments, all using distinct cohorts of mice. Proximity events throughout training presented as an inserted graph within each testing vs. control graphs. a) Average proximity events measured following a muscimol (vs Saline) infusion into dorsal CA1 after training in one context (Exp. 1a). b) Average proximity events during avoidance testing in a novel context (vs testing context) after training in one context (Exp. 1b). c) Average proximity events during avoidance testing in an unpaired familiar context (vs testing context) after training in one context (Exp. 1c). d) Average proximity events measured following muscimol (vs Saline) infusion into dorsal CA1 after training in multiple novel contexts (Exp. 1d). Colored bar on training graph indicates performance of successfully trained mice (expected performance) while object is moving.
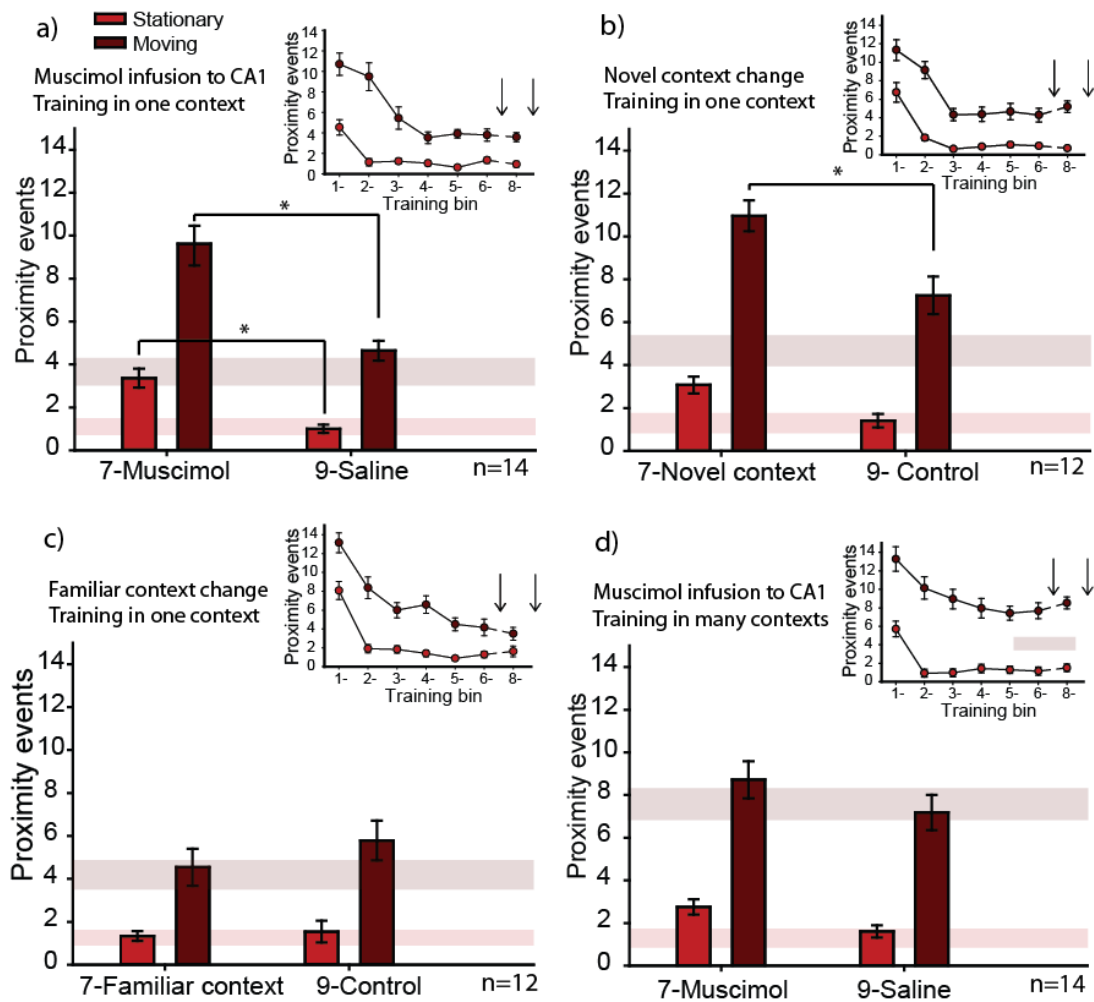
Figure 10. Behavioral performance in Enemy Avoidance experiments

Figure 11. Elevated Plus Maze anxiolytic measures following a Muscimol infusion into the dorsal CA1 region of the hippocampus. a) Top- Representative bilateral intra-hippocampal infusion sites within the CA1 region of the dorsal hippocampus. Bottom- Characteristic photomicrograph of the intra-hippocampal microinfusion site into the CA1. b) Average recorded velocity on the elevated plus maze following a local infusion of muscimol or saline into dorsal region of CA1. c) Average % time spent in open arms following an infusion of muscimol or saline into the dorsal region of CA1. d) Average % entries into open arms following an infusion of muscimol or saline into the dorsal region of CA1. e) Average distance traveled during the plus maze test following an infusion (n=8 Muscimol, n=10 saline) (Mean ±SEM).
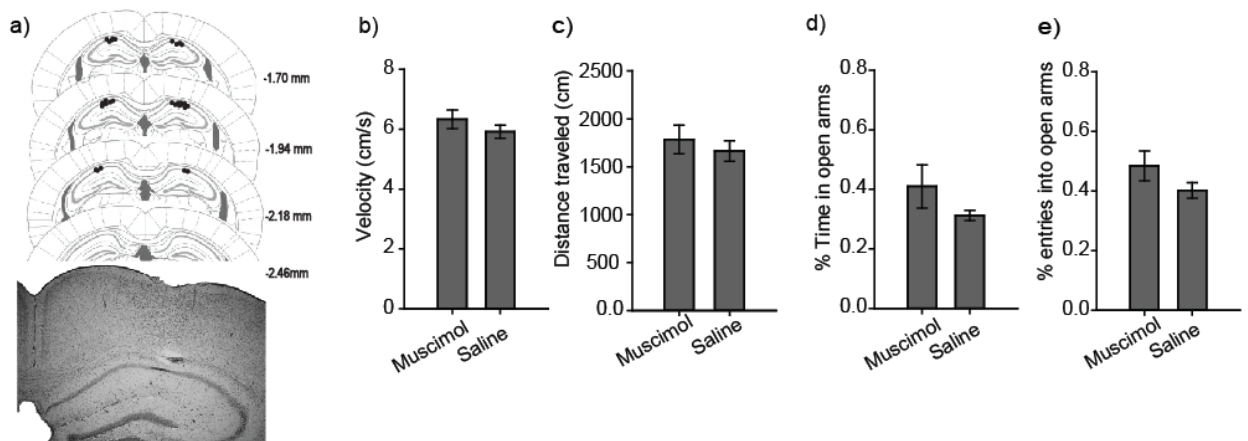
Plus Maze Muscimol infusion into ca1
Musciomol n=8
Saline n=10

Figure 11. Elevated Plus maze performance following local infusion of muscimol into CA1 of the dorsal hippocampus

Figure 12. Behavioral performance in Knowing Your Enemy following hippocampal inactivation after training within one context. Proximity events throughout training presented as an inserted graph within each testing vs. control graphs. a) Average proximity events measured while each object is stationary following a muscimol (vs Saline) infusion in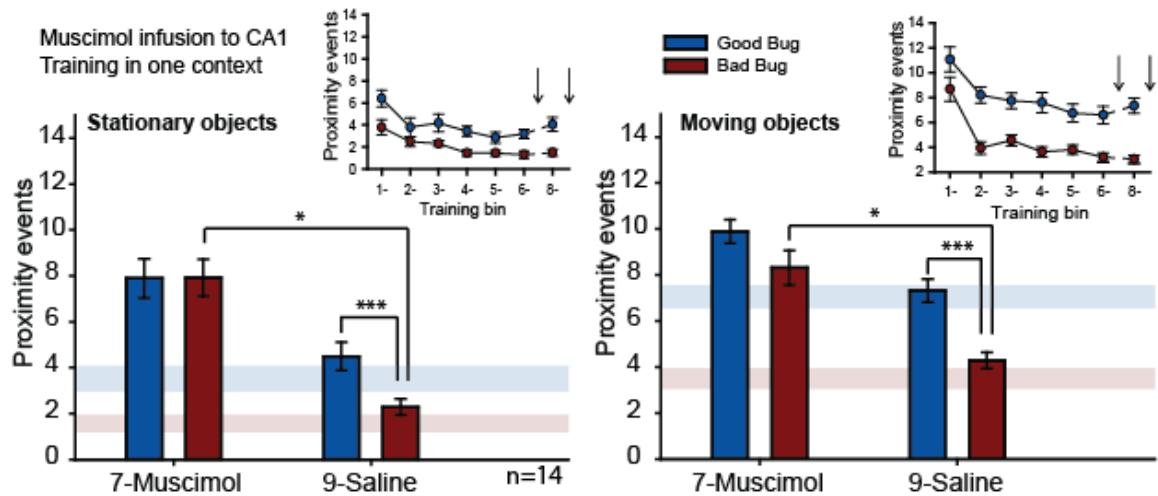to dorsal CA1 after training in one context. b) Average proximity events measured while each object is moving following a muscimol (vs Saline) infusion into dorsal CA1 after training in one context.

Figure 12. Knowing Your Enemy performance within one context CA1 inactivation (Exp. 3a)

Figure 13. Behavioral performance in Knowing Your Enemy during novel contextual change after training within one context. Proximity events throughout training presented as an inserted graph within each testing vs. control graphs. a) Average proximity events measured while each object is stationary, presented in a novel context (vs. training context) after training in one context. b) Average proximity events measured while each object is moving, presented in a novel context (vs. training context) after training in one context.
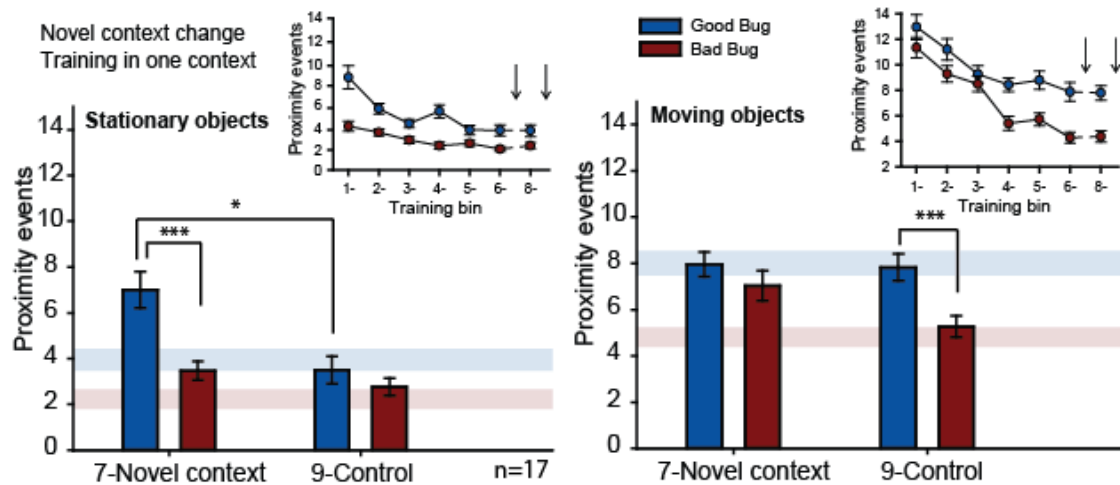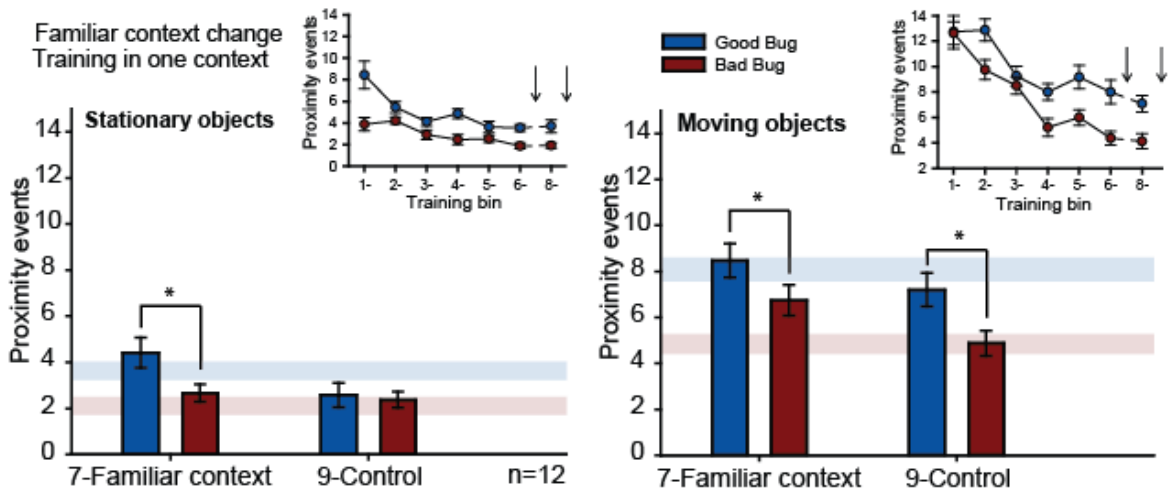

Figure 13 Knowing Your Enemy performance within novel context change (Exp. 3b)

Figure 14. Behavioral performance in Knowing Your Enemy during familiar unpaired contextual change after training within one context. Proximity events throughout training presented as an inserted graph within each testing vs. control graphs. a) Average proximity events measured while each object is stationary, presented in a familiar context (vs. training context) after training in one context. b) Average proximity events measured while each object is moving, presented in a familiar context (vs. training context) after training in one context.

Figure 14. K Knowing Your Enemy performance within familiar unpaired context change (Exp. 3c)

Figure 15. Behavioral performance in Knowing Your Enemy following hippocampal inactivation after training within multiple novel contexts. Proximity events throughout training presented as an inserted graph within each testing vs. control graphs. a) Average proximity events measured while each object is stationary following a muscimol (vs Saline) infusion into 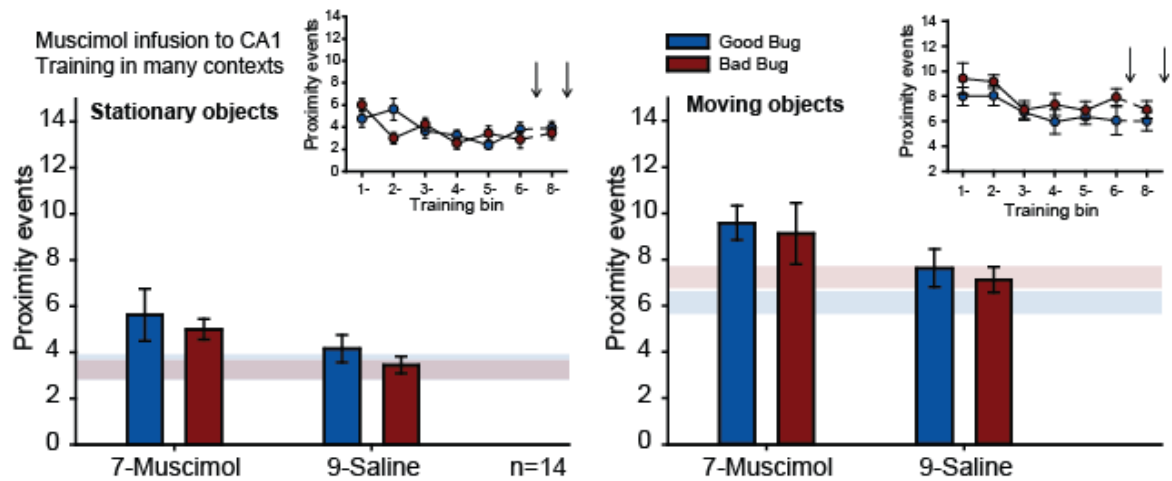dorsal CA1 after training in multiple novel contexts. b) Average proximity events measured while each object is moving following a muscimol (vs Saline) infusion into dorsal CA1 after training in multiple novel contexts.

Figure 15. Knowing Your Enemy performance within CA1 inactivation during novel context change (Exp. 3d)

Figure 16. Behavioral performance in Knowing Your Enemy following an IP injection of Diazepam after training within one context. Proximity events throughout training presented as an inserted graph within each testing vs. control graphs. a) Average proximity events measured while each object is stationary following an IP injection of Diazepam(vs Sal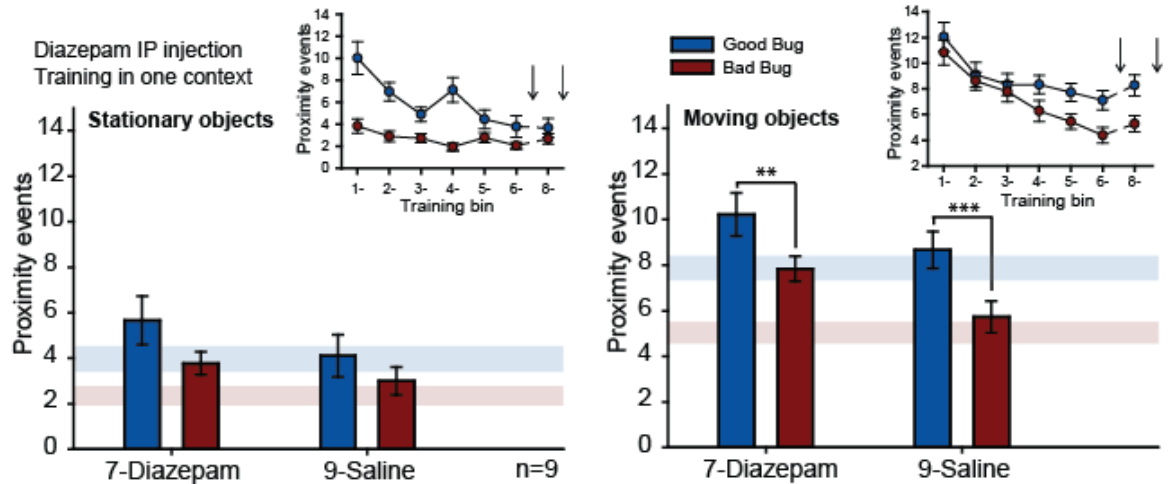ine) after training in one context. b) Average proximity events measured while each object is moving following an IP injection of Diazepam (vs Saline) after training in one context.

Figure 16. Knowing Your Enemy performance within IP injection of Diazepam (Exp. 3e)

Figure 17. Immunohistochemistry exposure groups. Mice (n=20) were exposed to one of four experimental groups prior to transcranial perfusion and tissue extraction 80 minutes later.
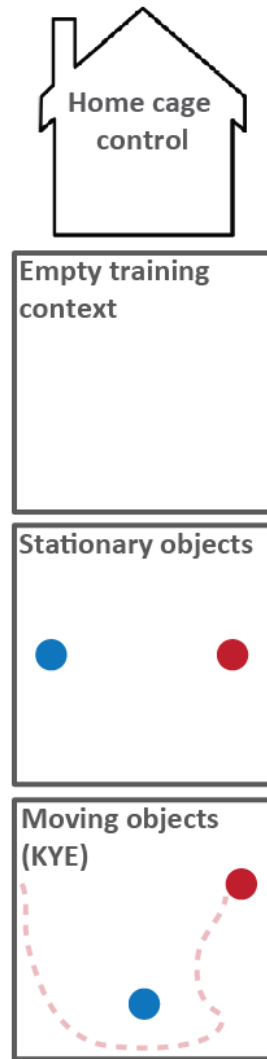

Figure 17. Exposure groups for immunohistochemistry

Figure 18. a) cFos expression within the CA1, CA3 and DG of the dorsal hippocampus in mice exposed to one of four experimental groups. b) cFos expression within the BLA in mice exposed to one of four experimental groups. c) cFos expression within the LEC in mice exposed to one of four experimental groups.
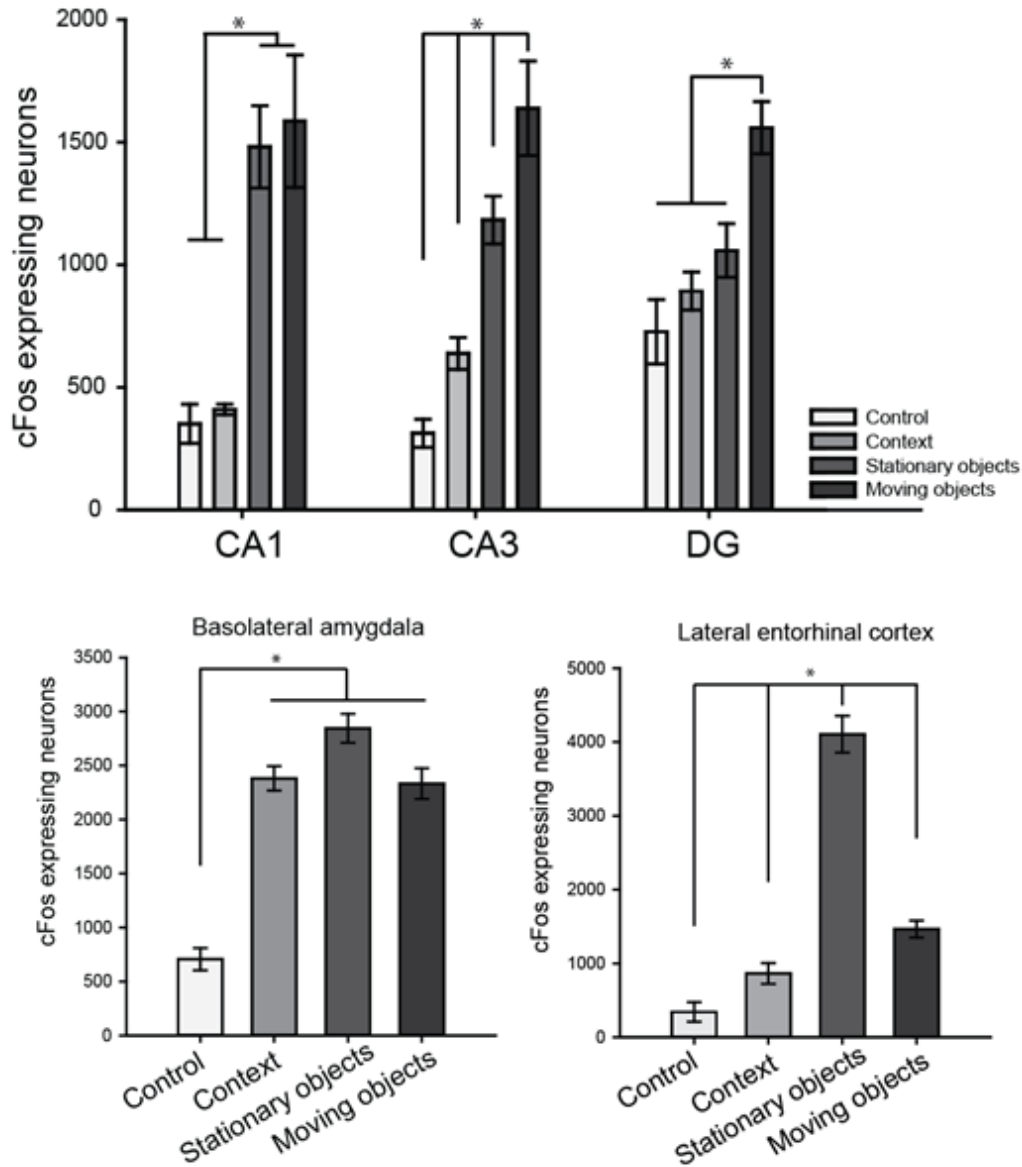

Figure 18. Quantified cFos expression per brain region

Figure 19. Object discrimination by mice exposed to objects prior to cFos quantification. a) Average proximity events measured from mice exposed to the stationary objects prior to cFos neuronal expression quantification. b) Average proximity events measured from mice exposed to the moving objects exposure (regular KYE testing bin) prior to cFos neuronal expression quantification. Discrimination also plotted for the objects while stationary.
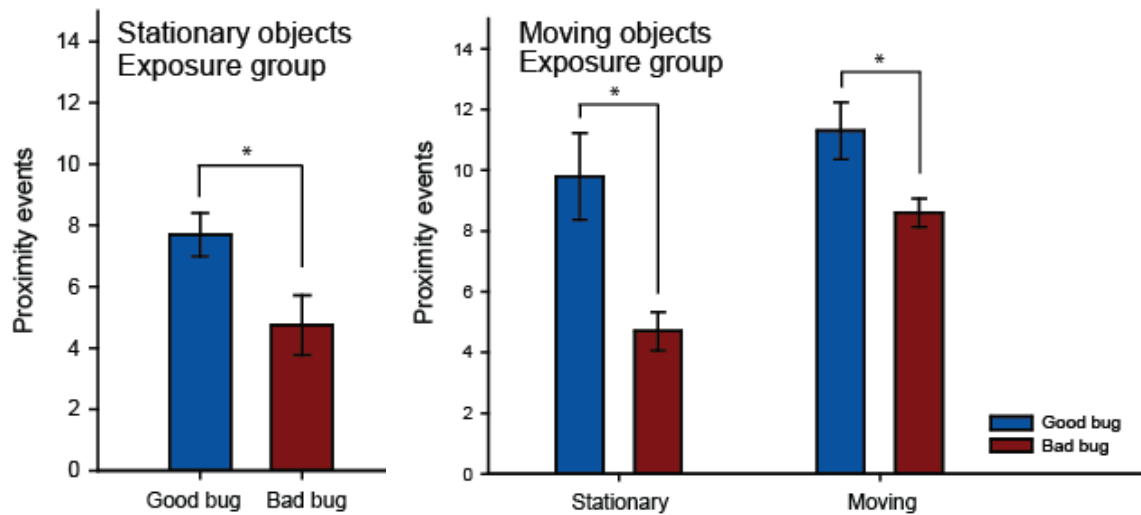

Figure 19 Object discrimination during Knowing Your Enemy prior to immunohistochemistry marker analysis

Figure 20. a) Distance traveled while mice were exposed to one of three conditions where behavioral analysis was possible (Empty context, Stationary objects, Moving objects). b) Relationship between the distance traveled during the exposure and number of cFos expressing neurons within CA1, CA3 and DG sub-regions of the dorsal hippocampus
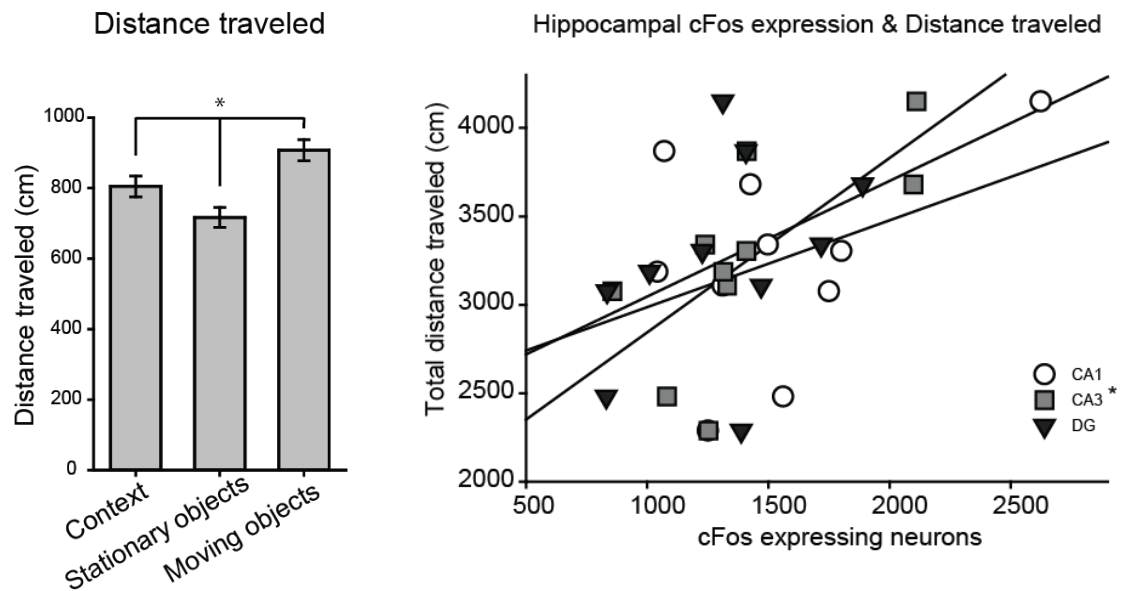

Figure 20. Distance traveled correlated with hippocampal cFos expression

Figure 21. Hippocampal CA1 place cells recorded in the presence of moving or stationary objects. a) Cue card rotation protocol commonly used to screen for place cells, used here to determine effects on stability measures in the presence of moving or stationary objects. Each session is 5 min long, with the mouse removed for few minutes in between while arena is cleaned and cue card rotated (East-North-East2). b) Micrograph and corresponding disruption of tissue above hippocampus demonstrating recording locations of the 4 mice used for these recordings. c) Averages stability measure (r), mean spatial coherence, mean information content, maximum firing frequency, and spike count calculated between the three cue card rotations with stationary or moving object, independent of mouse behavior. d) Liner regression demonstrating the relationship between information content and spatial coherence. Sessions containing a stationary object yield a significant relationship between the two measures.
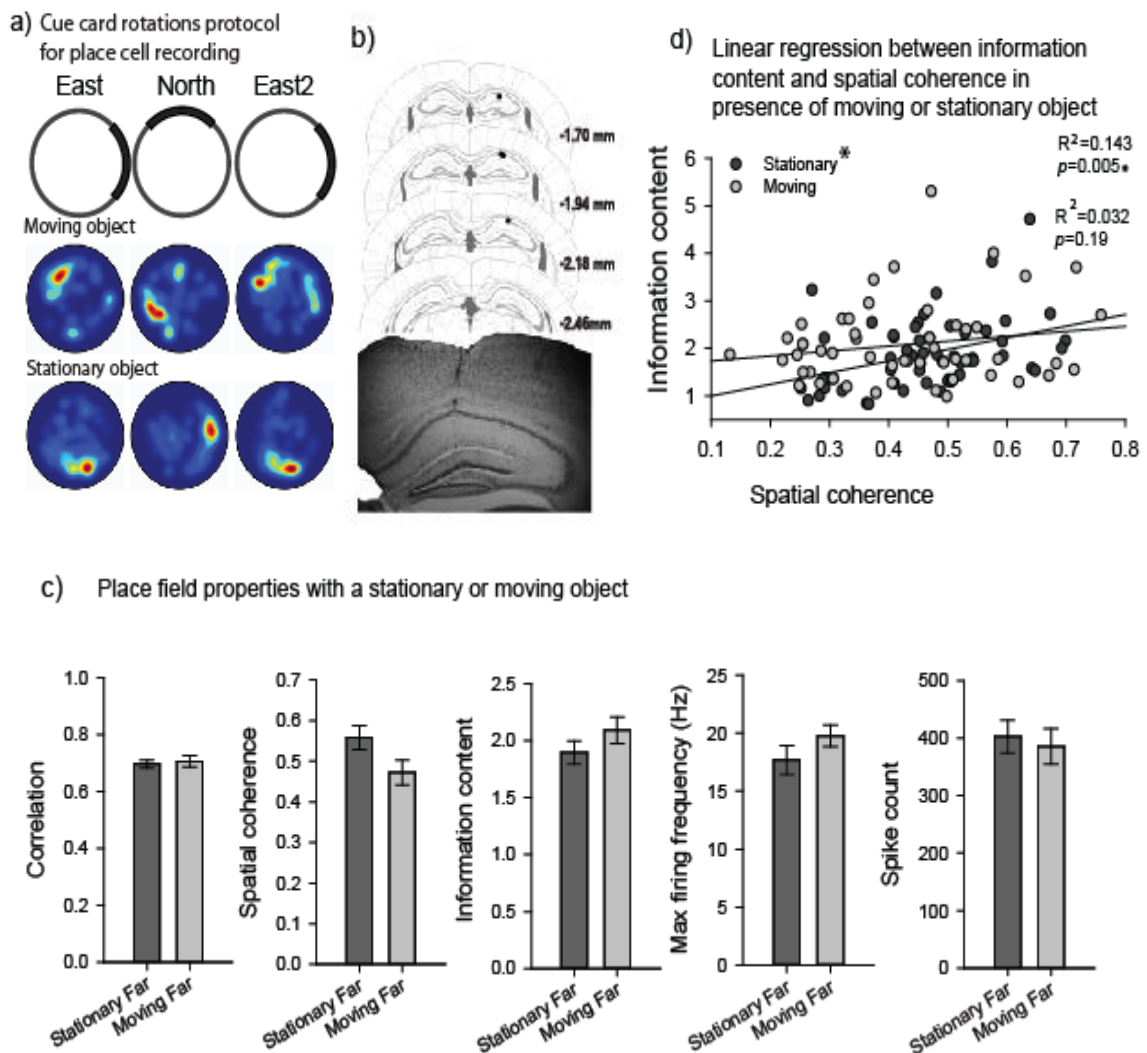

Figure 21. CA1 place cell activity recorded while mouse is in the presence of moving or stationary objects

174

Figure 21 continued. Hippocampal CA1 place cells recorded in the presence of moving or stationary objects. e) Time spent near or far from object when the object is either moving around the arena or stationary. These reduced time periods were used to plot the graphs shown below. f) Representative heat maps of cue card rotations plotted from the reduced sampling obtained from the manually coded "near-far" files. g) Averages stability measure (r), mean spatial coherence, mean information content, maximum firing frequency, and spike count calculated between the three cue card rotations with stationary or moving object, dependent on mouse behavior.
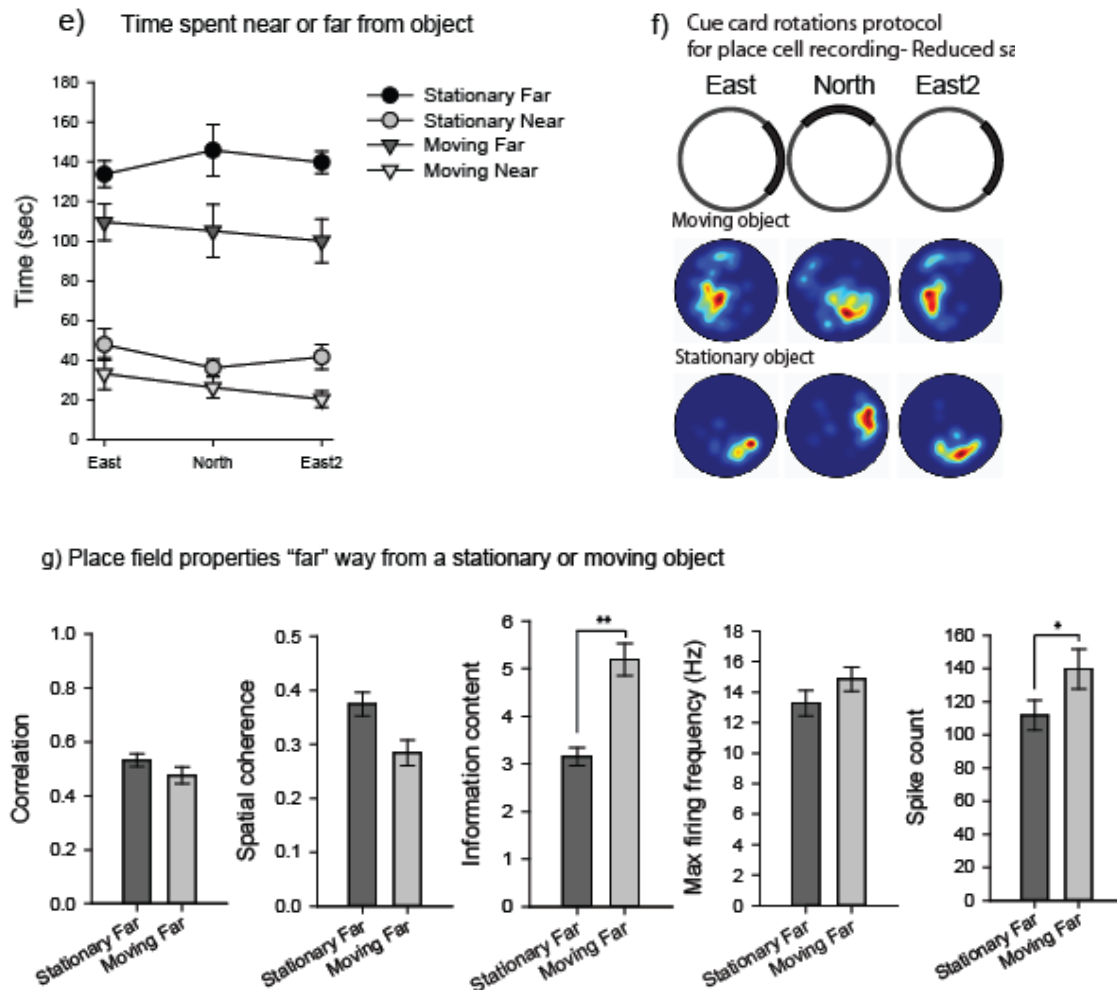

Figure 21 continued. CA1 place cell activity recorded while mouse is in the presence of moving or stationary objects

PART V: REFERENCES

Ainge, J. A., Heron-Maxwell, C., Theofilas, P., Wright, P., de Hoz, L., & Wood, E. R. (2006). The role of the hippocampus in object recognition in rats: examination of the influence of task parameters and lesion size. *Behav Brain Res, 167*(1), 183-195. doi: 10.1016/j.bbr.2005.09.005

Albasser, M. M., Poirier, G. L., & Aggleton, J. P. (2010). Qualitatively different modes of perirhinal–hippocampal engagement when rats explore novel vs. familiar objects as revealed by c-Fos imaging. *European Journal of Neuroscience, 31*(1), 134-147. doi: 10.1111/j.1460-9568.2009.07042.x

Andersen, P. (2007). *The hippocampus book*. Oxford; New York: Oxford University Press.

Antunes, M., & Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cognitive processing, 13*(2), 93-110. doi: 10.1007/s10339-011-0430-z

Arias, N., Méndez, M., & Arias, J. L. (2015). The recognition of a novel-object in a novel context leads to hippocampal and parahippocampal c-Fos involvement. *Behav Brain Res, 292*, 44-49. doi: 10.1016/j.bbr.2015.06.012

Asgeirsdottir, H. N. (2013). *Representation of object-in-context within mouse hippocampal neuronal activity*. M.A., Florida Atlantic University.

Atucha, E., Karew, A., Kitsukawa, T., & Sauvage, M. M. (2017). Recognition memory: Cellular evidence of a massive contribution of the LEC to familiarity and a lack of involvement of the hippocampal subfields CA1 and CA3. *Hippocampus, 27*(10), 1083-1092. doi: 10.1002/hipo.22754

Bannerman, D. M., Rawlins, J. N. P., McHugh, S. B., Deacon, R. M. J., Yee, B. K., Bast, T., . . . Feldon, J. (2004). Regional dissociations within the hippocampus—memory and anxiety. *Neuroscience & Biobehavioral Reviews, 28*(3), 273-283. doi: 10.1016/j.neubiorev.2004.03.004

Biegler, R., & Morris, R. (1993). Landmark stability is a prerequisite for spatial but not discrimination learning. *Nature, 361*(6413), 631.

Black, A. H., Nadel, L., & O'Keefe, J. (1977). Hippocampal function in avoidance learning and punishment. *Psychological Bulletin*.

Blanchard, R. J., & Blanchard, D. C. (1970). Dual mechanisms in passive avoidance: I. *Psychonomic Science*.

Bonnevie, T., Dunn, B., Fyhn, M., Hafting, T., Derdikman, D., Kubie, J. L., . . . Moser, M.-B. B. (2013). Grid cells require excitatory drive from the hippocampus. *Nat Neurosci, 16*(3), 309-317. doi: 10.1038/nn.3311

Bostock, E., Muller, R. U., & Kubie, J. L. (1991). Experience-dependent modifications of hippocampal place cell firing. *Hippocampus, 1*(2), 193-205. doi: 10.1002/hipo.450010207

Broadbent, N. J., Gaskin, S., Squire, L. R., & Clark, R. E. (2010). Object recognition memory and the rodent hippocampus. [Research Support, N.I.H., Extramural

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2006). Reversible hippocampal lesions disrupt water maze performance during both recent and remote memory tests. *Learning & memory (Cold Spring Harbor, N.Y.), 13*(2), 187-191. doi: 10.1101/lm.134706

Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience, 2*(1), 51-61. doi: 10.1038/35049064

Brun, V. H., Leutgeb, S., Wu, H.-Q. Q., Schwarcz, R., Witter, M. P., Moser, E. I., & Moser, M.-B. B. (2008). Impaired spatial representation in CA1 after lesion of direct input from entorhinal cortex. *Neuron, 57*(2), 290-302. doi: 10.1016/j.neuron.2007.11.034

Buffalo, E. A., Ramus, S. J., Squire, L. R., & Zola, S. M. (2000). Perception and recognition memory in monkeys following lesions of area TE and perirhinal cortex. *Learn Mem, 7*(6), 375-382.

Bullitt, E. (1990). Expression of C-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. *J Comp Neurol, 296*(4), 517-530. doi: 10.1002/cne.902960402

Burke, S. N., Maurer, A. P., Hartzell, A. L., Nematollahi, S., Uprety, A., Wallace, J. L., & Barnes, C. A. (2012). Representation of three-dimensional objects by the rat perirhinal cortex. *Hippocampus, 22*(10), 2032-2044. doi: 10.1002/hipo.22060

Burke, S. N., Maurer, A. P., Nematollahi, S., Uprety, A. R., Wallace, J. L., & Barnes, C. A. (2011). The influence of objects on place field expression and size in distal hippocampal CA1. [Comparative Study]. *Hippocampus, 21*(7), 783-801. doi: 10.1002/hipo.20929

Bussey, T. J., Duck, J., Muir, J. L., & Aggleton, J. P. (2000). Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of

the perirhinal and postrhinal cortices in the rat. *Behav Brain Res, 111*(1–2), 187-202. doi: https://doi.org/10.1016/S0166-4328(00)00155-8

Calton, J. L., Stackman, R. W., & Goodridge, J. P. (2003). Hippocampal place cell instability after lesions of the head direction cell network. *The Journal of ….*

Carpenter-Hyland, E. P., & Plummer, T. K. (2010). Arc expression and neuroplasticity in primary auditory cortex during initial learning are inversely related to neural activity. *Proceedings of the ….*

Cave, C. B., & Squire, L. R. (1991). Equivalent impairment of spatial and nonspatial memory following damage to the human hippocampus. *Hippocampus, 1*(3), 329-340. doi: 10.1002/hipo.450010323

Chan, E., Baumann, O., Bellgrove, M. A., & Mattingley, J. B. (2012). From objects to landmarks: the function of visual location information in spatial navigation. *Frontiers in psychology, 3*, 304. doi: 10.3389/fpsyg.2012.00304

Chawla, M. K., Guzowski, J. F., Ramirez-Amaya, V., Lipa, P., Hoffman, K. L., Marriott, L. K., . . . Barnes, C. A. (2005). Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience. *Hippocampus, 15*(5), 579-586. doi: 10.1002/hipo.20091

Cimadevilla, J. M., Wesierska, M., Fenton, A. A., & Bures, J. (2001). Inactivating one hippocampus impairs avoidance of a stable room-defined place during dissociation of arena cues from room cues by rotation of the arena. *Proceedings of the National Academy of Sciences, 98*(6), 3531-3536. doi: 10.1073/pnas.051628398

Clark, P. J., Bhattacharya, T. K., Miller, D. S., & Rhodes, J. S. (2011). Induction of c-Fos, Zif268, and Arc from acute bouts of voluntary wheel running in new and pre-existing adult mouse hippocampal granule neurons. *Neuroscience, 184*, 16-27. doi: 10.1016/j.neuroscience.2011.03.072

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *The Journal of neuroscience*.

Clarke, J. R., Cammarota, M., Gruart, A., Izquierdo, I., & Delgado-Garcia, J. M. (2010). Plastic modifications induced by object recognition memory processing. [Research Support, Non-U.S. Gov't]. *Proc Natl Acad Sci U S A, 107*(6), 2652-2657. doi: 10.1073/pnas.0915059107

Clarke, J. R., Rossato, J. I., Monteiro, S., Bevilaqua, L. R. M., Izquierdo, I., & Cammarota, M. (2008). Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the dorsal hippocampus impairs object recognition long-term memory. *Neurobiol Learn Mem, 90*(2), 374-381. doi: https://doi.org/10.1016/j.nlm.2008.04.009

Cohen, S. J., Munchow, A. H., Rios, L. M., Zhang, G., Asgeirsdottir, H. N., & Stackman, R. W., Jr. (2013). The rodent hippocampus is essential for nonspatial object memory. [Research Support, N.I.H., Extramural]. *Curr Biol, 23*(17), 1685-1690. doi: 10.1016/j.cub.2013.07.002

Cohen, S. J., & Stackman Jr, R. W. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behav Brain Res, 285*, 105-117. doi: https://doi.org/10.1016/j.bbr.2014.08.002

Collett, T. S., Cartwright, B. A., & Smith, B. A. (1986). Landmark learning and visuo-spatial memories in gerbils. *Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology, 158*(6), 835-851. doi: 10.1007/bf01324825

Corkin, S., Amaral, D. G., & González…, R. G. (1997). HM's medial temporal lobe lesion: findings from magnetic resonance imaging. *The Journal of …*.

Cressant, A., Muller, R. U., & Poucet, B. (1997). Failure of centrally placed objects to control the firing fields of hippocampal place cells. *The Journal of neuroscience*.

Cressant, A., Muller, R. U., & Poucet, B. (1999). Further study of the control of place cell firing by intra-apparatus objects. *Hippocampus, 9*(4), 423-431. doi: 10.1002/(sici)1098-1063(1999)9:4<423::aid-hipo8>3.0.co;2-u

de Lima, M. N., Luft, T., Roesler, R., & Schröder, N. (2006). Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. *Neurosci Lett, 405*(1–2), 142-146. doi: https://doi.org/10.1016/j.neulet.2006.06.044

Deshmukh, S. S., & Knierim, J. J. (2011). Representation of non-spatial and spatial information in the lateral entorhinal cortex. *Frontiers in behavioral neuroscience, 5*, 69. doi: 10.3389/fnbeh.2011.00069

Deshmukh, S. S., & Knierim, J. J. (2013). Influence of local objects on hippocampal representations: Landmark vectors and memory. [Research Support, N.I.H., Extramural]. *Hippocampus, 23*(4), 253-267. doi: 10.1002/hipo.22101

Dudchenko, P. A. (2004). An overview of the tasks used to test working memory in rodents. *Neuroscience & Biobehavioral Reviews, 28*(7), 699-709. doi: http://dx.doi.org/10.1016/j.neubiorev.2004.09.002

Eichenbaum. (2014). Time cells in the hippocampus: a new dimension for mapping memories. *Nature Reviews Neuroscience, 15*(11), 732-744. doi: 10.1038/nrn3827

Eichenbaum, H. (2000). A cortical-hippocampal system for declarative memory. *Nature reviews. Neuroscience, 1*(1), 41-50. doi: 10.1038/35036213

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu Rev Neurosci, 30*, 123-152.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res, 31*(1), 47-59. doi: https://doi.org/10.1016/0166-4328(88)90157-X

Ergorul, C., & Eichenbaum, H. (2004). The hippocampus and memory for "what," "where," and "when". *Learning & memory (Cold Spring Harbor, N.Y.), 11*(4), 397-405. doi: 10.1101/lm.73304

Fenton, A. A., Lytton, W. W., Barry, J. M., Lenck-Santini, P.-P. P., Zinyuk, L. E., Kubík, S., . . . Olypher, A. V. (2010). Attention-like modulation of hippocampus place cell discharge. *J Neurosci, 30*(13), 4613-4625. doi: 10.1523/jneurosci.5576-09.2010

Fonseca, R., Vabulas, R. M., Hartl, U. F., Bonhoeffer, T., & Nägerl, V. U. (2006). A Balance of Protein Synthesis and Proteasome-Dependent Degradation Determines the Maintenance of LTP. *Neuron, 52*(2), 239-245. doi: 10.1016/j.neuron.2006.08.015

Forwood, S. E., Winters, B., & Bussey, T. (2005). Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. *Hippocampus, 15*(3), 347-355.

Frick, K. M., Stillner, E. T., & Berger-Sweeney, J. (2000). Mice are not little rats: species differences in a one-day water maze task. *NeuroReport, 11*(16), 3461-3465.

Fusi, S., Miller, E. K., & Rigotti, M. (2016). Why neurons mix: high dimensionality for higher cognition. *Current Opinion in Neurobiology, 37*(Supplement C), 66-74. doi: https://doi.org/10.1016/j.conb.2016.01.010

Fyhn, M., Hafting, T., Treves, A., Moser, M.-B. B., & Moser, E. I. (2007). Hippocampal remapping and grid realignment in entorhinal cortex. *Nature, 446*(7132), 190-194. doi: 10.1038/nature05601

Gallitano, A. L., Satvat, E., Gil, M., & Marrone, D. F. (2016). Distinct dendritic morphology across the blades of the rodent dentate gyrus. *Synapse (New York, N.Y.), 70*(7), 277-282. doi: 10.1002/syn.21900

Gaykema, R. P. A., Nguyen, X.-M. T., Boehret, J. M., Lambeth, P. S., Joy-Gaba, J., Warthen, D. M., & Scott, M. M. (2014). Characterization of excitatory and inhibitory neuron activation in the mouse medial prefrontal cortex following palatable food ingestion and food driven exploratory behavior. *Frontiers in Neuroanatomy, 8*, 60. doi: 10.3389/fnana.2014.00060

George Paxinos, K. F. (2004). *The mouse brain in stereotaxic coordinates* (Second ed.): Academic press.

Ghosh, K. K., Burns, L. D., Cocker, E. D., Nimmerjahn, A., Ziv, Y., Gamal, A. E., & Schnitzer, M. J. (2011). Miniaturized integration of a fluorescence microscope. *Nature methods, 8*(10), 871-878. doi: 10.1038/nmeth.1694

Good, M. A., Barnes, P., Staal, V., McGregor, A., & Honey, R. C. (2007). Context- but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. *Behav Neurosci, 121*(1), 218. doi: 10.1037/0735-7044.121.1.218

Graves, A. R., Moore, S. J., Bloss, E. B., Mensh, B. D., Kath, W. L., & Spruston, N. (2012). Hippocampal pyramidal neurons comprise two distinct cell types that are countermodulated by metabotropic receptors. [In Vitro

Gundersen, H. J. G., Jensen, E. B. V., Kieu, K., & Nielsen, J. (1999). The efficiency of systematic sampling in stereology—reconsidered. *J Microsc, 193*(3), 199-211.

Guzowski, J. F., McNaughton, B. L., Barnes, C. A., & Worley, P. F. (1999). Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nat Neurosci, 2*(12), 1120-1124. doi: 10.1038/16046

Guzowski, J. F., Miyashita, T., Chawla, M. K., Sanderson, J., Maes, L. I., Houston, F. P., . . . Barnes, C. A. (2006). Recent behavioral history modifies coupling between cell activity and Arc gene transcription in hippocampal CA1 neurons. *Proc Natl Acad Sci U S A, 103*(4), 1077-1082. doi: 10.1073/pnas.0505519103

Hammond, R. S., Tull, L. E., & Stackman, R. W. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem, 82*(1), 26-34. doi: 10.1016/j.nlm.2004.03.005

Hardt, O., Migues, P. V., Hastings, M., Wong, J., & Nader, K. (2010). PKMζ maintains 1-day- and 6-day-old long-term object location but not object identity memory in dorsal hippocampus. *Hippocampus, 20*(6), 691-695. doi: 10.1002/hipo.20708

Hartley, T., Lever, C., Burgess, N., & O'Keefe, J. (2014). Space in the brain: how the hippocampal formation supports spatial cognition. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*(1635), 20120510. doi: 10.1098/rstb.2012.0510

Hitti, F. L., & Siegelbaum, S. A. (2014). The hippocampal CA2 region is essential for social memory. [Letter]. *Nature, 508*(7494), 88-92. doi: 10.1038/nature13028

Ho, S. A., Hori, E., Kobayashi, T., Umeno, K., Tran, A. H., Ono, T., & Nishijo, H. (2008). Hippocampal place cell activity during chasing of a moving object associated with reward in rats. *Neuroscience, 157*(1), 254-270. doi: 10.1016/j.neuroscience.2008.09.004

181

Hsu, D. (2007). Progress in Brain Research. *Section V. The Dentate Gyrus Network, 163*, 601-613. doi: 10.1016/s0079-6123(07)63032-5

Huff, N. C., Frank, M., Wright-Hardesty, K., Sprunger, D., Matus-Amat, P., Higgins, E., & Rudy, J. W. (2006). Amygdala regulation of immediate-early gene expression in the hippocampus induced by contextual fear conditioning. *Journal of Neuroscience, 26*(5), 1616-1623.

Hunsaker, M. R., Chen, V., Tran, G. T., & Kesner, R. P. (2013). The medial and lateral entorhinal cortex both contribute to contextual and item recognition memory: A test of the binding ofitems and context model. *Hippocampus, 23*(5), 380-391. doi: 10.1002/hipo.22097

Igaz, L. M., Vianna, M. R., Medina, J. H., & Izquierdo, I. (2002). Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. *J Neurosci, 22*(15), 6781-6789.

Jacobs, L. F., & Schenk, F. (2003). Unpacking the cognitive map: the parallel map theory of hippocampal function. *Psychological review, 110*(2), 285-315.

Jarrard, L. E. (1983). Selective hippocampal lesions and behavior: Effects of kainic acid lesions on performance of place and cue tasks. *Behav Neurosci, 97*(6), 873. doi: 10.1037/0735-7044.97.6.873

Jessberger, S., Clark, R. E., Broadbent, N. J., Clemenson, G. D., Consiglio, A., Lie, D. C., . . . Gage, F. H. (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem, 16*(2), 147-154. doi: 10.1101/lm.1172609

Jung, M. W., & McNaughton, B. L. (1993). Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus, 3*(2), 165-182. doi: 10.1002/hipo.450030209

Kang, H., & Schuman, E. M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science (New York, N.Y.), 273*(5280), 1402-1406. doi: 10.1126/science.273.5280.1402

Kelemen, E., & Fenton, A. A. (2010). Dynamic Grouping of Hippocampal Neural Activity During Cognitive Control of Two Spatial Frames. *PLoS Biol, 8*(6). doi: 10.1371/journal.pbio.1000403

Kesner, R. P., Gilbert, P. E., & Barua, L. A. (2002). The role of the hippocampus in memory for the temporal order of a sequence of odors. *Behav Neurosci, 116*(2), 286. doi: 10.1037/0735-7044.116.2.286

Kimura, D. (1958). Effects of selective hippocampal damage on avoidance behavior in the rat. *Canadian Journal of Psychology/Revue canadienne …*.

Kjelstrup, K. G., Tuvnes, F. A., Steffenach, H.-A., Murison, R., Moser, E. I., & Moser, M.-B. (2002). Reduced fear expression after lesions of the ventral hippocampus. *Proceedings of the National Academy of Sciences, 99*(16), 10825-10830. doi: 10.1073/pnas.152112399

Knierim, J. J., & Kudrimoti, H. S. (1995). Place cells, head direction cells, and the learning of landmark stability. *The Journal of …*.

Knierim, J. J., Neunuebel, J. P., & Deshmukh, S. S. (2014). Functional correlates of the lateral and medial entorhinal cortex: objects, path integration and local-global reference frames. [Research Support, N.I.H., Extramural]. *Philos Trans R Soc Lond B Biol Sci, 369*(1635), 20130369. doi: 10.1098/rstb.2013.0369

Komorowski, R. W., Manns, J. R., & Eichenbaum, H. (2009). Robust conjunctive item-place coding by hippocampal neurons parallels learning what happens where. *J Neurosci, 29*(31), 9918-9929. doi: 10.1523/jneurosci.1378-09.2009

Kveim, O., Setekleiv, J., & Kaada, B. R. (1964). Differential effects of hippocampal lesions on maze and passive avoidance learning in rats. *Experimental Neurology*.

Langston, R., & Wood, E. (2006). *Exploring the circumstances under which the hippocampus is required for spontaneous object-location recognition.* Paper presented at the SFN Abstr.

Larkin, M., Lykken, C., Tye, L. D., Wickelgren, J., & Frank, L. M. (2014). Hippocampal output area CA1 broadcasts a generalized novelty signal during an object-place recognition task. *Hippocampus*. doi: 10.1002/hipo.22268

Lavenex, P., & Amaral, D. G. (2000). Hippocampal-neocortical interaction: a hierarchy of associativity. *Hippocampus, 10*(4), 420-430.

Lee, I., & Park, S.-B. (2013). Perirhinal cortical inactivation impairs object-in-place memory and disrupts task-dependent firing in hippocampal CA1, but not in CA3. [Original Research]. *Frontiers in Neural Circuits, 7*(134). doi: 10.3389/fncir.2013.00134

Lepage, M., Habib, R., & Tulving, E. (1998). Hippocampal PET activations of memory encoding and retrieval: the HIPER model. *Hippocampus*.

Leroy, F., Brann, D. H., Meira, T., & Siegelbaum, S. A. (2017). Input-Timing-Dependent Plasticity in the Hippocampal CA2 Region and Its Potential Role in Social Memory. *Neuron, 95*(5), 1089-1102.e1085. doi: https://doi.org/10.1016/j.neuron.2017.07.036

Lisman, J. E. (2007). Role of the dual entorhinal inputs to hippocampus: a hypothesis based on cue/action (non-self/self) couplets. *Prog Brain Res, 163*, 615-625. doi: 10.1016/s0079-6123(07)63033-7

MacDonald, C. J., Lepage, K. Q., Eden, U. T., & Eichenbaum, H. (2011). Hippocampal "time cells" bridge the gap in memory for discontiguous events. *Neuron, 71*(4), 737-749. doi: 10.1016/j.neuron.2011.07.012

Manns, J. R., & Eichenbaum, H. (2009). A cognitive map for object memory in the hippocampus. [Research Support, N.I.H., Extramural

Maren, S. (1999). Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. *Trends in Neurosciences, 22*(12), 561-567. doi: 10.1016/s0166-2236(99)01465-4

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behav Brain Res, 88*(2), 261-274. doi: 10.1016/s0166-4328(97)00088-0

Marrone, D. F., Satvat, E., Shaner, M. J., Worley, P. F., & Barnes, C. A. (2012). Attenuated long-term Arc expression in the aged fascia dentata. *Neurobiology of Aging, 33*(5), 979-990. doi: 10.1016/j.neurobiolaging.2010.07.022

McGaugh, J. L. (2000). Memory--a Century of Consolidation. *Science, 287*. doi: 10.1126/science.287.5451.248

Minatohara, K., Akiyoshi, M., & Okuno, H. (2015). Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace. *Frontiers in Molecular Neuroscience, 8*, 78. doi: 10.3389/fnmol.2015.00078

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. [Research Support, Non-U.S. Gov't]. *Nature, 297*(5868), 681-683.

Moser, E., Moser, M. B., & Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *The Journal of neuroscience*.

Muller, R., Kubie, J., & Ranck, J. (1987). Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. *The Journal of neuroscience, 7*(7), 1935-1950.

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learning & memory (Cold Spring Harbor, N.Y.), 9*(2), 49-57. doi: 10.1101/lm.41302

Mumby, D. G., Tremblay, A., Lecluse, V., & Lehmann, H. (2005). Hippocampal damage and anterograde object-recognition in rats after long retention intervals. *Hippocampus, 15*(8), 1050-1056. doi: 10.1002/hipo.20122

Mura, A., Murphy, C. A., Feldon, J., & Jongen-Relo, A.-L. (2004). The use of stereological counting methods to assess immediate early gene immunoreactivity. *Brain Res, 1009*(1), 120-128. doi: https://doi.org/10.1016/j.brainres.2004.02.054

Naber, P. A., Witter, M. P., & Da Silva, F. H. L. (1999). Perirhinal cortex input to the hippocampus in the rat: evidence for parallel pathways, both direct and indirect. A combined physiological and anatomical study. *European Journal of Neuroscience, 11*(11), 4119-4133. doi: 10.1046/j.1460-9568.1999.00835.x

O'Keefe, J., & Conway, D. H. (1978). Hippocampal place units in the freely moving rat: why they fire where they fire. *Exp Brain Res, 31*(4), 573-590.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res, 34*(1), 171-175.

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

O'Brien, N., Lehmann, H., Lecluse, V., & Mumby, D. G. (2006). Enhanced context-dependency of object recognition in rats with hippocampal lesions. *Behav Brain Res, 170*(1), 156-162. doi: https://doi.org/10.1016/j.bbr.2006.02.008

Oliveira, A., Hawk, J. D., Abel, T., & Havekes, R. (2010). Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learn Mem, 17*(3), 155-160. doi: 10.1101/lm.1625310

Olton, D. S., & Samuelson, R. J. (1976). Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Processes, 2*(2), 97-116.

Pastalkova, E., Kelemen, E., & Bureš, J. (2003). Operant behavior can be triggered by the position of the rat relative to objects rotating on an inaccessible platform. *Proceedings of the National Academy of Sciences, 100*(4), 2094-2099. doi: 10.1073/pnas.0438002100

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods, 14*(3), 149-167. doi: 10.1016/0165-0270(85)90031-7

Pena, R. R., Pereira-Caixeta, A. R., Moraes, M. F. D., & Pereira, G. S. (2014). Anisomycin administered in the olfactory bulb and dorsal hippocampus impaired social recognition memory consolidation in different time-points. *Brain Res Bull, 109*, 151-157. doi: https://doi.org/10.1016/j.brainresbull.2014.10.009

Perrin-Terrin, A.-S., Jeton, F., Pichon, A., Frugière, A., Richalet, J.-P., Bodineau, L., & Voituron, N. (2016). The c-FOS protein immunohistological detection: a useful tool as a marker of central pathways involved in specific physiological responses in vivo and ex vivo. *JoVE (Journal of Visualized Experiments)*(110).

Ramírez-Amaya, V., Vazdarjanova, A., Mikhael, D., Rosi, S., Worley, P. F., & Barnes, C. A. (2005). Spatial Exploration-Induced Arc mRNA and Protein Expression: Evidence for Selective, Network-Specific Reactivation. *The Journal of neuroscience, 25*(7), 1761-1768. doi: 10.1523/jneurosci.4342-04.2005

Ranganath, C., & Blumenfeld, R. S. (2005). Doubts about double dissociations between short- and long-term memory. *Trends in Cognitive Sciences, 9*(8), 374-380. doi: http://dx.doi.org/10.1016/j.tics.2005.06.009

Riedel, G., Micheau, J., Lam, A. G., Roloff, E. L., Martin, S. J., Bridge, H., . . . Morris, R. G. (1999). Reversible neural inactivation reveals hippocampal participation in several memory processes. [Comparative Study

Rivard, B., Li, Y., Lenck-Santini, P.-P., Poucet, B., & Muller, R. U. (2004). Representation of Objects in Space by Two Classes of Hippocampal Pyramidal Cells. *The Journal of General Physiology, 124*(1), 9-25. doi: 10.1085/jgp.200409015

Save, E., Poucet, B., & Foreman…, N. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behavioral ….*

Shires, K. L., & Aggleton, J. P. (2008). Mapping immediate-early gene activity in the rat after place learning in a water-maze: the importance of matched control conditions. *European Journal of Neuroscience, 28*(5), 982-996. doi: 10.1111/j.1460-9568.2008.06402.x

Singer, P., Wei, C. J., Chen, J.-F., Boison, D., & Yee, B. K. (2013). Deletion of striatal adenosine A2A receptor spares latent inhibition and prepulse inhibition but impairs active avoidance learning. *Behav Brain Res, 242*, 54-61. doi: https://doi.org/10.1016/j.bbr.2012.12.024

Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological review*.

Squire, L. R. (2004). Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem, 82*(3), 171-177. doi: 10.1016/j.nlm.2004.06.005

Squire, L. R., Stark, C. E., & Clark, R. E. (2004). The medial temporal lobe. *Annu Rev Neurosci, 27*, 279-306.

Squire, L. R., Wixted, J. T., & Clark, R. E. (2007). Recognition memory and the medial temporal lobe: a new perspective. *Nature reviews. Neuroscience, 8*(11), 872-883. doi: 10.1038/nrn2154

Stackman, R. W., Clark, A. S., & Taube, J. S. (2002). Hippocampal spatial representations require vestibular input. *Hippocampus, 12*(3), 291-303. doi: 10.1002/hipo.1112

Stackman, R. W., & Taube, J. S. (1998). Firing properties of rat lateral mammillary single units: head direction, head pitch, and angular head velocity. *J Neurosci, 18*(21), 9020-9037.

Stupien, G., Florian, C., & Roullet, P. (2003). Involvement of the hippocampal CA3-region in acquisition and in memory consolidation of spatial but not in object information in mice. *Neurobiol Learn Mem, 80*(1), 32-41. doi: https://doi.org/10.1016/S1074-7427(03)00022-4

Suzuki, S., Augerinos, G., & Black, A. H. (1980). Stimulus control of spatial behavior on the eight-arm maze in rats. *Learning and Motivation, 11*(1), 1-18. doi: http://dx.doi.org/10.1016/0023-9690(80)90018-1

Taube, J. S., & Burton, H. L. (1995). Head direction cell activity monitored in a novel environment and during a cue conflict situation. *J Neurophysiol, 74*(5), 1953-1971.

Taube, J. S., Muller, R. U., & Ranck, J. B. (1990). Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations. *J Neurosci, 10*(2), 436-447.

Telensky, P., Svoboda, J., Blahna, K., Bures, J., Kubik, S., & Stuchlik, A. (2011). Functional inactivation of the rat hippocampus disrupts avoidance of a moving object. [Research Support, Non-U.S. Gov't]. *Proc Natl Acad Sci U S A, 108*(13), 5414-5418. doi: 10.1073/pnas.1102525108

Telensky, P., Svoboda, J., Pastalkova, E., Blahna, K., Bures, J., & Stuchlik, A. (2009). Enemy avoidance task: a novel behavioral paradigm for assessing spatial avoidance of a moving subject. [Research Support, Non-U.S. Gov't]. *J Neurosci Methods, 180*(1), 29-33. doi: 10.1016/j.jneumeth.2009.02.010

Teng, E., & Squire, L. R. (1999). Memory for places learned long ago is intact after hippocampal damage. *Nature, 400*(6745), 675-677. doi: 10.1038/23276

Thompson, L. T., & Best, P. J. (1990). Long-term stability of the place-field activity of single units recorded from the dorsal hippocampus of freely behaving rats. *Brain Res*.

Tian, J. B., & Bishop, G. A. (2002). Stimulus-dependent activation of c-Fos in neurons and glia in the rat cerebellum. *Journal of Chemical Neuroanatomy, 23*(3), 157-170. doi: https://doi.org/10.1016/S0891-0618(01)00153-3

Torterolo, P., Yamuy, J., Sampogna, S., Morales, F. R., & Chase, M. H. (2001). GABAergic neurons of the laterodorsal and pedunculopontine tegmental nuclei of the cat express c-fos during carbachol-induced active sleep. *Brain Res, 892*(2), 309-319. doi: https://doi.org/10.1016/S0006-8993(00)03264-9

Tsao, A., Moser, M.-B., & Moser, E. I. (2013). Traces of experience in the lateral entorhinal cortex. *Curr Biol, 23*(5), 399-405. doi: 10.1016/j.cub.2013.01.036

Vallence, A.-M., Schneider, L. A., Pitcher, J. B., & Ridding, M. C. (2014). Long-interval facilitation and inhibition are differentially affected by conditioning stimulus intensity over different time courses. *Neurosci Lett, 570*(Supplement C), 114-118. doi: https://doi.org/10.1016/j.neulet.2014.03.060

van Strien, N. M., Cappaert, N. L., & Witter, M. P. (2009). The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. *Nature reviews. Neuroscience, 10*(4), 272-282. doi: 10.1038/nrn2614

VanElzakker, M., Fevurly, R. D., Breindel, T., & Spencer, R. L. (2008). Environmental novelty is associated with a selective increase in Fos expression in the output elements of the hippocampal formation and the perirhinal cortex. *Learn Mem, 15*(12), 899-908. doi: 10.1101/lm.1196508

Wang, M. E., Wann, E. G., Yuan, R. K., Álvarez, M. M., Stead, S. M., & Muzzio, I. A. (2012). Long-Term Stabilization of Place Cell Remapping Produced by a Fearful Experience. *The Journal of neuroscience, 32*(45), 15802-15814. doi: 10.1523/jneurosci.0480-12.2012

West, M., Slomianka, L., & Gundersen, H. J. G. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec, 231*(4), 482-497.

Whishaw, I. Q., & Tomie, J.-A. (1996). Of Mice and Mazes: Similarities Between Mice and Rats on Dry Land But Not Water Mazes. *Physiology & Behavior, 60*(5), 1191-1197. doi: https://doi.org/10.1016/S0031-9384(96)00176-X

Wilson, D. I., Langston, R. F., Schlesiger, M. I., Wagner, M., Watanabe, S., & Ainge, J. A. (2013). Lateral entorhinal cortex is critical for novel object-context recognition. *Hippocampus, 23*(5), 352-366. doi: 10.1002/hipo.22095

Wilson, M., & McNaughton, B. (1993). Dynamics of the hippocampal ensemble code for space. *Science, 261*(5124), 1055-1058. doi: 10.1126/science.8351520

Winters, B. D., & Bussey, T. J. (2005). Transient Inactivation of Perirhinal Cortex Disrupts Encoding, Retrieval, and Consolidation of Object Recognition Memory. *The Journal of neuroscience, 25*(1), 52-61. doi: 10.1523/jneurosci.3827-04.2005

Winters, B. D., Forwood, S. E., Cowell, R. A., Saksida, L. M., & Bussey, T. J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *J Neurosci, 24*(26), 5901-5908. doi: 10.1523/jneurosci.1346-04.2004

Yoganarasimha, D., & Knierim, J. J. (2005). Coupling between place cells and head direction cells during relative translations and rotations of distal landmarks. *Experimental brain research. Experimentelle Hirnforschung. Expérimentation cérébrale, 160*(3), 344-359. doi: 10.1007/s00221-004-2016-9

Zinyuk, L., Kubik, S., Kaminsky, Y., Fenton, A. A., & Bures, J. (2000). Understanding hippocampal activity by using purposeful behavior: place navigation induces place cell discharge in both task-relevant and task-irrelevant spatial reference frames. [Research Support, Non-U.S. Gov't]. *Proc Natl Acad Sci U S A, 97*(7), 3771-3776. doi: 10.1073/pnas.050576397

Ziv, Y., Burns, L. D., Cocker, E. D., Hamel, E. O., Ghosh, K. K., Kitch, L. J., . . . Schnitzer, M. J. (2013). Long-term dynamics of CA1 hippocampal place codes. *Nat Neurosci, 16*(3), 264-266. doi: 10.1038/nn.3329

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *J Neurosci, 6*(10), 2950-2967. doi: 10.1093/neucas/2.4.259-aw

Zynyuk, L., Huxter, J., Muller, R. U., & Fox, S. E. (2012). The presence of a second rat has only subtle effects on the location-specific firing of hippocampal place cells. *Hippocampus, 22*(6), 1405-1416. doi: 10.1002/hipo.20977