UNIVERSITY OF CALIFORNIA, SAN DIEGO

Involvement of the hippocampus and medial entorhinal cortex in bridging discontinuous events

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Antonia Schonwald

Committee in charge:
Professor Stefan Leutgeb, Chair
Professor Robert Clark
Professor Jill Leutgeb

2017

The Thesis of Antonia Schonwald is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2017

DEDICATION

To my Dad

I can only hope that I will be able to give back all that you have sacrificed for me to be here. Until then, I want you to know that this dissertation is not only dedicated to you, but it is equally yours as it is mine. With all my love, thank you.

EPIGRAPH

- Antoine de Saint-Exupéry

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

MEC	Medial entorhinal cortex
LEC	Lateral entorhinal cortex
H	Hippocampus
DOUBLE	Double lesion
IACUC	Institutional Animal Care and Use Committee
NMDA	N-Methyl-D-aspartic acid
IBO	Ibotenic acid
AP	Anteroposterior
ML	Mediolateral
DV	Dorsoventral
LFP	Local field potential
DG	Dente Gyrus
SUB	Subiculum
PRESUB	Presubiculum
dPAS	Dorsal Parasubiculum
vPAS	Ventral Parasubiculum
PFC	Prefrontal cortex
mPFC	Medial prefrontal cortex

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to acknowledge Professor Stefan Leutgeb for all his support as the Chair of my committee. Thank you for providing me with constant support in both academia and research. Being a part of your research team has been one of the most invaluable and rewarding experiences of my student career.

I would also like to acknowledge Professor Jill Leutgeb and Professor Robert Clark for taking the time to be a part of my committee. Your support acts a constant reminder that research benefits most from collaboration.

Most importantly, I would like to acknowledge Professor Marta Collett who is the soul and brain of this project. I am humbled to have had you as my Professor at UCSD. Your love and excitement for research are still as contagious today as they were in the classroom. You are an infinite source of inspiration as a scientist, but your compassion is what really sets you apart as a teacher. Thank you for believing in me, advocating for me, and supporting me every step of the way. None of this would have been possible without you.

This thesis, in full, is currently being prepared for submission of publication of the material. Collett, Marta; Schonwald, Antonia. Professor Marta Collett is the primary investigator and author.

VITA

2015-2016	Instructional Apprentice, Department of Biological Sciences, University of California, San Diego
2016	Bachelor of Science, University of California, San Diego Bachelor of Arts, University of California, San Diego
2016-2017	Teaching Assistant, Department of Biological Sciences, University of California, San Diego
2017	Master of Science, University of California, San Diego

FIELD OF STUDY

Major Field: Biology

Studies in Neurobiology and Neurophysiology
Professors Jill Leutgeb and Stefan Leutgeb

ABSTRACT OF THE THESIS

Involvement of the hippocampus and medial entorhinal cortex in bridging discontinuous events

by

Antonia Schonwald

Master of Science in Biology

University of California, San Diego, 2017

Professor Stefan Leutgeb, Chair

The medial entorhinal cortex (MEC) projects directly to the hippocampus. It is considered the major contributor of spatial information to the hippocampal place cells (1-3). However, it remains unclear to what extent MEC spatial firing is necessary for memory-guided behavior. We found that complete single or double bilateral lesions to the MEC and the hippocampus impaired rats in the delayed alternation task. Single lesioned rats partially improved their behavioral performance in delay trials across time. However, physiological analysis of CA1 cells in the MEC lesioned rats indicated less precision in firing, as well as less discrimination between left and right trials. Double

lesioned rats did not present any behavioral improvement throughout trials, but in fact exhibited perseveration. These results could indicate that single lesions of either structure disrupt the function of the entorhino-hippocampal loop. Over time residual neurons of the intact structure are sufficient to compensate for the communication with the prefrontal cortex, as supported by partial behavioral recoveries. Double lesioned animals, however, are prevented from improving in memory related tasks.

INTRODUCTION

Learning and Memory

Learning is a process of acquiring new information, while memory refers to the presence of learning in a state that can be revealed at a later time (Squire 1987).

Memories differ in their content and can be separated into two major categories: declarative and nondeclaritive (Squire and Zola 1996). Declarative memories are related to facts and events, while nondeclaritive memories are related to procedures, priming, perceptual learning, conditioning and nonassociative learning. Declarative memories are considered conscious recollections that can be stated verbally or described as a mental image. Nondeclaritive memories are of a more reflexive nature and sometimes considered unconscious. Declarative and nondeclaritive memories recruit different brain systems and use different strategies for storage (Squire and Kandel 2009). For spatial declarative memories it is yet unclear which systems are responsible for its complete encoding. This thesis dissertation investigates how spatial events are encoded into declarative memories by different brain systems.

Declarative memories can differ in their capacity and duration. We distinguish between immediate, working and long-term memories. Immediate memory is the shortest in duration, the most limited in capacity, and it requires focused attention in order to persist in the stream of thought. However, if immediate memory is actively rehearsed it is able to extend to working memory. It has been found in monkeys that working memory is stored in prefrontal cortex in order to guide behavior and cognition (Goldman and Rakic 1995). Its capacity can vary and its duration and is temporary. Both

immediate memory and working memory are characterized as transient, limited in capacity and retained without anatomical rearrangement or synthesis of new proteins (Squire and Kandel 2009). They can work in parallel upon memory encoding. Some working memory is able to pass to long-term memory after prolonged exposure or repetition of information itself. Then, memory is no longer limited in capacity or duration, and its encoding becomes dependent of the medial temporal lobe. It has been found in rats that an increase in information load forces the medial temporal lobe into supporting the encoding of information as a long-term memory (Ainge et al. 2007). This research project focuses on spatial working and long-term memory, and explores which physiological events are needed for their interaction.

Memory Encoding and Consolidation

Ribot's law (Ribot 1881) indicates that long-term declarative memories are not fixed to the time of learning, but need additional time to develop and reorganize into their stable forms. This is consistent with the standard model of systems consolidation theory (Squire and Alvarez 1995), which describes the interaction of the medial temporal lobe with the neocortex upon formation of new long-term declarative memories. This process is also known as encoding. While, the hippocampal region is initially required for long-term memory encoding and recall, it is not the final repository of long-term memories. As time passes after learning, there is a steady reorganization of memory storage. Long-term memories become stored in more permanent cortical locations, while the necessity for the hippocampal region gradually decreases.

Eventually, long-term memories can be retrieved independently from the hippocampal region. This theory is furthered by observations of anterograde amnesias that have temporally retrograde components. Anterograde amnesia is the inability to form new long-term declarative memories (Scoville et al. 1957), usually due to damage to the medial temporal lobe. Temporally graded retrograde amnesia is often co-present in such cases and is described as the inability to retrieve long-term declarative memories around the time of the trauma, but being able to recall events and facts that are further away in the past. In a case study by Scoville et al. 1957, a patient called H.M. underwent a surgical lesion of the medial temporal lobe in order to prevent epilepsy seizures. Consequently, H.M. was unable to form any new long-term declarative memories, but was able to use his immediate and working memory. He was also unable to recall any memories around the time of the surgery, but was able to remember other declarative long-term memories from the past. This case study supports that it takes time for long-term memories to consolidate in the human cortex, but also that the medial temporal lobe is necessary for the encoding of new long-term declarative memories.

This finding has proven true among other well-studied mammalian species, such as rats and monkeys. In each species, damage to the hippocampus developed impairment in the ability to establish declarative memories (Squire and Kandel 2009). For instance, Eichenbaum et al. 1990 studied the effect of hippocampal lesions on spatial learning and memory in rats. Animals were trained to swim from the edge of a circular Morris water maze to a slightly submerged, hidden platform. The platform was always positioned at the same location and marked by the same external cues in the

environment. After both the control and the lesioned group learned how to perform the task, the animals were started from novel locations around the circumference of the pool. Control rats were able to find the platform relatively quickly, orienting themselves through a flexible representation of space. The lesioned rats, however, were unable to find the platform and their success was mainly due to chance. Control animals were able to declaratively learn the spatial relationship between the platform location and external cues in the environment (Squire and Kandel 2009), while the lesioned animals were relying on habit learning to perform the spatial task. Habit is a type of non-declarative procedural memory and is far less flexible to guide behavior in novel situations (Eichbaum et al. 1990). Therefore, the study further confirms the necessity of the hippocampus in declarative learning. However, while it seems clear that the hippocampus is necessary for encoding of spatial information, it is yet unclear which other anatomically related systems support spatial learning and memory. In this research project, bilateral lesions are introduced to the hippocampus and medial entorhinal cortex (MEC) in order to investigate their physiological necessity in encoding of new spatial memories.

Spatial Memory

The hippocampus is known to contain a high-level neural representation of spatial receptive fields (J. O'Keefe 1978). These spatial fields are created by firing of hippocampal place cells. Place cells are pyramidal cells that fire when an animal is in a certain location (Henze et al. 2000). Spatial fields created by place cells can be evaluated

for their stability and precision. When a place cell always fires for a specific place in the environment, its spatial field can be considered stabile for a particular location. If the cell also fires only over a confined area around the location, the spatial field may be considered precise (Hales et al. 2014). Distinct firing of place cells is most apparent in the CA areas (CA 1-3), but also present in other subfields of the hippocampus. While, the same place cells can fire for several different locations, their firing fields differ from one environment to the next (Moser et al. 2008). Therefore, each location can be represented through a place cell activity, ultimately creating an internal cognitive map.

While the hippocampal circuit plays a key role in spatial memory encoding, its place-modulated neurons receive additional spatial signals from perforant-path projections (Hales et al. 2014). Therefore, place signals are not exclusively formed within the hippocampal circuit itself, but external inputs are necessary for a complete spatial representation. As previously mentioned, the hippocampus interacts with the neocortex in order for long-term declarative memories to fully encode and consolidate (McClelland et al. 1995). The same applies to spatial memories. The Entorhinal cortex largely contributes to this interaction by bridging the two structures together (Witter and Amaral 1991). It is able to provide bidirectional inputs from both structures, and therefore it is believed to participate in encoding and consolidating memory.

The entorhinal cortex receives two major projections from the neocortex (Hales et al. 2014); one through the lateral entorhinal cortex (LEC) and the second through the medial entorhinal cortex (MEC). This thesis dissertation primarily focuses on the MEC, which is involved in spatial information processing (Fyhn et al. 2004). The MEC receives

projections from the postrhinal cortex and then directly projects to the dorsal hippocampus through the perforant-path projections (Hales et al. 2014). Spatial signals are conveyed to CA1 cells from MEC layers II and III. Therefore, the MEC may be a prominent structure that passes on spatial information to the hippocampus.

The most abundant cell type in the MEC is the grid cell which exhibits periodic firing fields that span the entire available area in a hexagonal pattern (Zhang et al. 2013). Grid cells are similar to place cells in their sharply tuned spatial firing, except that each grid cell has multiple firing fields (Moser et al. 2008). Grid cells seem to signal the animal's changing position, as well as a possible metric measurement system for spatial navigation (Hafting et al. 2005). There are three other cell types that are also encountered in the MEC. One is the head direction cell which conveys the direction the animal is facing regardless of its location in space. Second is the border cell which signals the presence of geometric boundaries within the environment. Lastly, there is the spatially periodic nongrid cell (Hales et al. 2014) which contains irregular spatial firing fields. Grid cells exhibit the most activity upon the MEC stimulation; however, other cells also display short-latency firing (Zhang et al. 2013). The function of cells present in the MEC furthers the argument that the structure is involved in processing of spatial and directional information. Additionally, it has also been observed that encoding and retrieval occur between the hippocampus and MEC through theta-gamma modulated oscillations (Igarashi et al. 2014). However, it remains unclear what is the significance of MEC firing. This thesis dissertation investigates the extent of MEC involvement in spatial memory encoding, as well as its influence on spatial firing within the hippocampus.

Spatial Memory Tasks

In this thesis dissertation a spatial alternation behavior task was used in order to assess hippocampal firing, and the behavioral implications related to bilateral lesions of the hippocampus and the MEC. The spatial alternation task involves the use of a modified T-maze that resembles the shape of the number eight. In this task animals are trained to run in an alternating pattern between the left and the right arms of the maze which are bisected by a central stem. The alternating pattern is behaviorally reinforced in order for the animals to learn the task. However, the task itself is not hippocampal dependent if run continuously (Ainge et al 2007). In fact, this task falls under procedural nondeclaritive memory that is independent of the medial temporal lobe. Its encoding and consolidation are independent of the hippocampus. Instead it is more reliant on the striatum, since it acts as a procedural habit or skill (Squire 2004). Therefore, animals with hippocampal and MEC lesions are expected to preform equally as well as controls in the non-delay task.

However, if a delay is introduced in the central stem the task requires context-dependent hippocampal activity. By introducing the delay, the animal comes to a stop and its automatic behavior is interrupted which increases its working memory load. The task now requires a recall of the previous route and a prediction the correct one in order to receive the reward. These are declarative memories of spatial events. By further increasing the length of the delay the working memory load keeps extending as well. However, working memory is limited in capacity and duration. Therefore, some of the working memory load is conveyed to long-term memory in order for the task to be

performed correctly (Lee et al. 2003). Long-term spatial memories are largely encoded by the hippocampus, while also accepting some contributions from the MEC. These long-term memories might not consolidate within the cortex due to the relatively short time span of the behavioral task, however, their encoding and recall are dependent of the hippocampus (Ainge et al. 2007). Therefore, only animals with intact hippocampi are expected to be able to perform the delayed alternation task. It is yet unclear to what extent MEC lesioned animals might be impaired in the task. Therefore, this research project will investigate to what degree the MEC might be implicated in encoding of spatial information for the delay alternation task.

Hypothesis

This thesis dissertation is guided through two different entities, one being physiological and the other behavioral. MEC can act as an upstream element to the hippocampus and possibly influence its firing. From the physiological aspect, it can be hypothesized that any impairment in the MEC can disrupt the spatial firing within the hippocampus. This physiological disruption is hypothesized to be reflected in behavior. Therefore, from a behavioral aspect, it can be hypothesized that MEC lesions would produce impairment in the delayed alternation task. The extent of impairment is hypothesized to be approximately the same as the one of hippocampal lesions.

CHAPTER 1: METHODS

Subjects

The care for all of animals was supervised by the Institutional Animal Care and Use Committee (IACUC) of University of California, San Diego. The subject group consisted of 31 male Long–Evans rats weighing up to 500g at the time of the lesion surgery. Rats were housed individually on a reversed 12 h light/dark cycle. All behavioral and recording sessions were obtained during the dark phase of the cycle. All of the subjects were experimentally naïve. All animals were randomly assigned to one of four groups: (1) a group with N-Methyl-D-aspartic acid (NMDA) lesions of the medial entorhinal cortex (MEC; n=6), (2) a group with ibotenic acid (IBO) lesions of the hippocampus (H; n=6), (3) a group with both IBO lesions of the hippocampus and NMDA lesions of the medial entorhinal cortex (Double; n=5) and (4) a Sham group (Sham; n= 6). A control group underwent the same initial surgical procedures as the other groups in order to control for confounding variables related to the surgical incision. However, for the Sham group the dura mater was not punctured, the syringe needle was not lowered into cortex, and excitotoxins were not injected into the brain. After a 4-week recovery period from surgery, rats were food restricted and maintained at or above approximately 85% of their ad libitum weight.

Lesion Surgery

All surgery was performed using aseptic procedures. Isoflurane gas was used for anesthesia and it was maintained throughout surgery (0.8%-2.0% isoflurane delivered in O2 at 1 L/min). The animal was positioned in a Kopf stereotaxic instrument, and the incisor bar was adjusted until bregma was level with lambda. The bone overlying the target site was removed using a high-speed drill. The Sham group underwent the initial surgical procedures, but no lesions were induced with the exotoxins. In the three experimental groups (MEC, H, Double), excitotoxic lesions were produced by ibotenic acid (IBO), an excitatory analog of glutamic acid, or NMDA. Ibotenic acid (Biosearch Technologies, San Rafael, CA) was dissolved in 0.1 M PBS to provide a solution with a concentration of 10 mg/ml, pH 7.4. NMDA (Tocris) was dissolved in aCSF (Harvard Instruments) to provide a solution with a concentration of 10 mg/ml. IBO or NMDA was injected at a rate of 0.1 µl/min using a 10 µl Hamilton (Reno, NV) syringe. The syringe was mounted on a stereotaxic frame and held with a Kopf model 5000 microinjector. The syringe needle was lowered to the target and kept there for 1 min before starting the injection. After the injection, the syringe needle was left in place for 2 min (IBO) or 1 min (NMDA) to reduce the spread of drug up the needle tract. For the H group, IBO was injected into 18 sites (total volume, 0.51 µl) on each side of the brain. The injection was intended to damage the dorsal and ventral hippocampus (all coordinates are in millimeters, anteroposterior is relative to bregma, mediolateral is relative to lambda): anteroposterior (AP) -2.4, mediolateral (ML) ± 1.0, dorsoventral (DV) -3.5; AP -3.2, ML ± 1.4, DV -3.1, -2.3; AP -3.2, ML ± 3.0, DV -2.7; AP -4.0, ML ± 2.5, DV -2.8, -1.8; AP -4.0, ML

± 3.7, DV -2.7; AP -4.8, ML ± 4.9, DV -7.2, -6.4; AP -4.8, ML ± 4.3, DV -7.7, -7.1, -3.5; AP -5.4, ML ± 4.2, DV -4.4, -3.9; AP -5.4, ML ± 5.0, DV -6.6, -5.9, -5.2, -4.5. For the MEC group, NMDA was injected into 8 sites (total volume 1.04 µl) on each side of the brain. The injection was intended to damage the complete area of medial entorhinal cortex. The AP coordinate was dictated by the location of the anterior border of the transverse sinus, and the needle was inserted at ML ± 4.6 with an angle of 22º moving from posterior to anterior at that location with DV values: -5.2, -4.7, -4.2, -3.7, -3.2, -2.7, -2.2, -1.7. For the Double group, IBO and NMDA were injected into the hippocampus and grid cell area of medial entorhinal cortex, respectively, at the same sites as were used for each lesion alone. After each lesion completion the animal was allowed to recover from anesthesia on a water-circulating heating pad. Once awake the animal was placed into a clean cage with surgical bedding and was monitored post-operationally for a minimum of 5 days. The total recovery after the lesion surgery was 4 weeks. During this time animals were not allowed to be trained for any behavior task or be food deprived.

Behavioral Apparatus

For the delayed alternation task the behavior was conducted in a modified T-maze, also known as figure-8-maze (Figure 1). The maze was constructed from interlocking hard, grey plastic runways 10 cm wide, each equipped with 2 cm tall walls. The center runway that formed the stem of the figure-8-maze was 150 cm long and 10 cm wide and so were the right and left return arms. A crosspiece 101 cm long and 10 cm wide connected the center arm to the right and left return arms at the top and at the

bottom of the maze. An automatic and a manual barrier were used to introduce delay intervals between some of the trials. The delay zone was comprised in the center arm and it was 25 cm long. Food-rewards were delivered at the distal extremes of each of the return arms after the animals made a correct choice. A punctured plastic container filled with Coco Puffs was placed below each reward site in order to avoid guidance through the olfactory system. The figure-8-maze was elevated 50 cm above the floor and positioned within an open environment with prominent and constant visual cues.

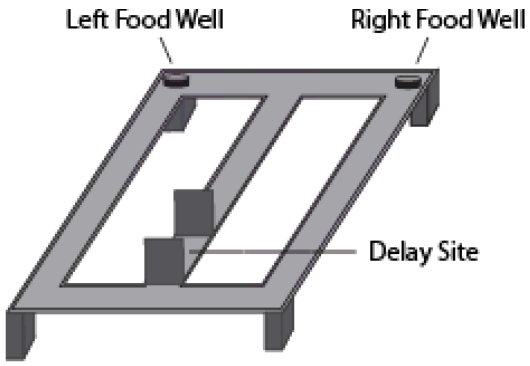

Figure 1. Spatial Alternation Task. All animals are initially placed at the delay site of the maze. Food wells were present on each arm to reinforce an alternating running pattern for the task.

Behavior: Habituation

Rats were given 4 weeks to recover from the lesion surgery before training for the behavioral task. After recovery from surgery, rats were handled and brought to the room where the behavioral testing would take place. Three day prior to the beginning of the training, rats were given Coco Puffs in their home cages. The first experimental day, “habituation”, rats were allowed to freely explore the maze for 10 min. Coco Puffs were

spread over the maze during the exploration. The animals were also given Coco Puffs in their home cage at the end of the session.

Behavior: Stage 1

The "first stage" began the second experimental day. All animals began food deprivation at this stage. Each rat was placed at the base of the center arm of the figure-8-maze and a manual barrier was used to force the rat to enter one of the two side arms where a reward was delivered. The rat was prevented from retracing his steps at any point. After consuming the reward, the rat was guided to return to the base of the center arm and was allowed to run to the opposite connecting arm in a figure-8-pattern. To force the correct choice upon the rat, the arm visited in the previous trial was blocked with the manual barrier. Each session was 20 min long or 30 trials whichever came first. This procedure was repeated to alternate arms, until the animals run the pattern consistently during two consecutive days.

Behavior: Stage 2

In the "second stage" the manual barrier at the choice point was phased out and the rats were able to enter either arm each time they reached the end of the stem. For the very first trial food was placed in both food wells, so that the animal was allowed to set its own running pattern. After the first trial the food was present only in one food well in order to ensure that reward was given for alternating runs only (Figure 2). Rats were prevented from retracing their steps. Each behavioral session in stage 2 was either 20 min long or composed of 30 trials (whichever came first). Rats were trained to a

criterion performance of at least 90% correct trials on two out of three consecutive days. After reaching that criterion they were allowed to move onto stage 3.

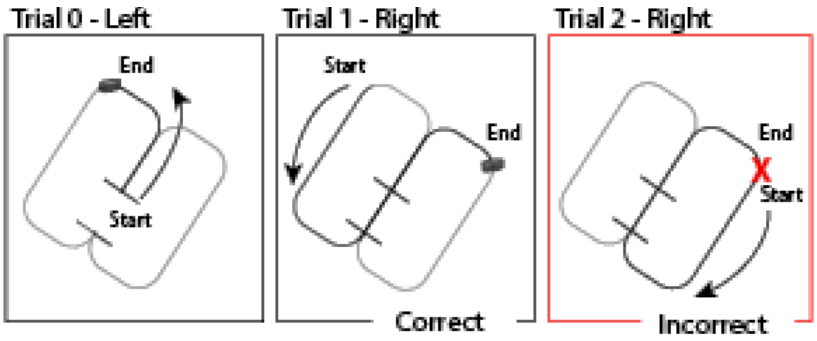

Figure 2. Alternation Pattern. All animals are trained to run an alternation pattern on the Figure 8 maze. The animal is initially placed at the base of the central arm and allowed to switch between the left and right sides of the maze. Reward is given only when the animal alternates between the arms on the maze.

Behavior: Stage 3

Delay testing "third stage" started once the rats reached this criterion. In each daily session, rats received 30 trials grouped into three blocks of 10 trials (no delay, 10 s delay and 60 s delay). The order of the three blocks was pseudorandomized every day. During delay trials, as the rat returned to the base of the stem after the last trial of the previous block, two barriers were placed to confine the rat to a 25 cm zone at the base of the stem. At the end of the delay interval, the barrier which allowed access to the center arm was removed and the rat was free to traverse the stem and make its next choice. After the rat made a choice and ate the reward, the barrier that gave access to the delay choice was removed and the one that blocked the pass to the center arm was reintroduced. This stage continued for 14 days that we called "phase 1" (see Figure 3).

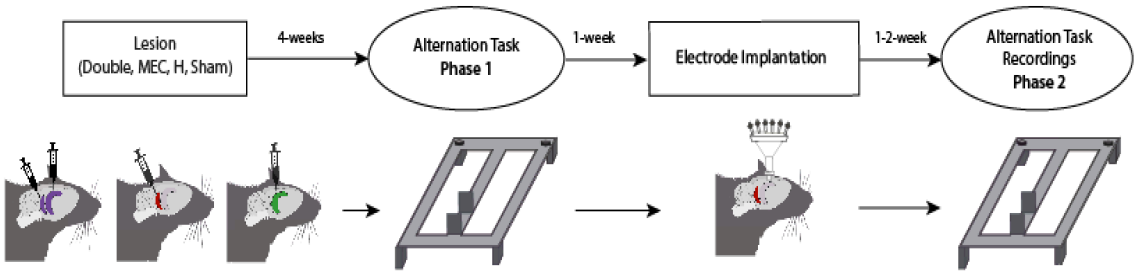

Figure 3. Experimental procedure. All experimental animals have undergone lesion surgery before any behavioral training. After recovering from surgery animals began learning the continuous spatial alternation task. Training of animals occurred in stages 1 and 2. In stage 1 animals ran a forced alternating pattern, while in stage 2 they were reinforced for making correct choices. After passing criterion for both stages 1 and 2, animals were allowed to move onto stage 3, also known as phase 1. Phase 1 had 30 trials that were composed of both delay (10s and 60s) and no delay runs. After running in phase 1 for 14 days, some animals underwent another surgery for electrode implantation. Those animals were allowed to recover from the surgery and were then moved onto phase 2. In phase 2, animals ran 60 trials composed out of delay (10s and 60s) and no delay runs. During those trials electrophysiological recordings have also been performed. Phase 2 lasted for at least 6 additional days.

Recording Device Implantation Surgery

Some subjects (4 MEC and 5 Sham) underwent a second surgery procedure in order to implant a fourteen-tetrode recording assembly. The second surgery followed the same protocol used for the lesion procedures. However, in this case a recording device was implanted in the cortex area lying above the dorsal hippocampus. Tetrodes were constructed by twisting four 17 µm polyimidecoated platinum-iridium (90 %/10 %) wires, and the electrode tips were plated with platinum to reduce the impedances to 200–300 kΩ at 1 kHz. The tetrodes were arranged into a bundle targeted to the hippocampus in the right hemisphere (AP: 4.0., ML: ± 2.8).

Recording

Nine animals (MEC=4 and SHAM=5) were fed ad libitum for a week after the recording device (14 tetrode-hyperdrive) was implanted in order to record from the CA1 cell layer. During the recovery period from surgery, tetrodes were slowly advanced into the CA1 area of the hippocampus. Three days after surgery rats started being allowed to run some trials without delays in order to get used to the cable. During tetrode advancement and recordings, the signals were preamplified with a unity gain headstage and then recorded with a data acquisition system with 64 digitally programmable differential amplifiers (Neuralynx, Tucson, AZ, USA). Spike waveforms above a threshold of 40-45 μV were time-stamped and digitized at 32 kHz for 1 ms. The rat's position was tracked at 30 Hz by recording the position of light-emitting diodes that were placed above the head. Local field potentials (LFP) were acquired by recording one channel of each tetrode with the filters set to the 1-450 Hz band. As expected (Hales et. al 2014), sharp wave ripples were not diminished by the MEC lesion and could therefore be used to guide electrode advancement into the cell layers in all rats.

Recording in the figure-8-maze began when tetrodes were stably positioned in the CA1 cell layer "phase 2". Spikes and LFP were also recorded while the rat was resting in a transparent holding chamber located in the same room for 1 hour at the beginning and 1 hour at the end of each recording day. The room had a light source on a corner at approximately 1 meter from the maze and 2 meters from the sleep chamber that kept the environment dimly illuminated. After the first sleep period, the animals run the delayed alternation task following the same protocol used during "stage 3 of

phase 1". Immediately after, the rat was put back in the resting holding chamber and the second sleep period began. Each animal run 1 session per day (sleep1, delayed alternation, sleep2) for an average of 6 days. Data collection and analysis were not performed blind to the conditions of the experiment.

Neurohistological Methods

Rats were administered an overdose of sodium pentobarbital and perfused transcardially with a phosphate buffered solution followed by 4% paraformaldehyde solution (in 0.1 M phosphate buffer). Brains were then removed from the skull and kept in a solution of 4% paraformaldehyde for 24 h. After this, brains were transferred to a 30% sucrose solution were they stayed for an average of 48 hours. Sagittal sections (40 μm) were cut with a freezing microtome beginning just lateral to the hippocampus and continuing medially through the length of the hippocampal region for each hemisphere. Every section was mounted and stained with cresyl violet. All sections were used to confirm the final locations of each tetrode. Through the guidance of the hyperdrive map all tetrodes could be identified and cross-checked with the data acquired during electrophysiological recordings. For MEC recordings the tetrode tracks were found in more lateral slices, while for the hippocampal recordings the tetrode tracks were always expected to be more medial. Every fourth section was used to quantify the lesion completion with the Cavalieri method, as previously described (Hales et al. 2014). For MEC lesions the volume of the spared tissue was estimated for the MEC layer II, MEC layer III, MEC deep layers, dorsal parasubiculum, ventral parasubiculum, and

hippocampus. For hippocampal lesions spared cell layer tissue was quantified for CAs, dentate gyrus (DG), subiculum and presubiculum. For double lesions both, MEC and H quantification protocols were followed. Damage to the brain areas other than the lesion targets were not substantial, as previously reported (Hales et al. 2014).

CHAPTER 2: RESULTS

Lesion Quantification

In order to confirm that the entire MEC, hippocampus, or both structures were included in the lesions, the extent of damage was evaluated in sagittal sections (Figure 4). The sections were cut at 40 nm and stained with cresyl violet to visualize any remaining neurons in the target of the lesion.

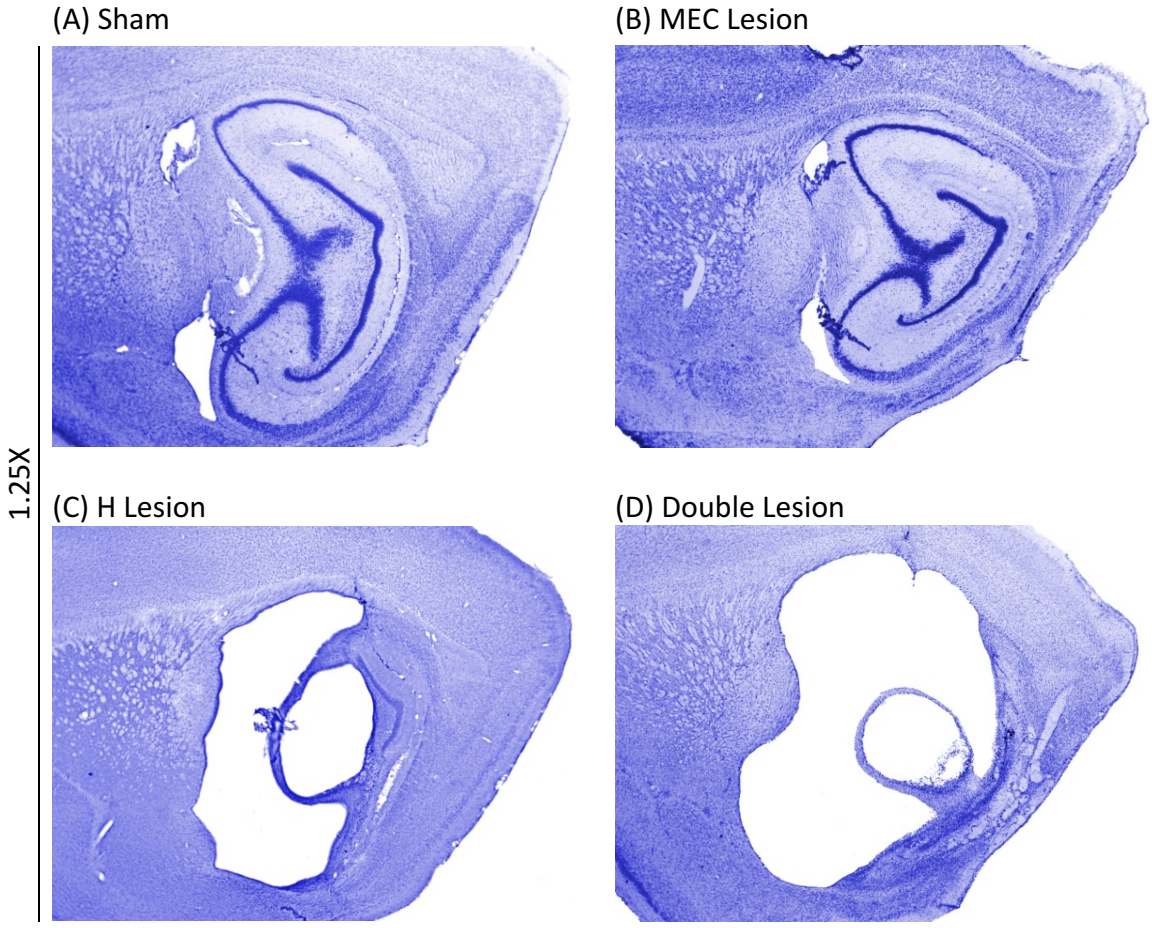

Figure 4. Lesion Quantification. 40 nm thick, sagittal sections of the right hemisphere stained using Cresyl Violet. Pictures were taken at 1.25X magnification. Sections presented include rats from (A) Sham, (B) MEC, (C) H and (D) Double lesions groups.

The lesion extent was quantified using the Cavalieri method that was described by Hales et al. 2014. Tissues were evaluated for possible spared areas and counted as grid points within relevant anatomical structures (MEC and hippocampus). A total volume of spared tissue was estimated by summing the total number of section thickness. The percent damage was calculated by dividing the volume of spared tissue by the average volume of tissue in Sham group rats, subtracting the division from 1 and then multiplying it by 100 (Figure 5).

$$\text{Percent damage} = \left(1 - \frac{\text{spared estimated volume}}{\text{average volume in Sham group}}\right) \cdot 100$$

Figure 5. Percent Damage Equation. Percent damage formula was used to evaluate the completion of lesions for all experimental groups.

In the MEC group (Figure 6), neurons were completely ablated in 95% of the total MEC volume (97.4% of layer II, 94.4% of layer III and 93.2% of deep layers), with the majority of the sparing in the most lateral extent of the MEC (Figure 9). Cell loss in adjacent cortical areas was predominantly in the parasubiculum (PAS) and the postrhinal cortex and was minor in the ventral hippocampus and the LEC. In the hippocampus lesion (H) group (Figure 7), the average damaged tissue included 72.7% of the total hippocampus (75.3% of the CA cell layers and the 70.2 % of the dente gyrus), with the majority of the sparing at the most posterior transition between the dorsal and ventral hippocampus (Figure 10). In the Double lesion group the total lesion was averaged to be 94.8% overall (Figure 8). The average completion of lesions in this group

was 95.6% for CA cell layers, 93% for the DG, 94.7% for MEC layer III, 96.0% for MEC layer II, and 95.0% for deep layers (Figure 11).

Damage per Structure (%)					Total MEC Damage (%)
Deep layers	Layer III	Layer II	dPAS	vPAS	95.06
93.3	94.4	97.4	57.3	61.1	

Figure 6. MEC Lesion Quantification. Table contains average percent damage per structure, as well as the total percent damage for the entire MEC. Total MEC damage was obtained as an average of damage in deep layers, layer III and layer II.

Damage per Structure (%)				Total H Damage (%)
CAs	DG	SUB	PRESUB	72.7
75.3	70.2	70.1	49.8	

Figure 7. Hippocampal Lesion Quantification. Table contains average percent damage per structure, as well as the total percent damage for the entire hippocampus. Total hippocampal damage was calculated as an average of damage in the CA layers and the dente gyrus.

Damage per Structure (%)									Total Double Damage (%)
Deep layers	Layer III	Layer II	dPAS	vPAS	CAs	DG	SUB	PRE SUB	94.8
95.0	94.7	96.0	63.2	88.5	95.6	93.0	89.0	85.4	

Figure 8. Double Lesion Quantification. Table contains average percent damage per structure, as well as the total percent damage for the Double lesioned group (MEC & hippocampus). Total Double damage was calculated as an average of damage in the CA layers, dente gyrus, deep layers, layer III and layer II.

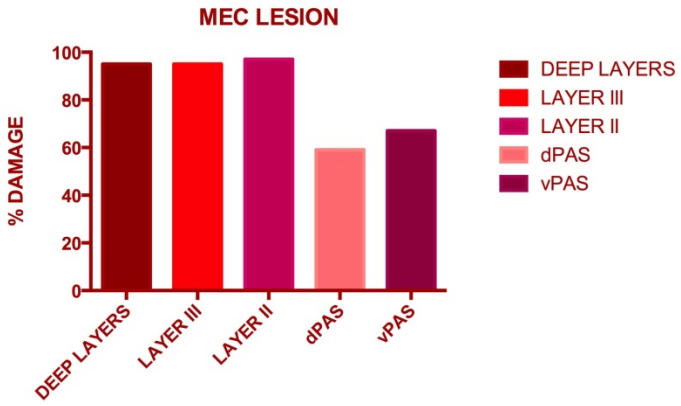

Figure 9. Percent Damage in MEC Lesions. Average percent damage per structure for the MEC lesioned group.

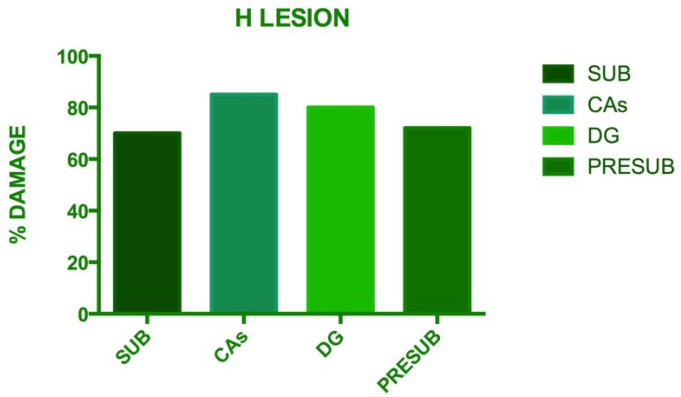

Figure 10. Percent Damage in H Lesions. Average percent damage per structure for the H lesioned group.

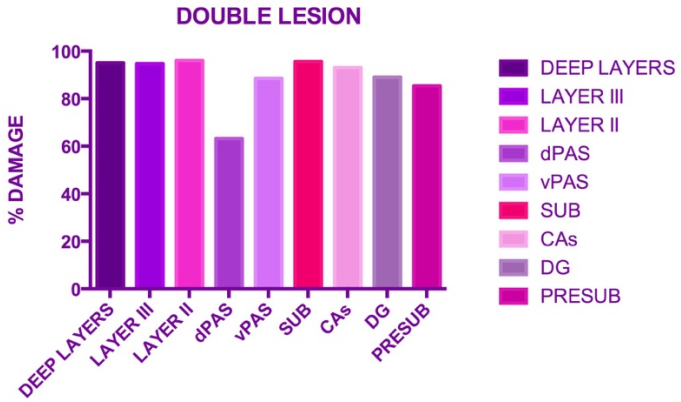

Figure 11. Percent Damage in Double Lesions. Average percent damage per structure for the Double lesioned group.

Tetrode Tacking

Cresyl violet stained sections were also used to determine the final location of recording tetrodes in the hippocampus (Figure 12). Tetrode tracks were observed as an apparent shift in damage between sections (Hales et al. 2014). The most ventral part of each tetrode track was considered the tetrode tip. The tip marks the final location of electrophysiological recordings for that tetrode. Recordings from tetrodes with final position in CA1 pyramidal cell layer were included in the data analysis. Tetrodes that recoded CA2-3 layer were not included.

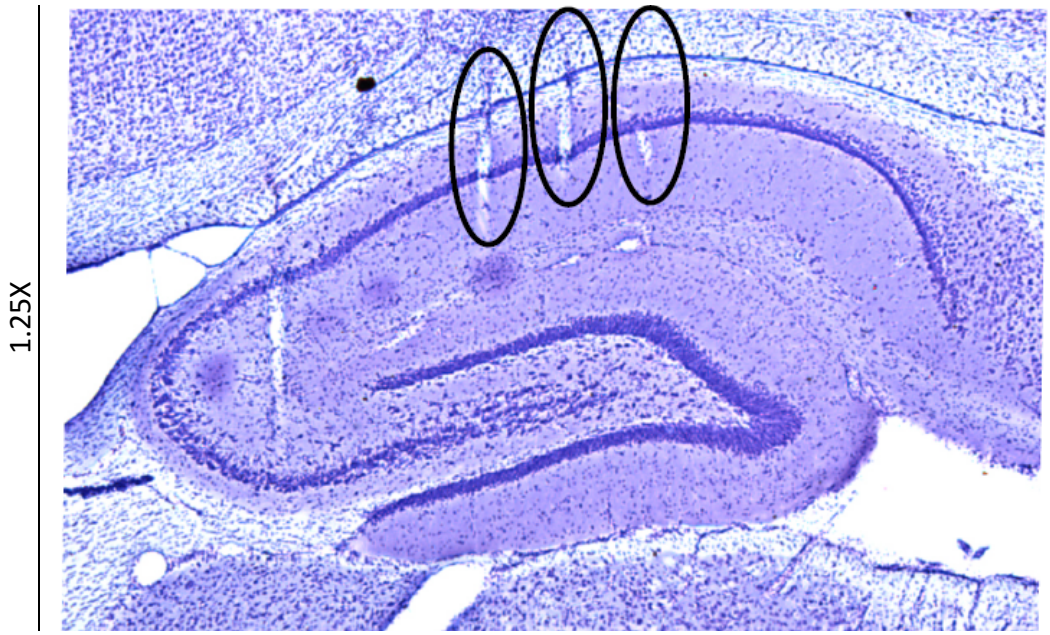

Figure 12. Tetrode tracking. 40 nm thick, sagittal section of the right hemisphere stained using cresyl violet of a Sham animal. Pictures were taken at 1.25X magnification. Tetrode tracks that terminated in the CA1 cell layer are marked with black circles.

Behavior

Rats with either bilateral hippocampal lesions (N=6), bilateral MEC lesions (N=6), double lesions of the hippocampus and the MEC (N=5) or Sham lesions (N=6) were food deprived and trained to perform a continuous spatial alternation task in which the animals alternated between left and right sides of a figure-8-maze on a trial-by-trial basis to receive food reward. When animals reached criterion (90% of correct trials in three out of 4 consecutive days), blocks of trials were introduced with 10-second and 60-second delays for a period of 14 daily sessions (phase 1). After surgery, tetrodes were used to obtain hippocampal recordings in MEC lesioned (N=4) and Sham animals (N=5). Recordings during the spatial alternation task (phase 2) began when tetrodes were positioned in the CA1 layer. Testing continued for additional six days. Some H (N=2) and Double lesioned (N=3) animals ran the alternation task during this phase as well.

In phase 1 (Figure 13), a mixed-model ANOVA (Session x Delay x Lesion) showed a Delay x Lesion significant interaction effect $F(6, 38)= 17.596$), $p<0.0001$. This interaction was accompanied by significant main effects of Session $F(13, 247)= 5.308$, $p<0.0001$ and Delay $F(2, 38)=365.468$, $p<0.0001$ (figure 3: Anova). To analyze the interaction (Delay x Lesion), the post-hoc Tukey's multi-comparisons test was conducted. All groups (N=23) performed similarly in the trials without delay. There was no significant difference in performance between groups for no-delay trials. However, when a delay was introduced all the lesion groups (MEC, H and Double) made significantly more errors than the Sham group in both 10-second and 60-second delay

conditions (all $p<0.0001$). Moreover, the Double lesion animals were more impaired in the task than single lesion ones (MEC and H) in both delay conditions (all $p<0.05$). The Double lesion group also performed below chance level (50% correct response) for both delay conditions.

In phase 2 (Figure 14), a mixed-model ANOVA (Session x Delay x Lesion) showed a Delay x Lesion significant interaction effect $F(4.108, 13.695)=22.685$, $p<0.0001$. The greenhouse-geisser correction was used due to the lack of sphericity. The main effect of delay was also found significant $F(1.369, 13.695)=244.954$, $p<0.0001$. The analysis of the interaction (Delay x Lesion) revealed that there was no significant difference between groups in the no delay condition. However, the lesion groups (MEC, H and Double) performed worse than Sham rats in trials with delay conditions (all $p\leq0.0001$). There was no significant difference in performance of MEC and H lesion groups on the delay conditions. However, the single-lesion groups (MEC and H) performed significantly better than the Double lesion animals in delay trials (all $p<0.06$). The Double lesion group preformed below chance level for both delay conditions.

In both phases, single lesions (H or MEC) derive a similar impairment level in memory performance. Lesion of either structure caused impairment in delay conditions, but not in non-delay ones compared to Sham animals. Double lesion of both structures produced more severe memory deficits. Double lesioned animals performed significantly worse than both Sham and single lesioned groups in delay trials, but not in no-delay ones. The memory deficit seems to be severe enough to cause the animals to perform below chance level in both phases of behavioral testing.

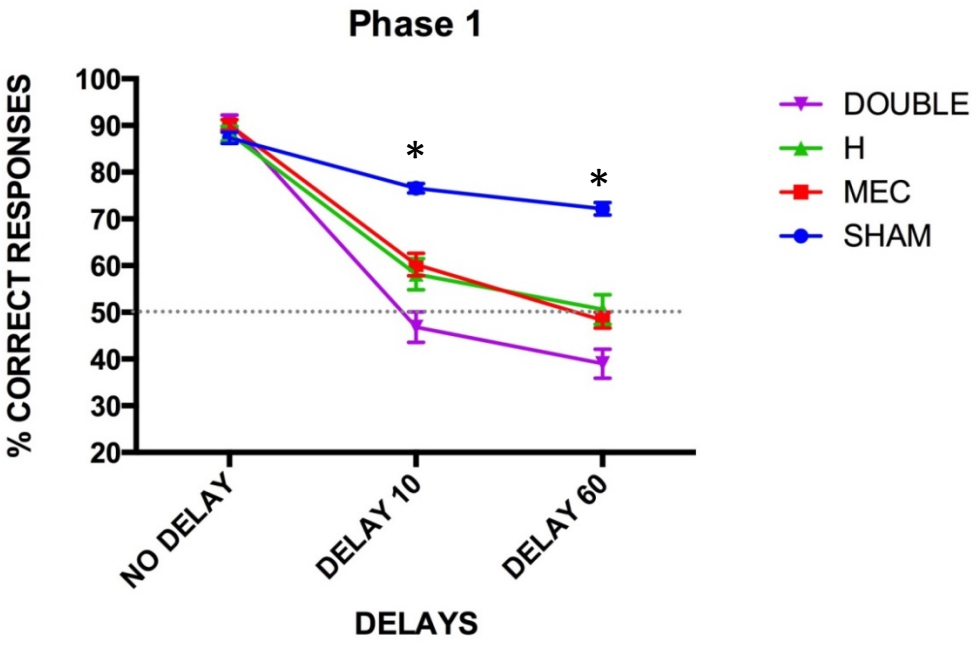

Figure 13. Phase 1 Behavior. Percent correct responses on the spatial alternation task for no delay, delay 10s and delay 60s conditions, across all groups.

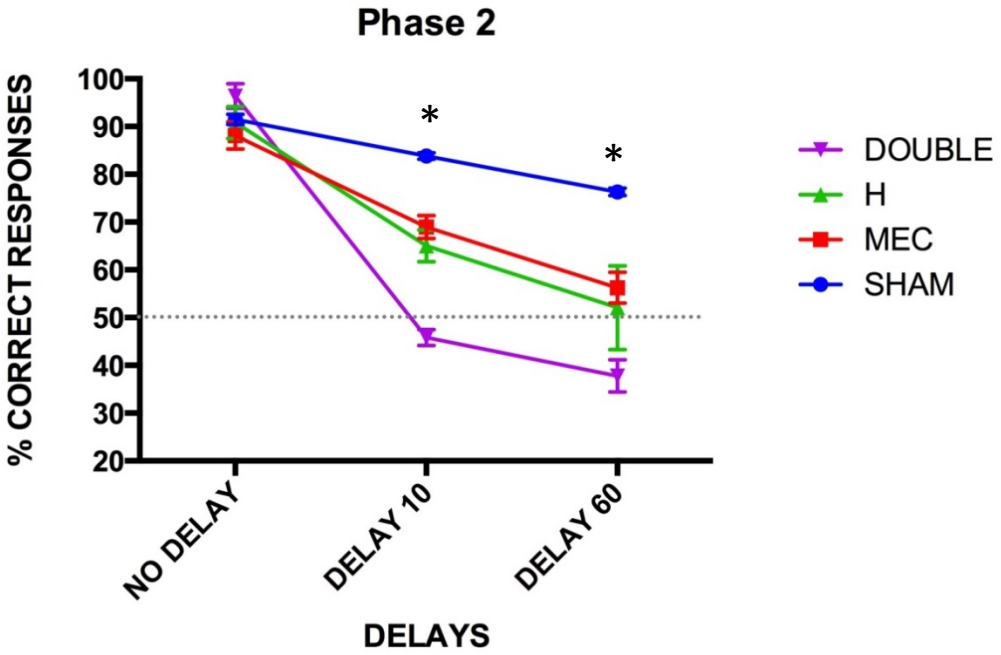

Figure 14. Phase 2 Behavior. Percent correct responses on the spatial alternation task for no delay, delay 10s and delay 60s conditions, across all groups.

Perseveration

MEC and H single-lesioned animals performed worse than the control in trials with delay conditions. However, the average number of errors that they made decreased after extended training. Double lesions were prevented from the same improvement in memory performance.

A regression analysis was performed in order to evaluate the relationship between correct responses and time spent training for both phases (Figure 15). For the no delay condition, the slope was significant for Sham, MEC lesion and Double lesion groups (SHAM Slope significant ≠ 0, r^2 =0.06; MEC Slope significant ≠ 0, r^2 =0.036; DOUBLE Slope significant ≠ 0, r^2 =0.09). However, in H lesion group no significant slope was observed (H Slope not significant = 0, r^2 =0.031). For 10s delay condition, slopes were significant for the Sham group and the single lesioned groups, but not for the Double lesion group (SHAM Slope significant ≠ 0, r^2=0.16; MEC Slope significant ≠ 0; r2 =0.13, H Slope significant ≠ 0; r^2 =0.19; DOUBLE Slope not significant = 0, r^2=0.001). For the 60s delay, the slope what again significant for the Sham and single lesioned groups, but not for the Double lesion group (SHAM Slope significant ≠ 0, r^2 =0.04; MEC Slope significant ≠ 0, r^2 =0.07; H Slope significant ≠ 0, r^2 =0.04; DOUBLE Slope not significant = 0; r^2 =0.006).

Positive r^2 value indicated a linear relationship between time and performance. All r^2 were positive for all groups across all conditions. A positive slope across time was interpreted as an improvement in performance on the spatial alternation task, while no slope was interpreted as absence of any improvement. The Sham group improved in

performance in all conditions. The MEC lesion group also experienced improvement across all conditions. The H lesion group improved in performance in delay conditions, but not in the no-delay one. The Double lesion group improved in performance only in the no-delay condition, but not in any of the delay ones. All improvements in experimental groups were partial and never reached the same criterion as the Sham group.

We decided to analyze what types of mistakes deteriorated the performance of the Double lesion group on the spatial alternation task. First we examined the number of errors occurring in each condition (Figure 16A). Tukey's Multiple Comparison Test was used to evaluate any significant difference across groups in the number of errors made for each condition. Across all trials single lesioned H and MEC animals were not significantly different from one another. For the no-delay condition the Double lesion group made significantly less mistakes than the Sham group ($p \leq 0.04$). All other groups for the no-delay condition were not significantly different in the number of errors made. The single lesioned groups performed worse ($p < 0.0001$) than the Sham group for both delay conditions, but were not as impaired as the Double lesion group. The Double lesion group made significantly more errors ($p < 0.0001$) than the Sham group and the single lesion groups (MEC and H) for both delay conditions.

Upon further examination of behavioral data, it was revealed that the Double lesion group was prone to making a string of consecutive mistakes, instead of intermittent ones. This type of behavioral inflexibility, also known as preservation, is often observed in frontal lobe syndromes (Izaki et al. 2001). To analyze if these

consecutive errors were in excess to chance (Figure 16B) we calculated the average number of consecutive errors on shuffle data (repeated 100 times) and then subtracted it from the average number of consecutive errors observed. The data set was then subjected to Tukey's Multiple Comparison Test. For the no-delay trials the Double lesion group made significantly more consecutive errors compared to the Sham group ($p<0.05$). Single lesioned groups (MEC and H) were not significantly different from one another or the Sham group for that condition. No significant difference among groups was observed for 10s delay trials. For 60s delay trials, the Double lesion group made significantly more consecutive errors than the Sham group ($p\leq0.003$) and both of the single lesion (MEC and H) groups ($p\leq0.01$). Other groups were not significantly different for the 60s delay condition.

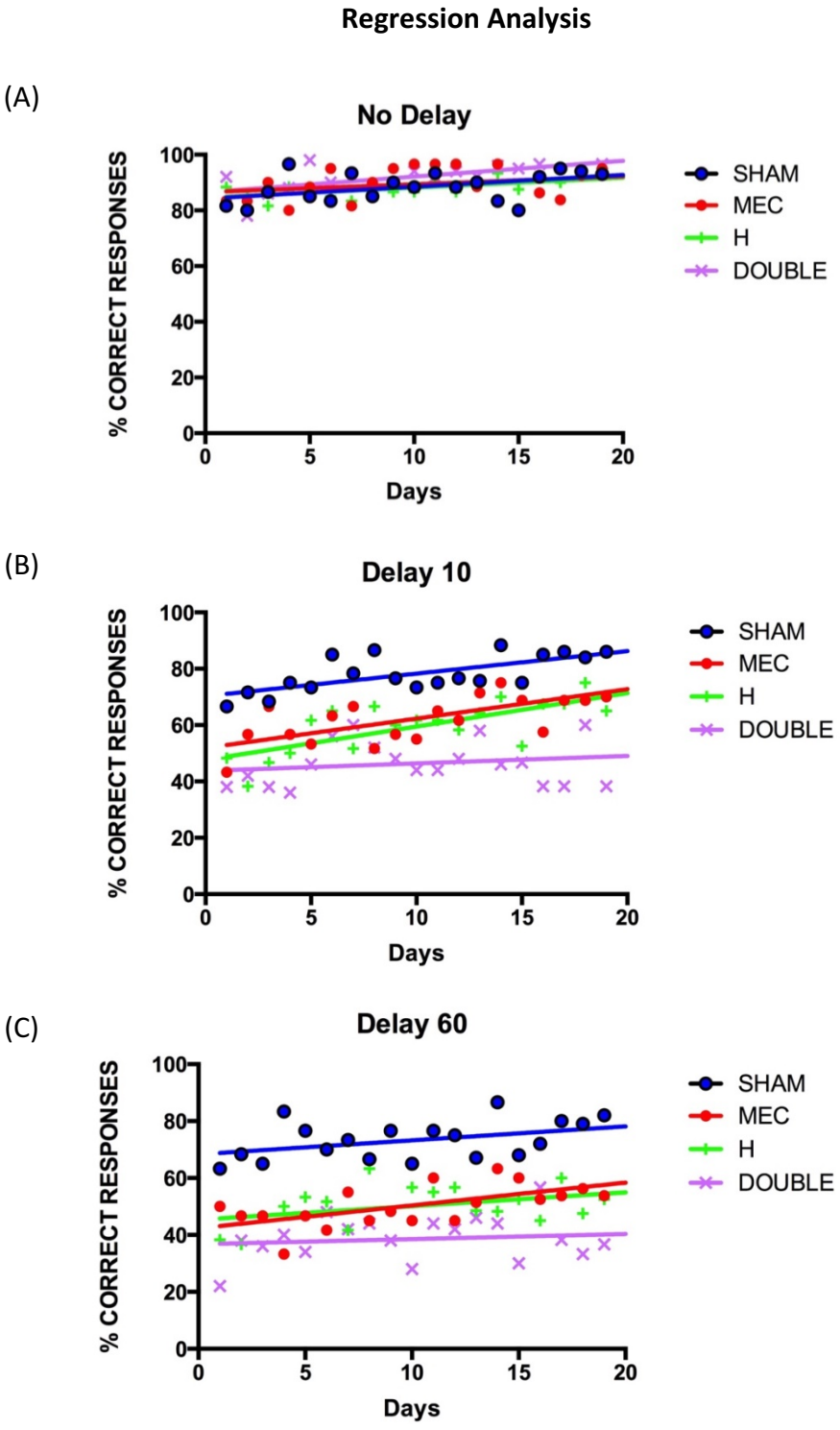

Figure 15. Regression Analysis. Regression of percent correct responses for (A) no delay, (B) delay 10s and (C) delay 60s conditions across all groups. Regression analysis encompasses behavior from both phase 1 and phase 2.

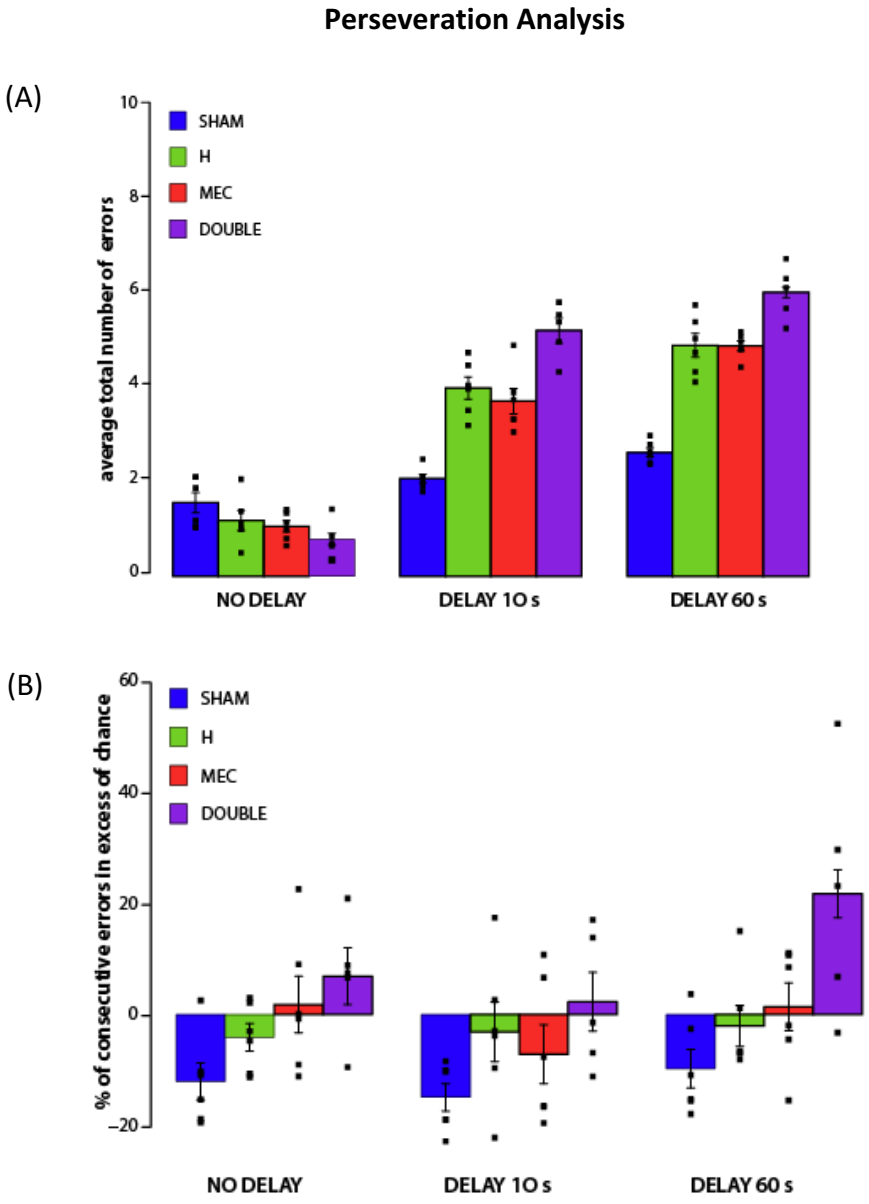

Figure 16. Perseveration Analysis. (A) Average total number of errors for no delay, delay 10s and delay 60s conditions across all groups. (B) Percent of consecutive errors in excess to chance (>50%) for no delay, delay 10s and delay 60s conditions across all groups.

Physiology

Previous behavioral analysis revealed that the MEC lesioned group performed significantly worse than the Sham group in the delay conditions on the spatial alternation task. In order to investigate if this behavioral impairment had a physiological basis, electrophysiological recordings of CA1 cells were performed in Sham and MEC lesioned animals (Figure 17).

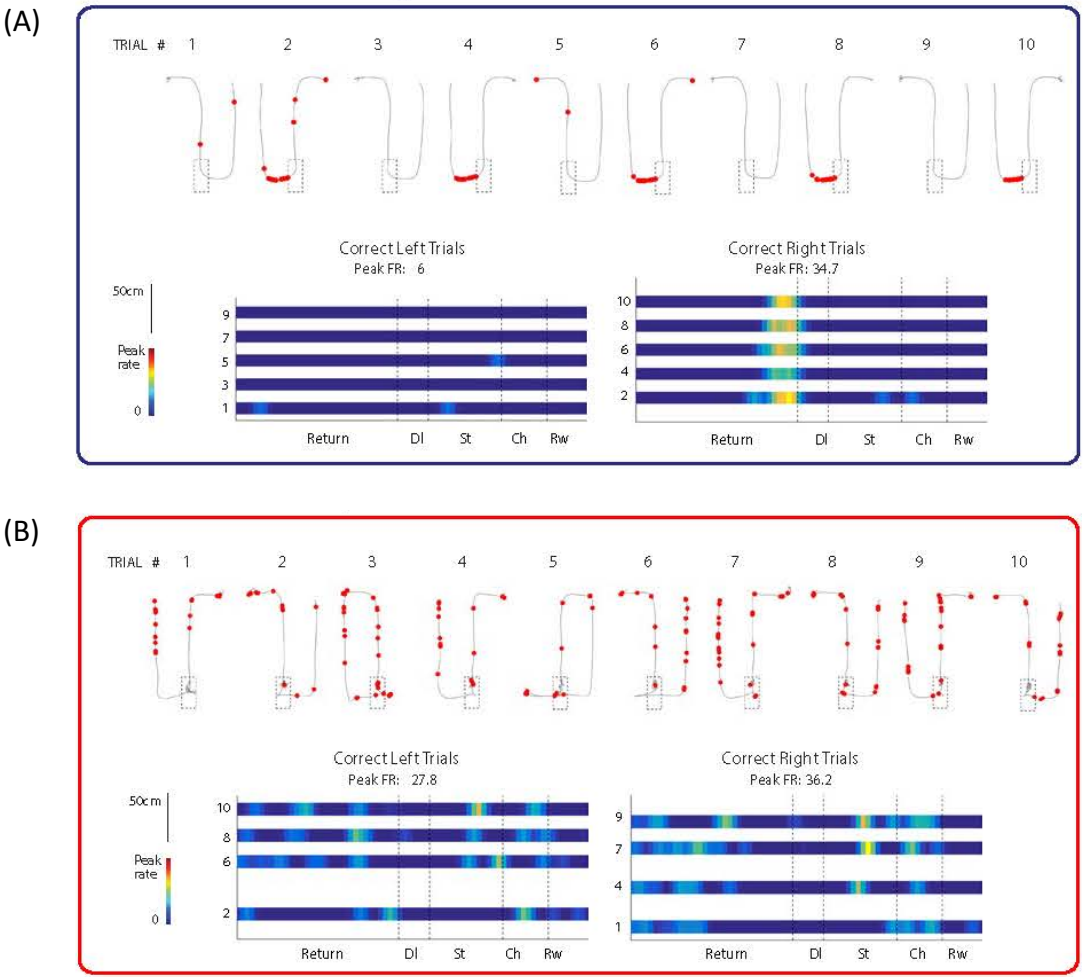

Figure 17. Examples of CA1 Firing on the Spatial Alternation Task. Examples of CA1 activity for correct trials on the spatial alternation task in (A) Sham and (B) MEC lesion group.

The analysis of electrophysiological recordings included 245 CA1 cells from the Sham group and 242 CA1 cells from the MEC lesion group. All cells were evaluated for their average mean firing rate, average peak firing rate, spatial information, across-sides spatial correlation and within-sides spatial correlation.

Each place cell codes for a place field through its firing. Therefore, each place field is an accumulation of firing, with higher frequency of firing in the center of the location and less firing on the outskirts. We observed the average firing frequency of each CA1 cell for a particular place field and calculated its average across events (when the location was revisited). CA1 cells were distributed into cumulative densities based on their average mean firing rate (Figure 19A). No firing rate threshold was used for this cumulative distribution; instead all cells were included in the analysis. Cells with average mean firing rates above 0.2 Hz were considered active. All the cells below that firing rate were deemed as silent. This criterion was set in order to ensure that the difference in firing did not emerge from a higher portion of cells firing at extremely low rates during behavior. The overall mean firing rate was calculated with the following equation:

$$\lambda = \int_x \lambda(x)p(x)dx$$

Figure 18. Overall Mean Firing Rate Equation. Overall mean firing rate equation, where x is the location, λ(x) is the mean firing rate at location x and p(x) is the probability density for the rat to be at that location (Skaggs et al. 1993).

Both the Sham and MEC lesion group produced similar cumulative density curves for the average mean firing rate. In fact, it seems that both groups experienced the

same percentage of silencing among CA1 cells that was approximately 40%. Therefore, any difference in firing between groups is not due to differences in the number of active and silenced cells. Furthermore, MEC lesions did not cause significant silencing of CA1 cells in the hippocampus. The average mean firing rate for both the Sham group and the MEC lesion group is approximately 0.5 Hz.

The peak firing rate is the maximum firing frequency of a place cell when the animal is in a particular location. The magnitude of the peak firing was observed on heat maps as a warm color, ranging from yellow to red (Figure 17). The presence of a high peak usually indicated a stable and precise place field, since the place cell would fire at its maximum frequency for that location. Cumulative density of CA1 cells was plotted for the average peak firing rate (Figure 19B). No firing rate threshold was used for this cumulative distribution; instead all cells were included in the analysis. We calculated the average peak firing rate by averaging the size of the peak (Hz) across all events for a particular location. It was found that the Sham group exhibited higher peak firing rate at lower cumulative densities, than did the MEC lesioned animals. This meant that even at smaller populations the CA1 cells in the Sham group were able to fire larger peaks than the MEC lesion group. This trend seems to be true for the entire cumulative distribution. The average peak firing rate for the Sham group was approximately 6 Hz, while the one for the MEC lesion group was approximately 5 Hz.

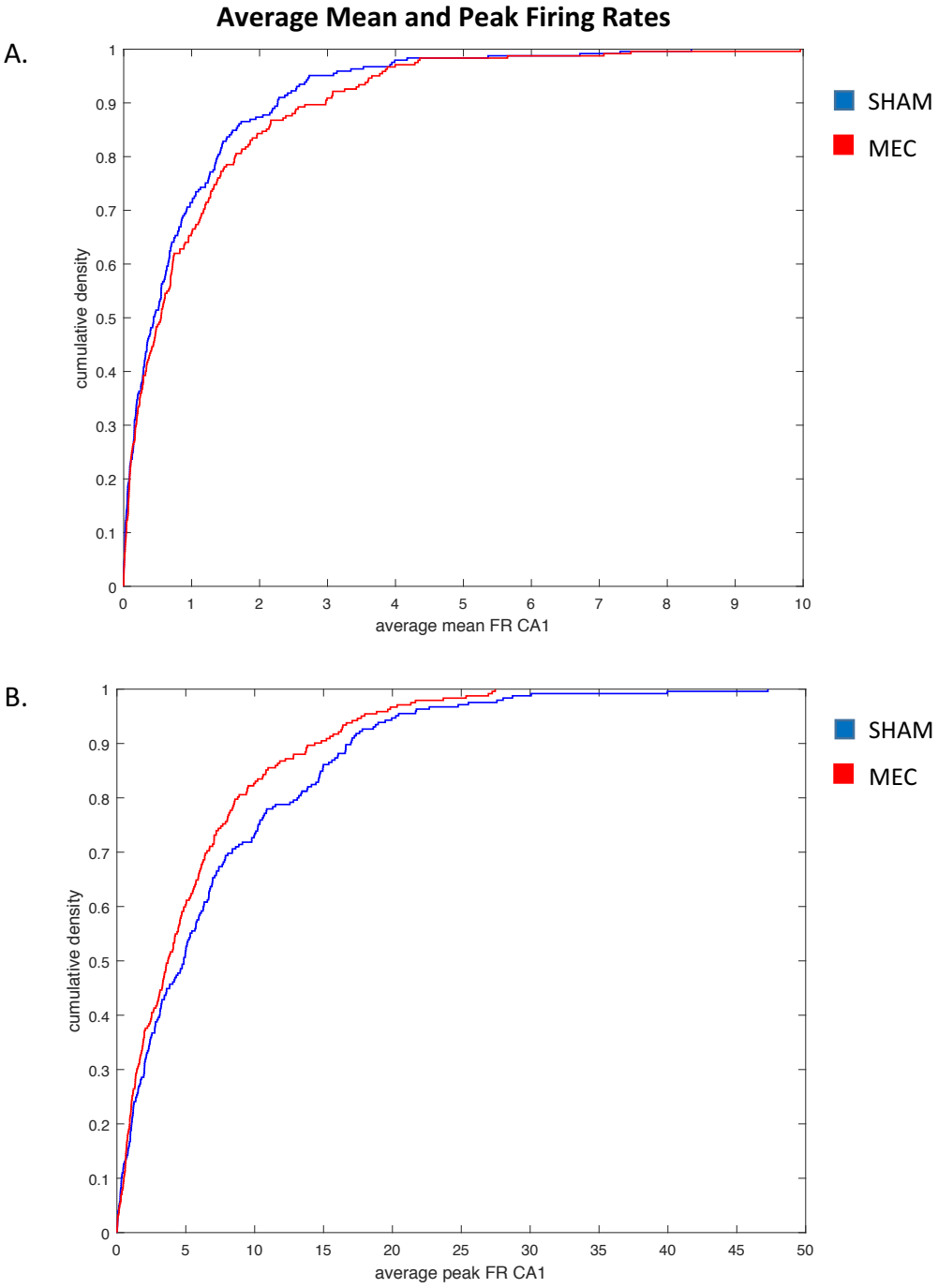

Figure 19. Firing Rates. (A) Cumulative density distribution of average mean firing rate (Hz) in CA1 cells, for Sham and MEC lesion animals. (B) Cumulative density distribution of average peak firing rates (Hz) in CA1 cells, for Sham and MEC lesion animals.

Compared to Sham animals, the firing of CA1 cells in the MEC lesion group was more scattered throughout the maze (Figure 17). This acted as a possible indicator the cells in MEC lesioned animals had an impairment in spatial information. Spatial information is a measurement of precision in firing. Therefore, place cells with high spatial information would fire over constricted areas around their peaks, while the cells with low spatial information would fire randomly over larger areas in the environment. Cells were evaluated for their ability to carry spatial information with the following equation:

$$I = \int_x \lambda(x)\, log_2 \frac{\lambda(x)}{\lambda} p(x) dx$$

Figure 20. Spatial Information Equation. Spatial information equation, where x is the location, λ(x) is the mean firing rate at location x and p(x) is the probability density for the rat to be at that location and λ is the overall mean firing rate of the cell (Skaggs et al. 1993).

CA1 cells from the Sham and MEC lesion group were distributed into cumulative densities based on their spatial information (Figure 21A). All cells got included in the analysis. The Sham group exhibited higher spatial information at lower CA1 population samples than did the MEC lesioned group. Furthermore, the Sham group was able to reach a higher maximum value of spatial information overall (approximately 3.5) than the MEC lesioned group (approximately 3.3). This trend was consistent for approximately 90% of the total population for both groups. The only exception arose at low cumulative densities, where the MEC lesioned animals had slightly higher spatial

information than the Sham group. The mean spatial information for the Sham group was above 1, while the one for the MEC group was below 1.

Compared to Sham animals, it was also noted that place cells in MEC lesion animals did not fire consistently for a particular location. In fact, the firing seemed random and inconsistent for every trial (Figure 17). This observation indicated a possible impairment in stability of place cells. Stability is a measurement of consistent firing for a particular location. Therefore, a place cell with high stability will always fire for the location it encodes, while an unstable one will be unpredictable in its firing. The stability of CA1 cells was analyzed through a within-sides spatial correlation. Within-sides spatial correlation observed any correlation in firing of a place call for the same location across all events. Place cells firing in the stem were excluded from this analysis. CA1 cells from the Sham and MEC lesion group were distributed into cumulative densities based on their within-sides spatial correlation (Figure 21B). Cells with firing rates lower than 0.25 Hz were excluded from this analysis. The Sham group exhibited higher within-sides spatial correlation across the entire population of CA1 cells, than the MEC lesion animal. Furthermore, the highest within-sides correlation in firing was observed at 0.9 for MEC lesion group, compared to approximately 1 for the Sham group. The mean within-sides spatial correlation for the Sham group was 0.7, while the one for the MEC group was 0.3.

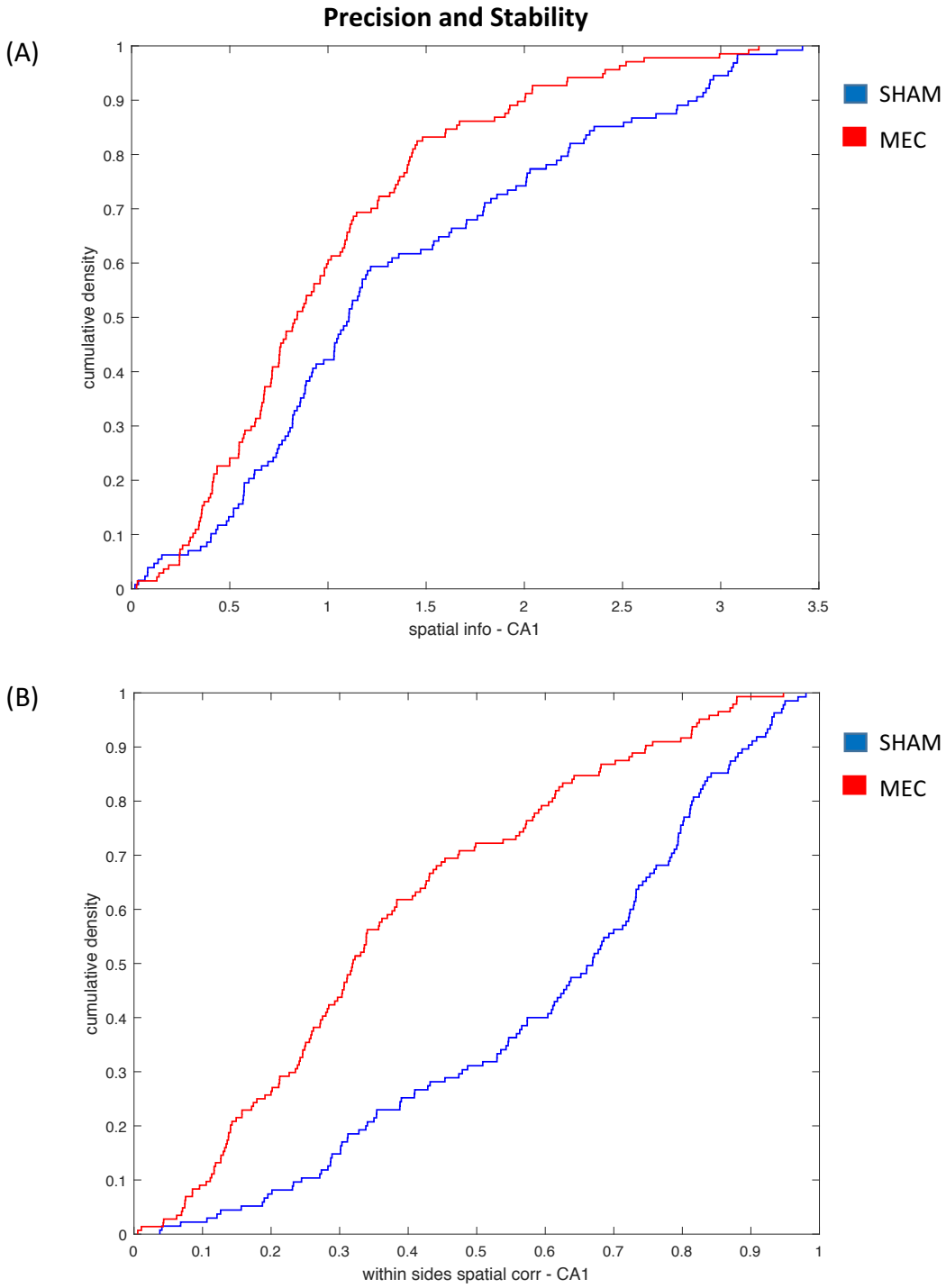

Figure 21. Precision and Stability. (A) Cumulative density distribution of spatial information in CA1 cells, for Sham and MEC lesion animals. (B) Cumulative density distribution of within-sides spatial correlation in CA1 cells, for Sham and MEC lesion animals.

To further investigate the stability of CA1 cells, but also their ability to differentiate between sides on the spatial alternation task, an across-sides correlation was observed. Across-sides spatial correlation noted any correlation in firing for both sides of the maze, across all events. Therefore, if the across-sides spatial correlation was high that meant that the same place cell would fire in the same pattern on both sides of the maze across all events. High across-sides spatial correlation indicated impairment in differentiating between the left and the right side of the maze. Place cells firing on the stem arm of the maze were excluded from this analysis. CA1 cells from the Sham and MEC lesion group were distributed into cumulative densities based on their across-sides spatial correlation (Figure 22A). Cells with firing rates lower than 0.25 Hz were excluded from this analysis. Higher across-sides spatial correlation overall was observed for the MEC lesion group. The highest across-correlation was the same for both groups and equaled to approximately 1. The mean across-sides spatial correlation for the Sham group was below 0, while the one for the MEC group was approximately 0.3.

A population distribution of CA1 cells was plotted in order to visualize any difference in clustering between groups (Sham and MEC) for the across-sides and the within-sides spatial correlations (Figure 22B). While the cells in MEC lesion group did not exhibit any significant clustering in a particular quadrant, the Sham group exhibited some clustering in the lower-right quadrant. The lower-right quadrant represents high within-sides spatial correlation (or high stability) and a low across-sides spatial correlation (or high differentiation between sides).

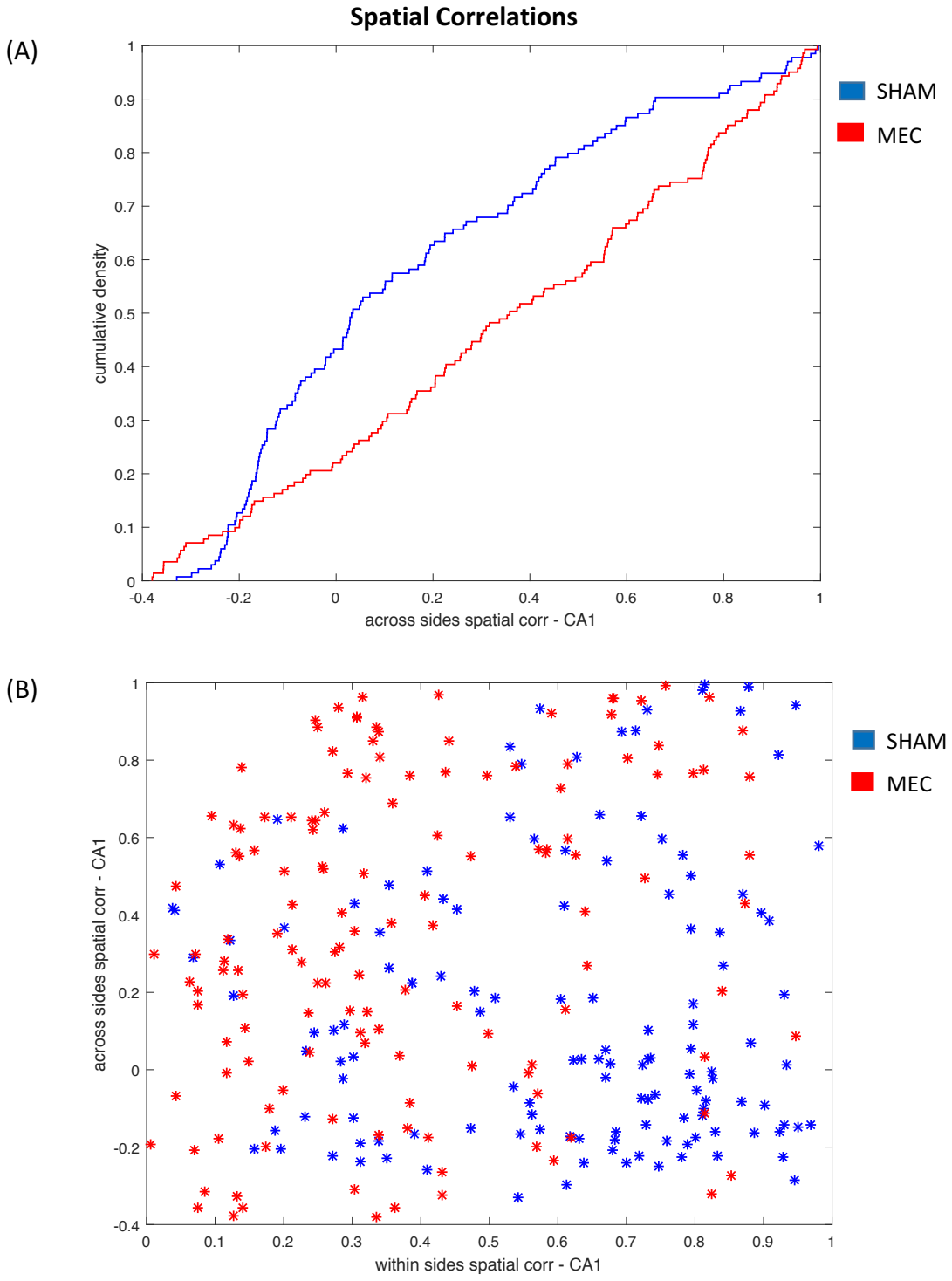

Figure 22. Spatial correlations. (A) Cumulative density distribution of across-sides spatial correlation in CA1 cells, for Sham and MEC lesion animals. (B) Population distribution of CA1 cells with across-sides and within-sides spatial correlations, for Sham and MEC lesion group.

CHAPTER 3: DISCUSSION

MEC participates in spatial memory encoding and consolidation through its direct interaction with the hippocampus (Krupic et al. 2012). Therefore, any impairment of the MEC might cause a substantial disruption in hippocampal spatial firing and memory encoding. We produced nearly complete bilateral single and double lesions of the MEC and the hippocampus, in order to investigate the impact of the MEC on behavior and physiology.

Animals were trained to run the spatial alternation task on the Figure 8 maze by alternating between left and right arms. When this task was run continuously (no delay), animals used their non-declarative habit memory in order complete the task (Ainge et al 2007). This type of memory is independent of the medial temporal lobe. As expected, the Sham group and all experimental groups (MEC, H and Double) performed well on the no delay task by scoring above 85% across all phases (Figure 13 and 14). In fact, regression analysis found that the Double lesion group performed slightly better over time on the no-delay task (Figure 15). The Double lesion group also made significantly less mistakes than the Sham group overall (Figure 16). This is consistent with other findings that habit learning is enhanced in animals with medial temporal lobe lesions, due to less interference between declarative and nondeclaritive memory encoding (Squire et al. 1993).

The animals also ran the delayed task that is of a declarative nature (Lee et al. 2003). The delayed task asks the animals to make a connection between past events and future predictions in order to receive the reward. For the delayed spatial alternation

task all experimental groups were significantly impaired, compared to the Sham group (Figure 13 and 14). Most impairment was evident in the longest delay condition (60s), since the animals were fully forced into using their long-term declarative memory (that is dependent of the medial temporal lobe). Both single lesioned groups exhibited an equal amount of impairment in the delayed tasks. The difference between them was not significant for all delay conditions, in both phases of behavioral assessment. Lack of difference in behavioral results between the H and MEC lesion groups implies that MEC might hold the same significance in spatial memory encoding as the hippocampus. The Double lesioned group performed worse than both single lesioned groups, in both phases. This severe behavioral impairment might be due to the absence of structures that support spatial memory encoding. This is also consistent with data observed in single lesioned animals that experienced less impairment in the task, since one structure was able to partially compensate for spatial encoding.

The regression analysis furthers this claim by indicating an increase in performance across time for both single MEC and H lesion groups, but not for the Double lesion group (Figure 15). The presence of partial improvement in performance of single lesion groups is consistent with Hales et al. 2014, who also observed that MEC lesioned rats eventually performed better on the Morris water maze after extended training. It is probable that residual neurons of the intact structure in single lesions are able to compensate for the communication with the prefrontal cortex (which holds working memory). Therefore, a link between spatial working and long term memory is still possible. Over time these anatomical connections strengthen, which results with an

improvement in behavioral performance. Double lesion animals are prevented from any such improvements due to the lack of anatomical structures. Therefore, in the Double lesion group the connection between working memory and long term memory is expected to be lost.

The absence of this link most likely caused the Double lesion group to perform below chance level for both delay conditions. Their mistakes typically occurred in strings of at least 4 errors. The number of consecutive mistakes was significantly larger than the one in the Sham group (Figure 16). Such behavioral inflexibility is categorized as perseveration. Perseveration is seen in frontal lobe syndromes and evaluated with motor tests. The prefrontal cortex (PFC) is critical for the ability to flexibly adapt established patterns of behavior in response to a change in environmental contingencies (Beas et al. 2017). We believe that the spatial firing patterns in mPFC are initially disrupted in single lesion groups, but can be regained by functional reorganization of the connectivity with the remaining structures. However, for the Double lesioned animals the spatial firing in mPFC is irreparable, which results in rigidity in behavior and inconsistent firing patterns.

To better understand the physiology behind spatial encoding, electrophysiological recordings of CA1 cells in Sham and MEC lesion animals were performed throughout phase 2. We observed that the MEC lesion did not impact the silencing of CA1 cells, since there was no significant difference between the groups (Figure 20). Therefore, differences in firing between groups were not due to any differences in the number of active and silenced cells. We observed approximately 40%

of CA1 silencing for both groups, which is consistent with the data reported in Hoelscher et al 2004. Furthermore, there was no significant difference in the average mean firing rates of CA1 cells between the two groups. However, slight differences were observed in average peak firing rates, with higher peaks occurring in the Sham group (Figure 20). Higher average peak indicates a more precise and stable place field, since the place cell fires at high frequencies only for a particular location over a constricted area. This observation prompted us to further investigate if CA1 cells in MEC lesioned animals had impairments in stability and precision. Additionally, Hales et al. 2014 also reported that spatial fields were broadened in MEC lesioned rats.

Precision was evaluated through spatial information, while stability was analyzed through within-sides spatial correlation (Figure 21). We found that CA1 cells in MEC lesioned animals were less precise and less stable in their firing rates. This impairment resulted in inconsistent and scattered firing over the maze. Their place cells were unable to produce stable place fields that would only occur at particular locations in the maze. They were also unable to fire over constricted areas, but rather fired throughout the entire environment. Therefore, the observed behavioral impairments in MEC lesion group were consistent with the physiological data. Compared to Sham animals, MEC lesioned animals had impairments in spatial encoding on the spatial alternation task. Therefore, these animals were less aware where they were in space. This pushed us to investigate if these animals were able to differentiate between the left and right side of the maze.

An across-sides spatial correlation was used in order to observe if place cells

fired at the same rate for both sides of the Figure 8 maze. Higher across-sides spatial correlation was observed in the MEC lesioned group, compared to the Sham group. The firing pattern of these cells occurred on both sides of the maze, instead of just one side. This correlation indicated that CA1 place cells in MEC lesioned animals were unable to differentiate between the right and left side of the maze. In order to achieve an overall comparison for stability of firing and differentiating between the two sides of the maze, a population distribution was plotted for a within and across spatial correlation. A significant portion of CA1 cells in Sham animals have high stability and high differentiation between the left and the right side. CA1 cell in MEC lesion group exhibited opposite qualities, with some clustering in the upper right quadrant.

Based on all of the physiological data, it can be concluded that MEC lesions cause a significant physiological impairment in hippocampal firing. Place cells of MEC lesioned animals were less precise and less stable in their firing. They were also impaired in differentiating between the left and right side of the maze. The physiological impairments in firing probably disrupted spatial memory encoding and therefore also caused impairments in behavior.

Some future directions could include recordings from the MEC, upon hippocampal lesions. It has also been found that the MEC can act independently in memory encoding, specifically through sharp wave-ripple associated replay (O'Neill et al. 2017). Our data also indicates that MEC and H lesions cause an equal amount of behavioral impairment. Therefore, it would be noteworthy to examine how H lesions might affect the physiology occurring in MEC. It would also examine how the MEC might

be sufficient to support encoding of spatial memories. Another future direction could also to observe how MEC and H lesions affect the firing in the medial prefrontal cortex. Our data already indicates an impairment through behavioral results, but physiological analysis is still needed in order to establish a direct link.

CHAPTER 4: CONCLUSION

We found that complete single and double bilateral lesions to the MEC and the hippocampus significantly impaired rats in the delayed alternation task. The Double lesion group was the most impaired and performed below chance level for the delay conditions. Single lesioned rats partially improved their behavioral performance in delay trials across time. Double lesioned rats did not present any behavioral improvement throughout the trials. In fact, they exhibited perseveration through behavioral inflexibility. Physiological analysis indicated no significant difference in silencing of CA1 cells in MEC lesioned animals. Further physiological analysis revealed that MEC lesioned rats indicated less precision in firing, less stability and less discrimination between left and right trials. These results indicate that single lesions of either structure disrupt the function of the entorhino-hippocampal loop. Over time residual neurons of the intact structure are sufficient to compensate for the communication with the prefrontal cortex, as supported by partial behavioral recoveries. Double lesioned animals, however, are prevented from improving in memory related tasks.

This thesis, in full, is currently being prepared for submission of publication of the material. Collett, Marta; Schonwald, Antonia. Professory Marta Collett is the primary investigator and author

REFERENCES

Ainge, J. A., van der Meer, M. A., Langston, R. F., & Wood, E. R. (2007). Exploring the role of context-dependent hippocampal activity in spatial alternation behavior. Hippocampus, 17(10), 988-1002.

Beas, B. S., McQuail, J. A., Banuelos, C., Setlow, B., & Bizon, J. L. (2017). Prefrontal cortical GABAergic signaling and impaired behavioral flexibility in aged F344 rats. Neuroscience, 345, 274-286.

Buzsáki, G., & Moser, E. I. (2013). Memory, navigation and theta rhythm in the hippocampal-entorhinal system. Nature neuroscience, 16(2), 130-138.

Eichenbaum, H., Stewart, C., & Morris, R. G. (1990). Hippocampal representation in place learning. Journal of Neuroscience, 10(11), 3531-3542.

Fyhn, M., Molden, S., Witter, M. P., Moser, E. I., & Moser, M. B. (2004). Spatial representation in the entorhinal cortex. Science, 305(5688), 1258-1264. Hafting, T., Fyhn, M., Molden, S., Moser, M.B., and Moser, E.I. (2005). Microstructure of a spatial map in the entorhinal cortex. Nature 436, 801–806.

Hafting, T., Fyhn, M., Molden, S., Moser, M. B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. Nature, 436(7052), 801-806.

Hales, J. B., Schlesiger, M. I., Leutgeb, J. K., Squire, L. R., Leutgeb, S., & Clark, R. E. (2014). Medial entorhinal cortex lesions only partially disrupt hippocampal place cells and hippocampus-dependent place memory. Cell reports, 9(3), 893-901.

Henze, D. A., Borhegyi, Z., Csicsvari, J., Mamiya, A., Harris, K. D., & Buzsáki, G. (2000). Intracellular features predicted by extracellular recordings in the hippocampus in vivo. Journal of neurophysiology, 84(1), 390-400.

Hoelscher, S., & Alderman, D. H. (2004). Memory and place: geographies of a critical relationship. Social & Cultural Geography, 5(3), 347-355.

Goldman-Rakic, P. S. (1995). Cellular basis of working memory. Neuron, 14(3), 477-485.

Igarashi, K. M., Lu, L., Colgin, L. L., Moser, M. B., & Moser, E. I. (2014). Coordination of entorhinal-hippocampal ensemble activity during associative learning. Nature, 510(7503), 143-147.

Izaki, Y., Maruki, K., Hori, K., & Nomura, M. (2001). Effects of rat medial prefrontal cortex temporal inactivation on a delayed alternation task. Neuroscience letters, 315(3), 129-132.

Krupic, J., Burgess, N., & O'Keefe, J. (2012). Neural representations of location composed of spatially periodic bands. Science, 337(6096), 853-857.

Lee, I., & Kesner, R. P. (2003). Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. Journal of Neuroscience, 23(4), 1517-1523.

Leutgeb, S., Leutgeb, J. K., Moser, M. B., & Moser, E. I. (2005). Place cells, spatial maps and the population code for memory. Current opinion in neurobiology, 15(6), 738-746.

McClelland, J.L., McNaughton, B.L., and O'Reilly, R.C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol. Rev. 102, 419–457.

Moser, E. I., Kropff, E., & Moser, M. B. (2008). Place cells, grid cells, and the brain's spatial representation system. Annual review of neuroscience, 31.

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

O'Neill, J., Boccara, C. N., Stella, F., Schoenenberger, P., & Csicsvari, J. (2017). Superficial layers of the medial entorhinal cortex replay independently of the hippocampus. Science, 355(6321), 184-188.

Ribot, T. (1881). Les maladies da la memorie. Paris: Alcan

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. Journal of neurology, neurosurgery, and psychiatry, 20(1), 11.

Skaggs, W. E., McNaughton, B. L., & Gothard, K. M. (1993). An information-theoretic approach to deciphering the hippocampal code. In Advances in neural information processing systems (pp. 1030-1037).Squire, L. R. (1987). Memory and brain.

Squire, L. R. (1992). Declarative and nondeclarative memory: Multiple brain systems

supporting learning and memory. *Journal of cognitive neuroscience*, *4*(3), 232-243.

Squire, L. R., Knowlton, B., & Musen, G. (1993). The structure and organization of memory. *Annual review of psychology*, *44*(1), 453-495.

Squire, L. R. (2004). Memory systems of the brain: a brief history and current perspective. Neurobiology of learning and memory, 82(3), 171-177.

Squire, L.R., and Alvarez, P. (1995). Retrograde amnesia and memory consolidation: a neurobiological perspective. Curr. Opin. Neurobiol. 5, 169–177.

Squire, L. R., & Kandel, E. R. (2000). Memory: From mind to molecules (Vol. 69). Macmillan.

Squire, L. R., & Knowlton, B. J. (1995). Learning about categories in the absence of memory. Proceedings of the National Academy of Sciences, 92(26), 12470-12474.

Squire, L. R., & Zola-Morgan, S. (1988). Memory: brain systems and behavior. Trends in neurosciences, 11(4), 170-175.

Witter, M.P., and Amaral, D.G. (1991). Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. J. Comp. Neurol. 307, 437–459.

Zhang, S.J., Ye, J., Miao, C., Tsao, A., Cerniauskas, I., Ledergerber, D., Moser, M.B., and Moser, E.I. (2013). Optogenetic dissection of entorhinal-hippocampal functional connectivity. Science 340, 1232627.