Investigating the Role of Hippocampal States in Episodic-like Memory Consolidation

By Zemina Meghji

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Neuroscience

University of Alberta

Abstract

Acquisition and storage of episodic-like information fundamentally contributes to quality of life. It is the type of information that allows us to remember meaningful events such as our graduation and our heartaches. The memory of these events allows us to adapt our behaviours to facilitate more of the good (graduation) and less of the bad (heartache). Though this form of mental time travel plays an important role in daily life, much of the way in which this type of information is stabilized is still under question. An avenue unto which we can investigate the stabilization of episodic-like information, a function that has been shown to rely on the hippocampus, is by looking at the one-to-one relationships previously established between brain and behaviour since generating episodes in our life often requires some sort of action/movement/presence of us. Specifically, researchers have demonstrated theta activity while a rat engages in active behaviours such as running, walking, swimming, and rearing. Large irregular amplitude (LIA) in addition to sharp-wave ripples (SPW-R) activity appears when a rat is grooming, drinking, or immobile. And finally, we observe slow oscillatory (SO) activity in a rat while in sleep behaviour, specifically during slow wave sleep (SWS). Here in this study, we wanted to determine how these behaviourally-associated hippocampal states contributed, if at all, to the solidification of episodic-like memory retrieval. To do this, we used a delayed matched to sample paradigm in the circular water maze. We provided rats four training trials inter-leaved with one-minute breaks. External cues were placed within the maze room to facilitate the learning of the platform location on that day. The four training trials were then followed by a one-hour delay during which specific behavioural conditions were encouraged. To test whether a difference in behavioural condition would affect the consolidation of episodic-like information, we then administered a probe trial. During this trial , the platform was not available. Therefore, rats were required to remember the location of the platform based on an allocentric map. An aggregation of our results demonstrated that when rats spent at least 40% of their time sleeping during the inter-leaved one-hour delay, their performance, measured in terms of latency, was

improved as compared to when they engaged in exploratory or grooming behaviours. In fact, performance appeared to be hindered when rats spent most of their time exploring. Thus, we have demonstrated differentiated affects on episodic-like memory recollection as a function of hippocampal state suggesting that hippocampal state may also need to be taken into consideration when investigating information solidification processes with respect to episodic-like memory.

Preface

In addition to the data I collected, this project was a continued effort from initial work done by Lisa Rimstad and Brandon Hauer.

In all other cases, the ideas, data, and analysis presented here are my own, developed in collaboration with my supervisor, Dr. Clayton Dickson, Tara Whitten, and Brandon Hauer. This thesis was part of a larger project that received ethics approvals from the University of Alberta Biosciences Animal Care and Use Committee (AUP00000092_AME1; Project name: Cellular and Network Dynamics of Neo – and Limbic – Cortical Brain Structures)

Dedication

We are what we repeatedly do

- Will Durant

Your now is not my now; and again, your then is not my then; but my now may be your then, and vice versa. Whose head is competent to these things?

- Charles Lamb

There is a theory which states that if ever anyone discovers exactly what the universe is for and why it is here, it will instantly disappear and be replaced by something even more bizarre and inexplicable.

There is another theory which states that this has already happened

- Douglas Adams

Acknowledgements

The work in this thesis was supported by a Natural Science and Engineering Council of Canada (NSERC) grant #249861 to Dr. Clayton Dickson. I was additionally supported by a Queen Elizabeth II Scholarship.

I would like to thank the critical assistance by Claire Scavuzzo, Brandon Hauer, and Tara Whitten without whom I would not have been able to complete this project. In addition, I would like to acknowledge and thank the patience, teachings, and critical guidance of my supervisor, Dr. Clayton Dickson; for giving a business student a chance at science. I am a more critical person because of it.

To my family and friends in both Toronto and Edmonton: you kept me (half) sane.

Thank you.

Table of Contents

Table of Figures

List of Abbreviations

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AP-5 = (2R)-amino-5-phosphonovaleric acid

BOSC = Better Oscillation

DMS = Delayed Match to Sample

EEG = Electroencephalogram

HPC = Hippocampus

LIA = Large Irregular Amplitude

nCTX = Neocortex

NMDA = N-Methyl-D-aspartate

NREM = Non-Rapid Eye Movement

REM = Rapid Eye Movement

SO = Slow Oscillation

SPW-R = Sharp Wave Ripple

SWA = Slow Wave Activity

SWS = Slow Wave Sleep

Chapter 1: Introduction

Importance of Memory

Imagine a device that allows us to encode, store, and retrieve different types of information which have been deemed important for survival. This information can consist of facts, people, and particular events that are worthy to note. One can easily think about the benefits of such a device. The encoding component allows us to assess the information, environment and context which would allow for further processing. The subsequent storing of such information would require some sort of mechanism by which it can be maintained. Finally, a different process would be required for the retrieval and use of said information. Gaining access to such a device would unequivocally provide evolutionary favour to the organism who possess it because they are able to store important information and use it at a later time. It would essentially be an experience-modulated system which would allow learning and growth, adjustment of behaviours and thoughts, storage of dangerous environments and/or foods or facts and information that would be beneficial. Such a system would be central to survival, revered in literature, and sought after in sciences. Luckily for us, imagining such a device is not necessary as such a system is already in place.

Memory is the treasury and guardian of all things.
Marcus Tullius Cicero

The etymology of the term "memory"

The term memory comes from the Latin word *memor* which is to remember or be mindful (Algeo, Barnhart, & Steinmetz, 1989). Superior memory capacity has always been thought to be characteristic of a higher being or form of man. In many Indian stories, the journey for the protagonist is to unveil their *maya*, which is the illusion that prevents them from remembering their true origin and purpose in life (Buxton, Bolle, & Smith, 2017). The importance of memory was also not lost on Greek mythology. Mnemosyne (memory) who is

thought to be the mother of Muses is said to know everything about the past, present, and future. It was thought to be beneficial for a performer to call upon the God Mnemosyne so as to remember their lines (Augustyn et al., 2017). Today, information and data are critical to the functioning of the economy, and people who have the ability to store and recall information are individuals who seem to find the most success and are among the most envied. The onset of neurodegenerative diseases such as Alzheimer's, then, is a dire consequence in society today. The affected individual loses not only knowledge and information accumulated throughout their life, but also, the memories of people and relationships that made both positive and negative impacts on them over time. It is no question, then, as to why many neuroscientists have sought to understand the nature and mechanisms of memory and memory stabilization.

Early studies of memory

Considering the importance of memory and the benefit this mechanism provides, it is only natural that scientists endeavoured to understand it. Learning and memory are often spoken in relation to each other because one naturally builds on the other. Where learning has been defined as the acquisition of skill or information, memory is the retention of this learned information (Okano, Hirano, & Balaban, 2000). At times, it is easy to lose sight of the fact that learning and memory themselves are theoretical concepts and are actually *assumed* to take place following behavioural modifications (Okano et al., 2000; Rudy, 2008). For instance, when a rat becomes sick following the exposure to novel food, it has been observed that the same rat will avoid consuming this food weeks after initial exposure. We can assume then, that this avoidance is the behavioural expression of the memory of becoming sick at a previous time point and has subsequently learned and remembered. It has been the job of both psychologists who look at the behavioural output of learning and memory, and neuroscientists who examine the neurobiological mechanisms behind learning and memory, to understand how both are realized in the brain.

Early contemplations of memory began in 1885 when Herman Ebbinghaus (1850-1909) produced his famous forgetting curve, essentially demonstrating that previously retained information reduces as a function of time when no effort is exerted in retaining said information (Rudy, 2008). Effort, in this case, was the active rehearsal of lists of items (in this case, nonsense syllables). These results would imply that the rehearsal of information was helpful in assisting with the longevity of information. Another key result from Ebbinghaus's 1885 study was the differential effects of massed versus spaced training. He found that memory retrieval would improve by distributing the learning of a list of nonsense syllables over time with delays between items or training bouts (spaced) as compared to attempting to learn a list with training bouts delivered all at once (massed) (Ebbinghaus, 1913; Sisti, Glass, & Shors, 2007; Spreng, Rossier, & Schenk, 2002). Forgetting was less dramatic for spaced versus massed learning trials. The results here imply that new information and its recall is advantaged by practise (i.e., rehearsal) and time (i.e., in terms of spacing the training).

The question of how time benefits memory was a question that Ebbinghaus himself posed: "how does the disappearance of the ability to reproduce, forgetfulness, depend upon the length of time during which no repetitions have taken place?" (Ebbinghaus, 1913). Furthermore, he also noted the apparent significance of repetition to concretizing information: "under ordinary circumstances, indeed, frequent repetitions are indispensable in order to make possible the reproduction of a given content" (Ebbinghaus, 1913). At this point in history, Ebbinghaus was constrained by available technology and appropriate experimental design to address these questions. In addition, his experiments had several issues – one of which being that Ebbinghaus was his own subject (Rudy, 2008). Despite these flaws, his study did spark interest in the study of memory.

Müller and Pilzecker were interested in understanding the requirements of memory solidification, or in other words, what made our memories permanent. More specifically,

relating to the work of Ebbinghaus, what was the difference between lost versus spared information? Through a series of experiments that used a modification of Ebbinghaus' lists of nonsense syllables, they were able to conceptualize the requirements of memory solidification. During their experiments, they unexpectedly found that their human participants had difficulty suppressing the seemingly spontaneous rehearsal of previously learned information between trials (Lechner, Squire, & Byrne, 1999). After noting this, Müller and Pilzecker developed a method to interrupt this rehearsal of activity by forcing one group of participants to learn additional information in the time period between learning and recall of the original list. What they found was "retroactive interference' whereby the group that was forced to learn additional information recalled fewer elements from the prior-learned list (Lechner et al., 1999). As the term suggests, it would appear that the information learned at later time points (information acquired on a more recent time scale) hindered the ability for participants to recall information learned previously. This suggests that information, within a certain time period of being learned, is labile and susceptible to interference and that memory requires time and perhaps this spontaneous rehearsal to solidify. With respect to the results of Ebbinghaus, information that was allowed more time to be rehearsed (either actively or even spontaneously - in the case of spaced training paradigms) is less labile than that which is not. This suggests that directly after encoding, memories have a sensitive period to be "consolidated", a term that Müller and Pilzecker coined (Müller & Pilzecker, 1900).

The work of Ebbinghaus, Müller, and Pilzecker drew on very similar themes that can be easily extracted from their findings: Rehearsal (both active and passive) of information over time contributes to the longevity of memory, thereby increasing the quality of its behavioural expression, meaning: successful recollection; and time spent in the brain: being short term (labile information vulnerable to interruption and amnesic agents) and long term (solidified information, less vulnerable). In summary, Müller and Pilzecker fell upon the

necessary and sufficient conditions that were required for effective consolidation of memory: rehearsal and time. Since this seminal study, a significant amount of research has been made towardsanswering questions which allow scientists to understand the nature, mechanisms, and organization of memory, however much of what we understand about memory today is largely in part by the tireless work of Scoville, Milner, Corkin and the gracious cooperation of patient H.M.

Neuropsychological Studies: Patient H.M and other observations

Henry Gustav Molaison (H.M, 1926 - 2008) had been knocked down by a bicycle at the age of 7. He began to experience mild seizures at the age of 10 and had his first major seizure at the age of 16. The episodes continued to increase in severity so that by the time he reached the age of 27, he was forced to leave the workplace (Squire, 2009). In an effort to control his seizures, William Scoville performed a bilateral resection of patient H.M.'s entire hippocampus, and other medial temporal lobe (MTL) structures. Although this operation greatly reduced his seizures, patient H.M. was left with profound memory deficits (Eichenbaum, 2011; Scoville & Milner, 1957).

Patient H.M.'s memory impairment was immediately acknowledged post-surgery. He did not recognize the hospital staff, could not recall his way to the bathroom, and seemed to lack recollection of day-to-day activities. However, he was able to hold information long enough to answer questions and participate in conversations (Scoville & Milner, 1957). In addition to a potential disassociation of temporal memory function, it also appeared that H.M.'s memory deficit exhibited a clear delineation of retrospective and prospective time from the point of his operation. Meaning, he suffered from amnesia in the both anterograde (the inability to form new memories) and partial retrograde (the inability to recall memories pre-surgery up to a certain age) directions. An example to illustrate the difference between amnesia types is patient H.M.'s inability to recall his most recent home address but seemed

to have full memory of his childhood home. Additional probing into H.M.'s memory impairment also led to the finding of a dissociation between memory types. H.M. was unable to answer direct questions about tasks he had just engaged in or whether he had lunch but was fully capable of being trained at the mirror drawing task and even the Gollins partial picture task**.** The mirror drawing task requires the participant to trace a shape and stay within the boundaries of a double line while only using the inverted reflection of their hand through a mirror (Julius & Adi-Japha, 2016). Errors are scored by the amount of times an outlined border is contacted. The Gollins partial picture task, on the other hand, requires the subject to recognize familiar common objects only by fragmented line drawings (Eichenbaum, 2011). What Scoville and Milner reported was that H.M. was able to learn these tasks and develop the required associations necessary to perform normally as compared to control subjects (Scoville & Milner, 1957; Squire, 2009). This suggests that although H.M. was not able to explicitly recall events, he was still able to learn implicit forms of associations and skills. Further testing demonstrated that H.M. had complete faculties in priming, skill learning, Pavlovian conditioning, and sequence learning while also maintaining an above-average I.Q, kind demeanor, and personality (Scoville & Milner, 1957). Therefore, the main deficit that H.M. initially seemed to possess was that of a declarative type of memory loss, meaning, the loss of explicit types of memory—both semantic and episodic-like which will be further discussed in the following sections.

Interestingly, the temporal gradient to H.M.'s amnesic deficits also highlights a disassociation between temporally distinct memory types, being short-term and long-term. His inability to form new long-term memories was distinct from his ability to maintain short-term information via rehearsal. As well, the relatively selective loss of recent memories prior to his surgery was distinguished from the intact nature of more remote ones (Corkin, 2002). This seems to parallel the findings of Ebbinghaus, Müller, and Pilzecker in terms of

an inability to stabilize new long-term memories as well as showing lability for prior encoded material.

This notion of time playing an important component to memory stabilization was acknowledged by Théodule Ribot in 1881. In his conception of time and memory, he postulated that recent memories are more likely to be lost than remote ones in the event of trauma or amnesia (Murre, Chessa, & Meeter, 2013; Ribot, 1882). The disproportionate loss of recent memories which is characteristic of retrograde amnesia could be due to a reduction of specific neural activity that needs to take place in order for a memory trace, or *engram*, to solidify. The loss in being able to create short-term memories altogether could be traced back to the lack of appropriate brain regions which allow for the formation and temporary storage of short-term memory. Since H.M. exhibited both anterograde and partial retrograde amnesia after the resection of his MTL, this may signify that in addition to his loss of declarative memory function, he also lost the capacity for long-term memory storage, preventing any information to be exchanged between the HPC and other cortical structures.

Building on all of this work, several researchers have endeavoured to understand the mechanisms behind what solidifies mnemonic information. What exactly is it that happens directly after a learning event, specifically in relation to time (i.e., the difference between short- and long-term memory) and brain operations (i.e., rehearsal or some other process)? In order to attempt to answer these questions, however, we would need to identify a model which allowed for the modification or change in the structure of connections between neurons to permit the formation of a memory trace and to maintain it. This model, of course, would come in the form of synaptic plasticity, or long-term potentiation.

Cellular models of Learning and Memory

Santiago Ramon Y Cajal is considered to be the father of neuroscience because of his extraordinary contributions to cell theory and being the champion of the "Neuron Doctrine". This doctrine can be summed up in his quote: "the relationship between nerve cells [is] not one of continuity but rather, contiguity" (Lopez-Munoz, Boya, & Alamo, 2006). Cajal came to these conclusions by updating Camillo Golgi's silver chromate technique to silver nitrate staining. Using this technique, he concluded (in direct contrast to Golgi) that nerve cells were autonomous and were in fact separated by small gaps. He also concluded that a neuron can be divided into three main sections: a cell body or soma, an axon, and dendrites and he hypothesized that these subdivisions and the polarity of neurons was based on specialization for communication, with the axon carrying information to distant regions and the dendrites receiving this information from axons of different neurons.

Charles Sherrington, who actually named the junction between neurons at the level of axons and dendrite a *synapse*, hypothesized that this was the specialization by which nerve cells transmitted information and communicated with one another (Levine, 2007). The corollary of this idea was that these synapses could also be the sites of modifications that could fundamentally alter information processing, and thus suggestively creating the core idea of synaptic plasticity in learning which still holds currently.

Indeed, it was the Canadian Psychologist, Donald Hebb who proposed a theoretical mechanism by which synaptic modifications could take place in order to produce an engram. A major part of his model of nervous function relied on the concept of the cell assembly, a collection of neurons whose contiguous activation formed a code for the neural substrate of experience – a neural representation of the external sensory world. Indeed, he also conceptualized that the re-activation of these ensembles could also constitute the actual engrams of those experiences. Maintenance of activation via either external sensory or internal means would form a closed-loop system which allowed for the recapitulation of

activity, thereby initiating the required metabolic and structural changes to create a memory trace. This continued activity post-experience would allow for further solidification of information. The reverberation or replay of this activity is what would allow for structural changes to take place at the synaptic level. The activity of this cell assembly working together becomes an associative process:

When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased (Hebb, 1949).

Here, again, we note that the rehearsal of information or in this case, repeated neural ensemble activity plays an important role in solidifying the memory engram. Cessation of repetitive activity or repetitive activity cut short would, theoretically, prevent the formation of a memory engram. In studies subsequent and continuing to this day, Hebb's predictions turned out to portend actual neurobiological mechanisms of synaptic plasticity as well as perhaps, memory solidification.

Bliss and Lømo characterized the phenomenon of functional synaptic plasticity within the circuits of the *in vivo* rabbit hippocampus. They observed that high frequency stimulation (or tetanus) of a given input resulted in increased synaptic efficacy in that very circuit, a process that came to be known as long-term potentiation (LTP) (Bliss & Lomo, 1973). LTP proved to be a sound model to understand what the synaptic underpinnings of learning and subsequent development of memory might look like in the HPC. This is because LTP was found to demonstrate three critical factors: specificity, cooperativity, and associativity (Bliss & Lomo, 1973; Eichenbaum, 2011). These components allowed for information to avoid overlap, provided a framework of the role of cell assemblies, and also demonstrated the neurobiological basis of creating mnemonic memories; much of which was theorized by

Hebb (Hebb, 1949). Specifically, cooperativity suggested that LTP had a threshold – in terms of tetanizing stimuli, when the collective output of the stimulated fibres would be large enough to produce a suprathreshold response in the target neurons which could then induce LTP. For instance, a weakly stimulated input would only excite a few excitatory synapses and would therefore fail to induce LTP, whereas a strong input would successfully induce LTP because of the increased activity of more excitatory synapses working together. Input specificity was also demonstrated by the fact that LTP would not occur on other, unstimulated, synaptic pathways and would only be observed in the tetanized pathway. Finally, associativity was demonstrated by showing that a weak stimulation that was paired with a stronger independent stimulation would allow LTP to be expressed in the weak pathway (Bliss & Lomo, 1973; Eichenbaum, 2011; Hebb, 1949; Nicoll, 2017). These properties were exactly those predicted and specified by Hebb.

The exact mechanisms of the induction of LTP were of substantial interest. Mayer Westbrook and Gutherie (Mayer, Westbrook, & Guthrie, 1984) were able to identify the type of receptors in the HPC that mediated LTP. Specifically, excitatory hippocampal synapses were found to be glutamatergic, and there are different receptors for glutamate that were named for their preferred synthetic pharmacological agonists. The two main varieties were α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. Both act as ionotropic receptors to depolarize the post-synaptic cell but NMDA receptors were found to be conditional. Essentially, their activation required both the binding of the ligand and sufficient depolarization of the post-synaptic membrane. AMPA receptors, on the other hand, only required glutamate. Tetanizing stimuli were found to massively activate AMPA receptors, which then depolarized the post-synaptic membrane sufficiently for NMDA receptors to be activated. NMDA channels are permeable to Ca^{2+} which acts as an important second messenger that ultimately results in the increased efficacy of AMPA-mediated transmission at that very synapse (Bliss & Lomo, 1973; Eichenbaum, 2011; Nicoll, 2017).

On the other hand, activity-dependent synaptic weakening (known as long term depression, LTD) is thought to be the reversal or opposite of the previously explained LTP. Onset of LTD is normally observed when low-frequency stimulation (LFS, ~0.5-3Hz) is applied to the Schaffer collateral projections from CA3 to CA1 area of the HPC. Interestingly, researchers have demonstrated that LTD also requires the activation of NMDA receptors at the postsynaptic cell, and involves the influx of Ca^{2+}, however, at much lower levels. The LTD process reduces net synaptic potentiation and thereby weakens or eliminates connections (Purves, 2001). An early criticism of LTD was that the LFS initiated damage to the cells and the reduced potentiation was a result of this damage. To ensure that the changes observed under LTD are not a result of permanent damage to target neurons due to LFS, Dudek and Bear (1992) showed that LTP was still able to occur at the very sites LTD took place suggesting that the integrity of the synapses remained after LFS. Furthermore, they also varied stimulation frequency and observed differential effects. For instance: 900 pulses at 3Hz caused LTD, while the same amount of pulses at either 10Hz or 50Hz caused either no change or potentiation at the same targets (Dudek & Bear, 1992). Like LTP, LTD has also shown to be NMDA-receptor dependent, input specific, cooperative, associative, and, in addition: frequency dependent, saturable and reversible (Bear & Abraham, 1996).

Although these discoveries of both LTP and LTD were quite exciting as they paralleled the components required to effectively model learning and memory, its behavioural significance was still questioned and required further examination

The Behavioural Relevance of LTP

So far, we have discussed LTP in terms of controlled, laboratory settings; disjointed from real-life applications. It is not clear as to whether this type of synaptic potentiation would apply to actual settings of behavioural learning and memory. In fact, a term was used to convey the idea of synaptic plasticity evoked by learning in an LTP-like fashion: behavioural

LTP. Several expectations might be made for behavioural LTP: 1) that learning would evoke observable synaptic potentiation in relevant brain circuits; 2) that manipulations that modified LTP would modify learning; and that 3) learning and the induction of LTP might compete with each other.

In order to test these ideas, what is required is a test of learning and memory that is specific to a particular brain region which could then allow for localized manipulations of potentiation to assess whether an LTP-like process might underlie the behavioural plasticity itself. Not surprisingly, the hippocampus, being the first locus of study of LTP itself, became the first region to be tested in this way. The type of learning and memory tasks used to test hippocampal function were spatial in nature, as these are most prominently affected by hippocampal damage (Morris, 2006; Morris, Garrud, Rawlins, & O'Keefe, 1982).

One of the first behavioural test of hippocampal function was the circular (or Morris Water Maze: MWM, named for its inventor, Richard Morris). This is a spatial task for rodents that requires the hippocampus (Morris, 2006; Morris et al., 1982). In this task, rats are placed in a circular pool filled with murky water. The goal of the task is for the rat to locate a submerged (and thus hidden) platform placed in any one of the quadrants of the maze using only the landmarks available around the maze to navigate. In relatively few trials, rats learn to directly navigate to the platform location, thus indicating both allocentric navigational abilities and a memory for the location of the platform. Decreasing latencies to locate the platform thus indicate both learning and memory. What has been found is that lesions or disruptions to the structure of the HPC results in performance deficits in these tasks (Morris, 2006; Morris et al., 1982), thus, it was important to evaluate the specific conditions onto which learning, and specifically spatial learning, occurs in the HPC.

Moser, Moser, and Andersen systematically tested the affects of spatial learning in the HPC by specifically dissociating learning from other factors such as movement, temperature, and arousal. In a version of a novel object recognition task, they found greater net synaptic

potentiation in rats post-hoc as compared to conditions in which movement, temperature, and arousal were manipulated (Moser, Moser, & Andersen, 1994). Here, we have a clear demonstration that the HPC is in fact, affected by learning, and specifically to spatial learning. Our next step then is to determine whether LTP, as discussed above, has any relevance to spatial learning in the HPC. Direct manipulation of LTP processes during the MWM have been found to lead to behavioural deficits. Morris et al made intrahippocampal infusions of D,L-2-amino-5-phosphonopentanoic acid (AP-5), an NMDA receptor blocker, and compared the performance of these rats to a control group. This manipulation was previously shown to allow for continued synaptic transmission via AMPA receptors and was specific in blocking NMDA and LTP. Theoretically, if LTP and its processes were relevant to learning, we would observe performance deficits in the rats, or, in other words, we would not see any decrease in latency across trials. Indeed, Morris found that blocking NMDA receptors had negative consequences on the rats' capacity to effectively perform this task, thereby justifying the use of LTP as a model for learning and memory (Morris, 1989, 2006). What might also be interesting to note here is the idea that both LTP and LTD are NMDA receptor dependent. Thus, the case can be made that the learning deficit might be due to the disruption in both LTP and LTD processes (Kemp & Manahan-Vaughan, 2007). For instance: Etkin et al (2006) found that spatial learning in HPC-dependent tasks appeared to be impaired in a knockout mice strand missing the required NMD-receptor component, as compared to control. The battery of behavioural tasks tested in this study included the MWM (Etkin et al., 2006). These results are interesting as it begins to allow us to conceptualize how blocking behavioural LTP (and perhaps, even LTD) results in observed dysfunctions of memory expression, and, even more broadly, to dysfunctional expressions of episodic-like memory.

The MWM task itself can be a test of episodic-like-like memory as complex multimodal associations are made between the external cues in the room and the egocentric location of

the animal in relation to the projected location of the platform. A rat is able to orient itself based on these cues and, subsequently forms a mental representation to facilitate navigation. Thus, each trial in the maze becomes an episode linking the experiences relevant to the task at hand (D'Hooge & De Deyn, 2001). Experiences are organized and linked via episodes across multiple domains of information into a cognitive map for the purposes of generating well-informed actions and behaviours (Tolman, 1948), a theory proposed by Edward Tolman. The objective of a cognitive map, then, would be to facilitate the planning of future trajectories, determining the most efficient routes, and managing expectations based on goals and outcomes (Moser, Moser, & McNaughton, 2017; Tolman, 1948). O'Keefe and Nadel further elaborated on this theory implicating the HPC as the brain region which facilitates this formation of a cognitive map (O'Keefe & Nadel, 1978).Naturally, understanding the exact mechanisms of how this cognitive map is formed is of great interest.

The discovery of place cells in the HPC provided first-level validation of Tolman's theory (O'Keefe, Burgess, Donnett, Jeffery, & Maguire, 1998; O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978). Cells located in CA1 regions of the dorsal HPC fired in relation to a specific spatial location. These cells, as the name suggests, were initially thought to be place-dependent. Essentially, specific cells (place cells) would fire when a rat entered a particular place field. Place cells are not topographically organized but rather, are distributed, meaning neighbouring cells are just as likely to fire for a distant spatial location as they would for a location that would be closer (Marozzi & Jeffery, 2012). One of the most interesting properties of place cells is that they show stability for that same environment for long periods of time after they form. This stability appears to be related to the actual spatial abilities of a given rat and may reflect episodic-like memory itself (Rosenzweig & Barnes, 2003). Specifically, Kentros et al (1998) demonstrated that the long-term stability of place cell representation of a familiar environment would be disrupted by specifically blocking

NMDA receptors. Meaning that a new map, formed by place cells, would be observed to represent an environment a rat was already exposed to following the blocking of NMDA receptors (Kentros et al., 1998). LTD may also be playing a role here with respect to differentiating novel and familiar objects in a given environment as it has been shown that LTP is dominant within completely novel locations but LTD appears to occur more frequently in a familiar environment but with novel objects. This would may lead to the speculation that LTD may facilitate the sharpening of the cognitive map (Kemp & Manahan-Vaughan, 2004). This suggests that the formation and subsequent stability and sharpening of the cognitive map by place cells is NMDA-receptor dependent and requires LTP and LTD-like mechanisms, thus, manipulations of LTP result in dysfunctional expressions of memory.

Finally, it is important to look at how learning and LTP specifically relate to one another. Ishihara et al (1997) have demonstrated higher population spike activity within the mossy fiber-CA3 pathway of rat HPC who learned a radial arm maze task. LTP induction, normally measured by the slope of the excitatory post synaptic potential (EPSP), was also higher in this experimental group as compared to the control after tetanizing stimulation of the same pathway, suggesting that learning in of itself causes a net gain of synaptic potentiation that may raise the future threshold for further potentiation (Ishihara, Mitsuno, Ishikawa, & Sasa, 1997). In this respect, it would appear that there might be such a phenomenon as "too much learning" or "too much synaptic potentiation". This has been observed in the form of synaptic saturation whereby LTP initiation creates a ceiling effect. Interestingly, saturation of hippocampal LTP by repeated stimulation leads to learning deficits in the MWM (Bliss & Richter-Levin, 1993), here, again, suggesting the competitive yet overlapping relationship between learning and LTP. Saturation control is thought to rely on LTD mechanisms which are thought to help with the signal to noise ratio (Braunewell & Manahan-Vaughan, 2001). The reduction of noise, or rather, removal of irrelevant information has been found to lead to better learning and subsequent performance (Braunewell & Manahan-Vaughan, 2001).

The direction unto which synaptic plasticity may occur (potentiation or depotentiation) appears to be determined by an activity-dependent process wherein the precise timing of pre and post synaptic activity may either strengthen or weaken connections. This process is known to be spike timing dependent plasticity (STDP) (Feldman, 2012). Potentiation occurs when a pre synaptic neuron fires before a post synaptic neuron (Bi & Poo, 1998). This form of plasticity is thought to align with Hebbian learning principles as it has been observed that the repeated pairing of pre and post synaptic activity is both input specific and often leads to potentiation of synaptic connections (Bi & Poo, 1998; Hebb, 1949). Though some studies noted that 100ms between pre and post synaptic activity should be sufficient to induce potentiation (Gustafsson, Wigstrom, Abraham, & Huang, 1987), researchers found that potentiation can occur within much shorter time windows. Specifically, the sequential activation of pre synaptic input and subsequent synchronous post synaptic output can occur ~10ms within one another and this also induces plasticity changes (Gerstner, Kempter, van Hemmen, & Wagner, 1996). It should also be noted that depotentiation has also been observed in accordance to STDP principles. Specifically, LTD occurs when post synaptic activity occurs before pre synaptic activity (Bi & Poo, 1998; Levy & Steward, 1983). Just like potentiation can occur through the sequential activation of pre synaptic input, depotentiation can be observed when there is a sequential and synchronous activation of post then pre synaptic activity. This precise order of activity might be determined and organized by the ongoing background rhythms by becoming phased-locked to them (Mehta, Quirk, & Wilson, 2000).

Here, we have demonstrated the relevance of LTP processes to learning and memory during ongoing behaviour and its important role in stabilizing a cognitive map or mental representation of our environment. This map then, is thought to facilitate ongoing planning and decision making. The formation of this cognitive map is thought to be formed by the collective activity of multitudes of specific place-coding cells, perhaps as originally

suggested by Donald Hebb. It is important to note that these place cells seem to also be temporally coordinated with the ongoing background theta rhythm (4-12 hz) (Frank, Brown, & Wilson, 2000) elicited by the movement of the rat (theta as a state and its speculated functional relevance is further elaborated in the following section). It is thought that the temporal relationship between theta phase and place cell activation facilitates the creation of a cognitive map (Moser et al., 2017; O'Keefe, 1976; O'Keefe & Nadel, 1978; Tolman, 1948). Furthermore, Huerta and Lisman (1996) found that LTP and LTD can occur at theta frequencies furthering the notion that place cells may be facilitating the initial learning of the environment in order to allow for ongoing navigation. This would suggest that the condition or state of the HPC facilitates the gathering of relevant information to inform ongoing, real-time experience, and, in this case, episodic-like memory in the form of spatial navigation. If this is in fact the case, then it would logically follow that the state may also have a role to play in the consolidation of memory and should therefore be considered as a potential facilitator.

State-dependent memory consolidation

Based on the discussion above, it would appear that hippocampal state may influence the information solidification process during ongoing behaviour. By this, one might be inclined to consider the possibility that ongoing collective activity patterns (e.g. theta) could be an operational state used by the brain to facilitate and organize the expression of complex behaviour based on previously stored information as well as updating it with incoming sensory inputs. Furthermore, the oscillatory and continuous nature of rhythmic patterns might be facilitating the concretization required to make memories permanent. Specifically, coordinated rhythms in the HPC might be the mechanism by which a closed loop system is formed to allow for the reverberation of activity that could reinforce and thus promote behavioural synaptic plasticity and consequently solidify relevant memory engrams, as Hebb

postulated (Hebb, 1949). Interestingly, the HPC exhibits behaviourally differentiated rhythms or states and each of these have been implicated in memory functions.

Theta

Theta, or rhythmic slow activity (RSA: as described by Case Vanderwolf), is a systematic 4-12Hz rhythm generated by the hippocampal network (Bland & Colom, 1993; Vanderwolf, 1969; Whishaw & Vanderwolf, 1973). It is thought to be mediated by interactions of hippocampal and medial septal (MS) cells which project extensively throughout the hippocampus via the fornix. Lesions to either the MS or fornix resulted in a significant attenuation of the theta rhythm (Buzsaki, 2002; Hasselmo, Bodelon, & Wyble, 2002). Due to these lesioning studies, and the presence of theta-rhythmic cells, the MS is considered to be a "pacemaker" of the hippocampal theta rhythm (Buzsaki, 2002).

Assessment of the pharmacological nature of the theta rhythm has revealed interesting distinctions. Two separable types of theta have been suggested to exist: atropine sensitive and atropine resistant theta. Atropine sensitive theta has been found, consistent with the anti-muscarinic action of atropine, to be cholinergic in nature. It is eliminated by high doses of both centrally-acting atropine or scopolamine. Interestingly, cholinergic agonists like eserine promotes this type of theta (Bland, 1986; C. H. Vanderwolf, 1975). Atropine resistant theta, on the other hand, has been found to be sensitive to most anesthetics, and specifically to ether and urethane, although, again as its name suggests, resistant to muscarinic antagonism (Bland, 1986; C. H. Vanderwolf, 1975). As mentioned previously, lesions to the medial septum and fornix have resulted in the attenuation of both types of theta. This suggests that cholinergic projections alone from the medial septum may not be the final arbiter of HPC theta but perhaps some other neurotransmitter or modulator. Indeed, immunohistochemical studies have shown that non-cholinergic septal projections use both gamma-aminobutryic acid (GABA) and glutamate, although they in themselves

may be excited by cholinergic mechanisms within the MS. Another postulate is that although the MS might be a final frequency adjuster/synchroniser for hippocampal theta, the two separate pharmacological systems may simply converge there or somewhere further upstream (i.e., supramammilary nucleus) (Quirk & Stewart, 1988). Regardless, in addition to the pharmacological distinctions of these types of theta, they have also been differentiated with respect to behaviour (Bland, 1986).

Specifically, Cornelius (Case) Vanderwolf was the first to characterize, in fine detail, the behavioural correlates of theta. In rodents, rabbits, and dogs, theta (in a bandwidth of 7-12 Hz) always accompanied "voluntary" movements (also known as type-1 behaviours). These included things like walking, jumping, swimming, and postural shifts. This theta was not sensitive to atropine and was interestingly scaled in terms of faster frequencies to the vigour of movement (Vanderwolf, 1969; C. H. Vanderwolf, 1975). Other stereotyped and fixed action pattern (involuntary or type 2) behaviours (like grooming, licking, eating, and also fixed immobility) were accompanied by non-rhythmic irregular hippocampal activity (LIA –see below). In certain cases, however, theta of a lower frequency range (6-7Hz) could be observed during immobility especially during intense and relevant sensory stimulation – especially in rabbits (Sainsbury, Heynen, & Montoya, 1987; C. H. Vanderwolf, 1975). Here too, when ultimately preceding a movement, this theta scaled in terms of frequency to the eventual vigour of the movement produced. This theta, like that when animals were immobilized with urethane or ether, was completely dependent on muscarinic neurotransmission (Vanderwolf, 1969; C. H. Vanderwolf, 1975; Whishaw & Vanderwolf, 1973). Atropine sensitive theta is also observed during REM sleep, also known as paradoxical sleep, but at lower frequencies as compared to when movement-related theta appears (Colgin, 2016).

In addition to characterizing the theta rhythm, it is also important to discuss its functional relevance, especially with respect to behavioural assays of learning and memory. Studies

have demonstrated that the attenuation of the theta rhythm can affect behavioural expressions of memory as eluded to in the previous section with respect to its relationship to place cell organization. Eichenbaum et al (1990) have shown that lesions to the fornix result in an inability for a rat to develop a direct path to a hidden platform when starting locations change across trials in the MWM task. This results in higher latencies as compared to control group and suggest that the theta rhythm may contribute to flexible decision making (Eichenbaum, Stewart, & Morris, 1990). Though this study assessed the hippocampal-dependent nature of spatial learning in the MWM task, an alignment can be made with respect to the findings in this study and what we can speculate about the functional role of theta to be.

It has been established that lesions to the fornix results in the attenuation of the theta rhythm. Studies have demonstrated that attenuation of theta rhythm leads to inflexible and perseverative-like behaviours (Hasselmo et al., 2002; Kunec, Hasselmo, & Kopell, 2005). It has also been established that place fields have the capacity to remap themselves based on changes of the environment and the failure to do so results in dysfunctional coding of the environment and therefore poor behavioural expression of spatial memory (Marozzi & Jeffery, 2012). Furthermore, swimming behaviour corresponds to a theta state, therefore, one can assume that the combinatorial action of the theta rhythm and place cell activity are working together to form the necessary cognitive map. Thus, based on the loss of the ongoing theta rhythm due to fornical lesions, we could hypothesize that it is this that leads to spatial impairment shown by the experimental group (Eichenbaum et al., 1990). These results strongly suggest that the theta rhythm is important in maintaining flexible spatial behaviour during navigation that also requires interfacing with spatial memory. Thus, it is a palpable notion to consider theta to be an important platform in forming and developing information regarding the environment and the interactions of a navigating entity. In

summary, it would be well suited to play the role of an encoder (Hasselmo et al., 2002; M. E. Hasselmo, Hinman, Dannenberg, & Stern, 2017).

Theta has also been observed to play a role in the sequential replay of previous experiences and in the coding of future/prospective behaviours (Ferbinteanu & Shapiro, 2003; Frank et al., 2000; Sereday, Marti, Damiano, & Moser, 1994). The sequential replay of individual cells with respect to representing an event, and not necessary a place, has been observed in the HPC in the form of episodic-like fields or cells. These episodic-like-fields appear to fire consistently despite the absence of a changing environment (i.e., when a rat is running at a constant speed in a running wheel) (Pastalkova, Itskov, Amarasingham, & Buzsaki, 2008). The sequential replay of these episodic-like fields are organized at different phases of the theta cycle and can be used to predict past and future behaviours depending on where these cells fire in relation to the theta phase in each respective theta cycle (O'Keefe & Recce, 1993). These results would suggest that the HPC uses temporally-precise coding during ongoing behavioural experiences in a time compressed manner which are not environmentally driven, but rather, are internally generated.

This temporal organization of single units could also provide a mechanism unto which LTP and subsequent memory consolidation processes can occur. For instance, in the study described above, Pastalkova et al also tested the nature of episodic-like-like activity in relation to memory and non-memory loaded tasks. It was observed that, although there were no differences found in the actual firing of these cells, transient unit activity during non-memory related tasks were less temporally organized according to theta cycle and also appeared to have significantly reduced population spiking activity. The synchronous discharge of cells in a temporally organized fashion has the ability to create the optimal environment for LTP as this ensemble of neuronal activity encourages associative and cooperative processes (Buzsaki, 1989; Eichenbaum, 2011; Hebb, 1949). This not only suggests that unit activity is modulated by the theta rhythm, but that also, the temporal

organization of episodic-like fields in accordance to the theta cycle may promote LTP-like mechanisms to promote further memory consolidation processes and Hebbian synaptic plasticity rules.

In summary then, theta appears to be both an encoder of initial experience influenced by the information it receives by external environmental inputs and internally generated episodic-like-sequences to inform ongoing behavioural decisions. Place cells phased locked to the theta rhythm suggest that theta works to temporally organize ongoing experiences. This could allow for the tagging of important neuronal assemblies throughout waking experiences for further memory consolidation by using Hebbian synaptic plasticity rules (Buzsaki, 1989; Hebb, 1949). Though this role is quite important, as one can imagine, without the appropriate encoding of experience, there is no information that can be processed (a circumstance we observed with patient H.M), the process of memory solidification requires more than initial encoding of external and internally driven events.

LIA and Sharp-Wave Ripples

The previous section explored the notion of the theta rhythm playing a role as an encoder of initial learning and experience. It has also been demonstrated that theta may assist with retrieval and updating of information during ongoing behavioural experiences (i.e., reversal learning, aka contingent learning). Unit activity of the hippocampus (i.e., theta and place cells) are phase locked with the theta rhythm suggesting that theta assists with the packaging and organizing of present environmental experiences to develop a cognitive map (O'Keefe et al., 1998; O'Keefe & Nadel, 1978; O'Keefe & Recce, 1993; Tolman, 1948). The development of the cognitive map and the inherent characteristics of the rhythm allow for the planning of online future trajectories by replaying a sequence of place cells (Ferbinteanu & Shapiro, 2003; Frank et al., 2000; Pastalkova et al., 2008). Interestingly, theta is not the only rhythm which uses sequential replay of activity to facilitate behavioural decisions.

Large Irregular Activity (LIA) is a non-rhythmic broadband signal with high power in the 0.01-3Hz band (Colgin, 2016) and is also a carrier of fast oscillatory events known as ripples (150-250 Hz) which themselves are coupled with transient large amplitude events known as sharp-waves. Sharp waves (SPW) are generated in the CA3 region of the HPC and transmit activity to the stratum radiatum layer of CA1 via Schaffer collaterals. They are a result of summed postsynaptic depolarization caused by the synchronous discharge bursting of large populations of CA3 pyramidal neurons, and are observed at the level of CA1 (Buzsaki, 1989). Ripples, on the other hand, are generated locally within the CA1 network itself by perisomatic basket interneurons. Ripples are fast, emergent, transient events in which pyramidal cell activity is exquisitely timed by this fast inhibition (Buzsaki, 2015). SPWs can occur independently of ripples, however, ripples are always associated with SPWs and both are a preserved mechanism across mammalian species including humans (Buzsaki, 2015). This preservation of activity suggests a commonality of function across mammalian species. Many neuroscientists suspect that SPW-R have a role in the memory consolidation process.

Initial studies of SPW-Rs began with the observation that these events occurred in the sleeping animal. These SPW-Rs were detected during non-REM (NREM) stages of sleep, specifically during slow wave sleep (SWS). Interestingly, SPW-R events are observed in relation to other fast transient events such as thalamocortical spindle activity (8–15 Hz), during NREM stage 2 (Buzsaki, 2006). In general, spindles have been found to precede SPW-Rs, however, peak SPW-R activity has also been observed just prior to spindle peak activity, suggesting a potential bidirectional relationship between these two forms of activity and thus across the hippocampus and cortex (Buzsaki, 2006; Latchoumane, Ngo, Born, & Shin, 2017). SPW-Rs are also observed to co-occur during the ON or UP phase of the neocortical (nCTX) slow oscillation (SO)—a high voltage, slow rhythm which has been implicated in memory consolidation and will be elaborated upon in the following section

(Buzsaki, 2006; Colgin, 2016; Steriade, Nunez, & Amzica, 1993b). On the other hand, LIA and SPW-R events can also be observed in the rat HPC during waking periods; specifically during immobility, drinking, eating, grooming, and other stereotypical species-specific and fixed action pattern behaviours (Buzsaki, Leung, & Vanderwolf, 1983). At a physiological level, wake and sleep SPW-R events do not seem to be particularly different from one another (Carr, Jadhav, & Frank, 2011), however, they may be playing different roles with respect to controlling behaviour as well as in regards to memory solidification.

Sharp-waves and ripples are thought to help with the memory consolidation process. While it has been proposed that the synchronous bursting of population neurons during SPWs is an ideal medium for LTP (Buzsaki, 1989), ripple complexes riding on the peak of these SPWs have been observed to replay the sequential activation of hippocampal place cells that represent previously learned experiences that occur during awake state (Carr et al., 2011). Replay can occur in both forward and reverse modes within a compressed timescale in both the waking rest and sleeping animal (Buzsaki, 2015). These events may provide the necessary conditions to create a closed-loop system required to promote the reverberation of activity which would subsequently allow for the solidification of a memory engram (Buzsaki, 1989; Hebb, 1949). While sleep SPW-R events are thought to assist with creating a chain of remote memories acquired throughout the waking experience of an animal via forward replay, wake SWP-Rs are thought to help reinforce information and experiences as they occur, and are most often, but not always, in reverse sequence (Foster, 2017). This is not to be confused with the role of theta during ongoing behaviour and in fact, the states in which theta and SPW-Rs occur are mutually exclusive to one another (Buzsaki, 1989).

Theta may provide an ongoing encoding of environment and real-time updating of experiences, while SPW-Rs may allow for remote experiences to be replayed or rehearsed for better behavioural modifications. Essentially, these two states work in sequence to one

another yet are contiguous which allows for a reinforced learning paradigm (Buzsaki, 1989). The interleaved nature of both theta activity and SPW-Rs can easily be understood by observing a rat's behaviour. For instance: in a freely moving rodent, you might observe a rat to explore (theta), pause (LIA), resume exploration (theta), eat (LIA), be immobile (LIA), explore again (theta), etc. (my own observations). In these behavioural sequences, it is easy to understand how theta and LIA/ SPW-Rs are mutually exclusive. Their EEG signals as well as their behavioural profiles are also quite distinct from one another (Buzsaki, 2002; Colgin, 2016). Essentially, the mutual exclusive nature of both theta and SPW-Rs may actually work in favour of solidifying information rather than harming the process.

Buzsaki has suggested a two-stage memory consolidation model between theta activity and SPW-Rs. While theta is thought to weakly "tag" neuronal assemblies during the encoding process via inputs from dentate granule cells, subsequent sequential reactivation of these cell assemblies in the form of SPW and ripples, at remote time points, allow for further consolidation (Buzsaki, 1989). What appears to differentiate theta and SPW-R reactivation events is the context unto which they seem to occur. Theta sequential play appears to be motivated by ongoing behavioural experiences in order to adapt and change real-time episodes , whereas SPW-R events appear to be exclusively internally guided and promote memory retrieval (Carr et al., 2011; Foster, 2017).

It would appear then that the brain uses several methods to rehearse, replay, and recapitulate information to inform behaviours. It has been noted that sequential activation/reactivation of experience is encoded both during theta and SPW-R events. Where theta allows for guided trajectories via the sequential activation by using both external and episodic-like-based information, SPW-R events appear to be more internally generated, an ideal model for memory retrieval (Carr et al., 2011). This replay or rehearsal of information appears to be fundamental to the memory consolidation process as it has

been shown that selective suppression of ripple events produced performance deficits in rats during declarative types of memory tasks (Girardeau, Benchenane, Wiener, Buzsaki, & Zugaro, 2009). Furthermore, their importance to information solidification is reiterated by the presence of rebound ripple events upon their suppression. This is in addition to the behavioural rehearsal/replay observed during the studies of Ebbinghaus (active rehearsal) and Müller and Pilzecker's (passive rehearsal) (Ebbinghaus, 1913; Lechner et al., 1999).

The discussion on the presumed role of SPW-Rs and their behavioural correlates position SPW-R events to play an intermediary role to memory consolidation. They are both influenced by incoming cortical information as well as pass information over to cortical areas as they have been found to be temporally coupled to both spindle activity and the nCTX slow oscillation (Buzsaki, 2015; Colgin, 2016). They occur in sequential order to theta activity both in terms of EEG and behavioural outputs (Buzsaki et al., 1983). Their emergent properties in addition to their behavioural correlations also makes them an ideal model for memory retrieval (Buzsaki, 2015; Buzsaki et al., 1983; Carr et al., 2011).

Considerations around the potential role and contribution of theta and SPW-Rs have begun to outline what may be happening in the process of forming and solidifying information, however, the picture is still incomplete. Theta and SPW-R events are bounded within their local circuits and may be limited in how far the information they carry, can travel. A slow oscillation, however, may help with organizing this information across longer distances which may help in the exchange of information across brain structures.

Hippocampal Slow Oscillation

The role of SPW-R events has been postulated to play an intermediary role in transferring information from the HPC to the nCTX due to their ordered appearance during ongoing wake

and sleep behaviours. Theta activity has also been discussed in relation to its presumed role in encoding initial experiences of learning. Both have been observed to utilize a form of sequential replay to allow for the ongoing updating of behaviours and planning for future trajectories. Activity patterns during these states may function towards the memory consolidation process by promoting synaptic potentiation (Buzsaki, 1989; Huerta & Lisman, 1996). However, these events are also quite local and may be limited in terms of how far the information they carry can travel. In order to mitigate this issue, a slower rhythm that has the ability to propagate over brain-wide distances may be required in order to exchange information across many structures, thereby solidifying information more concretely.

In 1993, Steriade and colleagues introduced the neocortical (nCTX) slow oscillation (SO) to the scientific community. This rhythm was described to be alternating in its activity between long lasting depolarizations separated by long periods of neuronal silence, which would eventually be termed as ON/UP phase and OFF/DOWN phase, respectively, during SWS (Steriade et al., 1993b). The nCTX SO appears to be an endogenously generated event as it survives both thalamic and callosol lesions (Steriade, Nunez, & Amzica, 1993a). In addition, the nCTX SO has been shown to persist in differentiated cortical slab preparations (Timofeev, Grenier, Bazhenov, Sejnowski, & Steriade, 2000). Since its discovery, many neuroscientists have sought to determine whether the SO is simply a result of the recurrent neocortical architecture, or actually serves a functional role in the memory consolidation process.

Several studies have demonstrated benefit to hippocampal-dependent memories after SWS, which is dominated by slow wave activity (SWA). Studies have shown that SWA during SWS increases as a result of experiences that occur during wake. Furthermore, in the same study, it was shown that this increase in SWA subsequently also led to an increased expression of plasticity-related genes (Huber, Tononi, & Cirelli, 2007). This observed increase may help explain why many studies have shown SWS benefits to hippocampal-

dependent memories. Diekelmann et al (2012) have demonstrated benefit to visuo-spatial memories in human participants after a 90 minute sleep period compared to a 40 minute sleep period suggesting that increased time spent in SWS may lead to protection of previously learned information (Diekelmann, Biggel, Rasch, & Born, 2012). Furthermore, in an important study conducted by Marshall and Born (2006), it was demonstrated that the application of transcranial stimulation at 1 Hz frequency in healthy humans during NREM sleep, resulted in an increase of declarative memory performance. Alternatively, stimulation of 5 Hz, theta frequency, which is found to predominate REM sleep, resulted in no difference in performance. In addition, slow oscillatory stimulation at 1 Hz also resulted in an increase of time spent in NREM sleep as well as sleep spindles (Marshall, Helgadottir, Molle, & Born, 2006). How precisely the SO facilitates this benefit is still under debate.

Studies have suggested that the SO serves as a base frequency unto which faster and more transient oscillatory events, such as spindles and SPW-Rs may interact together in the name of information processing across multiple brain regions (Neske, 2015). For instance, Luczak et al (2007) demonstrated that neurons displayed a unique spike pattern (sequential activation) during the first ~ 100ms of the initiation of the UP state (Luczak, Bartho, Marguet, Buzsaki, & Harris, 2007). It is suggested that facilitating the organization and grouping of these faster events may leave the cortex in a depolarized state, possibly opening itself up to LTP induction (Sirota & Buzsaki, 2005). On the other hand, it has also been suggested that synaptic weakening occurs during the time of the UP phase (Bartram et al., 2017). Studies have demonstrated that subthreshold inputs activated by extracellular stimulation during the UP-phase results in synaptic weakening. However, when presynaptic activity precedes postsynaptic activity (classic spike-timing dependent plasticity, STDP) this results in potentiation or the opposite of synaptic weakening (Bartram et al., 2017). This process of synaptic weakening is thought to promote the retuning and reorganizing of experiences which would allow for the elimination of irrelevant information and the potential

strengthening of more important memories. Though there is still uncertainty around the mechanisms by which the SO may use to solidify information, its importance is reiterated by the discovery of another slow oscillation, except this time in the HPC.

The hippocampal SO has been described as a large voltage, highly rhythmic slow rhythm which spontaneously and periodically appeared under urethane-anesthetized and natural sleeping conditions (NREM, SWS), similar to the previously described nCTX SO (Wolansky, Clement, Peters, Palczak, & Dickson, 2006). Specifically, a triad order of state alternations in the HPC during SWS was detected: REM-like (predominantly theta), and NREM-like (LIA, and then the hippocampal SO) (Wolansky et al., 2006). The hippocampal SO was observed to only transiently overlap (and follow) the nCTX SO and was only moderately coherent with it. In fact, SO coherence between intracortical and intrahippocampal sites, respectively, were consistently higher than cortico-hippocampal coherence, suggesting that the hippocampal SO is not a result of volume conductance of the nCTX SO (Wolansky et al., 2006).

The discovery of the HPC SO provides a basis unto which bidirectional synaptic scaling between the HPC and nCTX could occur (Dickson, 2010; Wolansky et al., 2006). Whereas the recruitment and grouping of oscillatory activity can help promote reactivation and strengthening of waking experiences, the removal and retuning of less salient information also facilitates the retrieval of important information. With respect to the HPC SO, however, it is highly speculative since studies of its functional role do not exist. That being said, however, we are interested in determining its contribution to declarative memory. If our assumptions are correct with respect to the HPC SO and its role in being able to group and organize information, either through potentiation or depotentiation, then it would follow that the promotion of the HPC SO would assist with solidifying information and allow for more accurate recollection of information when required. Therefore, since the SO is observed

during NREM, particularly SWS, promotion of sleep should, theoretically, assist with declarative forms of memory.

This section has provided a brief but important outline on the three states which have been observed in the HPC. Although these states (theta, LIA, and SO) are differentiated in terms of their behavioural correlates, there is a certain degree of overlap, as well as in terms of their assumed functional role with respect to memory. Because of this, we are interested in parsing out the contributions of each of these states to memory retrieval for declarative types of memory. We have developed an episodic-like-like paradigm to apply the theoretical and empirical notions presented in previous sections which we hope will provide us with insight into the specific contributions these states play with respect to declarative types of memories. The upcoming chapter and sections will outline my research project, detail the methods and results and end with an important discussion.

Chapter 2: The examination of state-dependent memory consolidation

Abstract

Understanding the process by which our memories become permanent (consolidation) is a fundamental question in psychology and neuroscience. From the very outset of memory research, the period directly following a learning episode was recognized as being important for future recall since manipulations that either enhanced or impaired neurobiological function during this time were shown to affect memory in either a beneficial or detrimental fashion, respectively. A critical factor for this time-dependent process involves state-dependent neural activity, often coupled to a particular behavioural state. In this study we took advantage of the tight coupling of hippocampal state and overt behaviour in rats to test for influences on memory expression following an episodic-like-like learning task. Rats were trained on the novel spatial location version of the circular water maze and were subsequently placed in one of three conditions to encourage exploring (a state in which theta activity predominates), grooming (a state showing large-amplitude irregular activity and sharp-wave ripples) and sleeping (a state showing large amplitude slow rhythms). Our behavioural assay demonstrates that when total time spent in each condition was evaluated, sleep appeared to have the largest benefit for subsequent memory on a probe test of the novel location whereas time spent in explore or groom condition have the opposite or no effect on performance.

Significance Statement

Different patterns of collective neural activity in the hippocampus are thought to play a role in memory fixation/consolidation. By encouraging rats to engage in different behavioural states like running/foraging, grooming, and sleeping, which are all known to evoke different patterns of collective activity (theta, ripples, and slow oscillation, respectively), we could

test how each behavioural state might influence the future retrieval of a newly learned hippocampal-dependent spatial task. Our results indicate that sleep, and the patterns expressed therein, is most beneficial for memory.

Introduction

The earliest studies of memory, initially performed by Ebbinghaus (Ebbinghaus, 2013) and subsequently championed by Müller, and Pilzecker (Lechner et al., 1999), demonstrated two critical conditions to memory permanence: time and activity after learning. Since then, many neuroscientists have sought to explain these conditions through a reductionist approach aimed at the level of intracellular and molecular processes within nerve cells that might mediate mnemonic longevity. Here instead, we revisit the role of behaviour and brain state in the period directly following acquisition of a hippocampal-dependent spatial memory task, since state and more importantly, state-dependent activity, has been suggested to play an important role in its own right (Buzsaki, 2006; Buzsaki et al., 1983; Colgin, 2016; Dickson, 2010; Hasselmo et al., 2002; Winson, 1978)

In the hippocampus, there are three different electrographic states that couple with specific behavioural states in a mutually exclusive fashion: theta (a large amplitude rhythmic oscillation between ~4-10Hz), large amplitude irregular activity (LIA, a broadband signal with little rhythmicity and higher power at lower frequencies), and the slow oscillation (SO, a large amplitude ≤ 1Hz rhythm) (Buzsaki et al., 1983; Vanderwolf, 1969; Wolansky et al., 2006). During wakefulness, theta is apparent during spatial displacement movements (exploration and running) as well as during postural changes, whereas LIA is observed during stereotypical patterned behaviours such as drinking and grooming as well as during awake immobility (Buzsaki et al., 1983; Kramis, Vanderwolf, & Bland, 1975; Vanderwolf, 1969; Whishaw & Vanderwolf, 1973). While sleeping, theta is observed during rapid eye movement (REM) stages, while LIA occurs during the initial (light) stages of NREM (Buzsaki,

1989). The HPC SO, thus far, has only been observed during slow-wave sleep (SWS) and is found to coordinate with the nCTX SO dynamically and intermittently (Wolansky et al., 2006).

Each of these EEG states has been differentially implicated in contributing to the formation and retrieval of episodic-like forms of memory, but all three have also been implicated in memory solidification (Buzsaki, 2002, 2015; Skelin, Kilianski, & McNaughton, 2018; Wolansky et al., 2006). Theta has been found to play an important role in the organization of place and episodic-like-like cell activation in the hippocampus (Ferbinteanu & Shapiro, 2003; Frank et al., 2000; Pastalkova et al., 2008) but has also been suggested to be important for spatial memory consolidation (Boyce, Glasgow, Williams, & Adamantidis, 2016). LIA enables the replay of cell activity sequences relevant to previous episodic-like experiences in a compressed time-format which are observed as sharp-wave ripples (SPW-R; 150-250Hz) (Colgin, 2016; Foster, 2017). The SO has also been shown to be important for consolidation, and is typically thought to contribute to the global re-organization process required for the stable fixation of memories (Born, Rasch, & Gais, 2006; Dickson, 2010; Steriade et al., 1993b; Wolansky et al., 2006).

Given these differentiated neural patterns of activity during sleep and wake states, as well as their role in the consolidation of memories, we sought to understand the role that these individual states have on memory consolidation during a one-hour delay period between learning and memory testing.

In order to test the idea that a particular pattern of post-learning hippocampal activity might affect the retention of previously-learned hippocampal specific information, we implemented two elements: 1) a situation-unique hippocampal-dependent memory task with a delay between training and testing, and 2) a way to influence behavioural state in the delay period directly following training to encourage each of the three main patterns of hippocampal state-dependent activity previously described. To fulfil our first requirement,

we elected to use the delayed match to place version of the Morris water maze (MWM) spatial memory task using test-unique locations. The MWM has been shown to be both a reliable measure of spatial memory and directly dependent on the integrity of the hippocampus (D'Hooge & De Deyn, 2001; Morris et al., 1982; Vorhees & Williams, 2006) . The delayed match to place version allows for the testing of unique spatial memory on each exposure since the location of the platform is changed quasi-randomly before training on every day (D'Hooge & De Deyn, 2001). To fulfill our second requirement, we implemented situations to encourage theta-, LIA-, and SO-correlated behaviours (run/forage, grooming, and sleep, respectively). We show that sleep is the state which most benefits memory, likely by the coordinating effect of SO.

Methods and Materials

Subjects

Data were obtained from a total of 32 male Sprague Dawley rats weighing between 200-300g. 21 were run in the behaviour-only condition with the 11 remaining being additionally implanted for electrophysiological monitoring. Based on our learning performance criteria we removed 19 rats from the final analysis. All rats were group housed with up to 3 litter mates except for implanted rats which were housed singly after implantation. Rats were kept on a 12-hour light/dark cycle (lights on 6am, lights off 6pm) and were tested late morning to late afternoon (11:00am – 3:00pm) All methods used conformed to the guidelines established by the Canadian Council on Animal Care, the Society for Neuroscience, and were approved by the Biosciences Animal Policy and Welfare Committee of the University of Alberta.

Overview of Experimental Procedures

The timeline for behavioural training and testing is shown in Figure 1. In brief, following habituation and training periods described in more detail below, the testing procedure

involved using the delayed match to sample (DMS) spatial version of the Morris water maze (MWM) task in order to test the effects of hippocampal state on spatial memory retrieval. Animals were first trained over four trials using the hidden (submerged) platform version with room cues to guide their swim navigation behaviour. The position of the platform was changed day-to-day. Following learning and after a one-hour delay period, memory was tested by using a probe trial in which no platform was present. During this delay period, we encouraged rats to adopt behaviours that would promote specific hippocampal states of activity. These behaviours were: 1) running/foraging to induce hippocampal theta rhythm (rats were given access to a running wheel from their home cage or they were placed in a cage that contained small pieces of flavoured cereal hidden in the bedding throughout the cage), 2) grooming to induce LIA and SPW-R activity (rats were re-immersed in water and were then rewetted following the first 30 minutes), and 3) sleeping to induce SO activity (rats were housed in a standard home cage without any other stimulus or manipulation). Any differences between groups in the memory probe in terms of latency or dwell time allowed us to assess if this interposed state had any effect on memory stabilization.

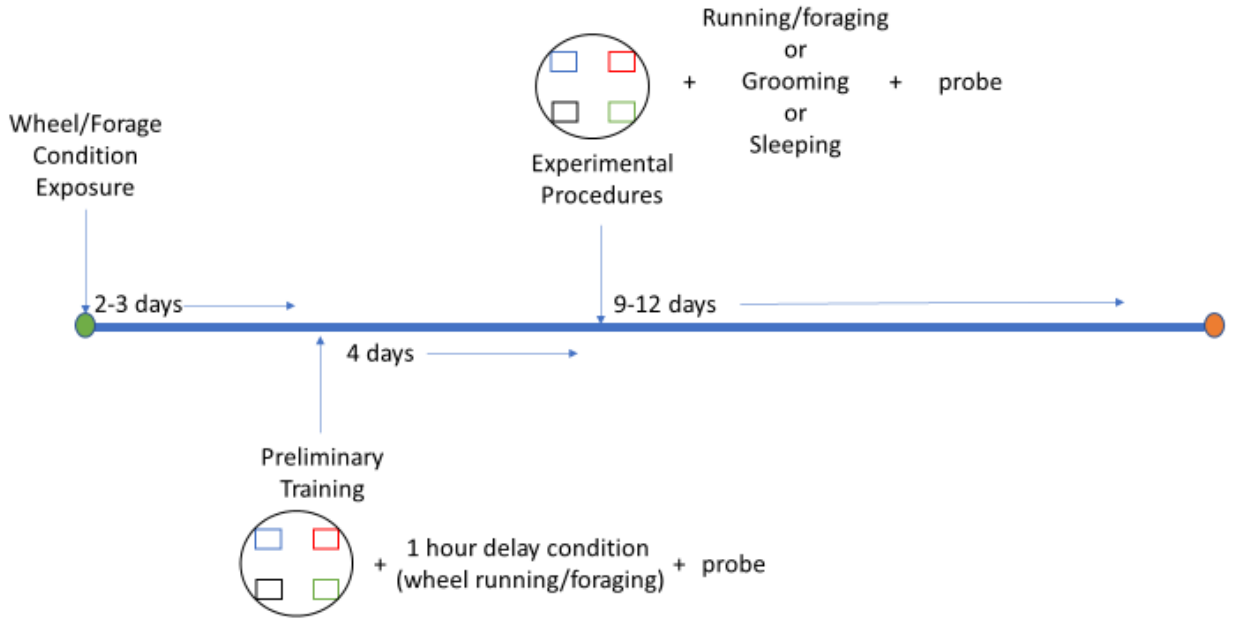

FIGURE 1: Time-line representation of procedures.

Rats are first exposed to the running wheel or foraging conditions. Running wheel habituation lasts for three days while foraging training occurs over two days. Once complete, preliminary standard training in the circular water maze is initiated. The location of the platform is quasi-randomly selected by the experimenter for the day and is consistent across daily trials (n=4) but explicitly varies across the 4 days of training so that each quadrant position is used by day 4. Across daily trials, rats are released at predetermined (and also quasi-random) locations. A one-hour delay with wheel/foraging access follows the four training trials which is subsequently followed by a probe trial in the water maze. Subsequent to training, experimental procedures consisted of similar water maze training and the one-hour delay would consist of all three behavioural states in running conditions. For foraging experiments ran 6 times a week over a period of three weeks also with a one-hour delay in all three behavioural states. Assignment to state was set by the experimenter prior to the beginning of experimental procedures.

Detailed Experimental Procedures

Habituation and Training for Run/Forage Conditions. Naïve rats were habituated to running wheels by providing them with unrestricted wheel access attached to a regular housing cage for three full days. This process allowed the rats to familiarize themselves with the wheel and to develop an expectation for and engagement with it. A minimum of 800 revolutions was set as a requirement for training and interaction with the running wheel during this time. In the foraging condition, rats were trained to expect food rewards hidden in a larger-than-normal (60.96 cm x 43.18 cm x 20.30 cm tall) home cage over a period of 4 days. This condition was used since the movements necessary for active foraging also encourage theta activity and would thus be an appropriate replacement for the running condition (Vanderwolf, 1969). This was initiated by presenting quarters of broken flavoured cereal (Fruity Hoops, Compliments Brand, Mississauga, ON) in a petri dish in the foraging cage on the first day to habituate the rats to the novel food. Subsequently, on the second day, the cereal pieces were placed in equal amounts across each of the four corners of the cage without the petri dish. On the third day, the cereal pieces were scattered about the cage, under shallow bedding material, and on the fourth, this was repeated under deeper bedding material. We determined that 20 whole pieces of cereal (each divided into quarters) were sufficient to maintain foraging for a full hour.

Training for MWM task. Following habituation to the running wheel/foraging condition, rats commenced pre-training in the DMS MWM task. As briefly described above, this task involved finding a hidden (submerged) location for an escape platform that had a fixed position on any particular day, but a novel position across days. Rats were released in the water maze (2m wide circular pool filled with at least 30 cm of water slightly below 20 degrees C) at pseudo-randomly selected locations and their trajectories and latencies to locate the fixed location were assessed using an overhead camera (Basler scA 1300-60gm, Basler AG, Ahrensburg, Germany) connected to a PC computer running a tracking software

system (Noldus Ethovision XT11, Wageningen, The Netherlands). Rats were given 4 trials per day to learn the position of the platform according to the distal cues placed on the walls of the MWM room, with a maximum time limit of 60s. If at the 60s mark, the rat did not locate the platform, they were gently guided by the experimenter to its location.

Following the four training trials and prior to the probe test, we continued our hour-long pre-training schedule in either the run or forage conditions as above. For rats in the run condition, they were placed in the same cages attached to the running wheel as previously described. Thus, rats were conditioned to expect the presence of the wheel during this time. Rats in the forage condition were similarly placed in the associated cage with access to hidden cereal. After the one-hour delay, a 30 second probe trial in the water maze took place. As stated previously, during the probe trial there was no escape platform available. The latency to cross the platform location, the amount of time spent in the training quadrant, and the number of crosses over the original location of the platform during training were evaluated. These measures were taken as an indicator of effective hippocampal-dependent spatial memory for the platform location (Vorhees & Williams, 2006, 2014). By the end of the four-day training period, rats were well trained in the nuances of the task and were also habituated to the run or forage conditions during the intervening period between the training and test swims.

Experimental Assessment Conditions. Following the training procedures as above, the actual experimental trials were run. The learning procedures across the first four trials were identical to those described in the pre-training condition, however, we then randomized rats across the three different delay behavioural conditions of run/forage, groom, or sleep. Rats who were assigned to the run condition were placed in the same wheel-attached cage in a separate room (as previously described) and were video monitored. For the forage condition, rats were placed in a large home cage with hidden pieces of cereal (as previously described) which was also located in a separate room and again video monitored. Rats

assigned to the grooming condition were transported directly from the water maze (i.e., still wet) to their home cage which was located in the same room as the rats in the running/forage condition. In addition to being video monitored, their fur was also re-wetted by re-immersion in a water bath after an initial 30-minute period. Rats who were assigned to the sleeping condition were placed in their home cages located in an entirely separate and quiet room from the other rats. They were also video monitored.

All rats had ad libitum access to water but not chow during this one-hour delay and were otherwise unconstrained to behave as they pleased. Following the one-hour period, each rat was tested using a probe trial in the MWM as previously described. Following completion, all animals were returned to their regular housing conditions until the next day. Each rat was tested across at least three different instances of each behavioural condition (i.e., each rat was tested at least 9-12 times/days in total).

Surgery for Electrographic Monitoring In an additional two rats, we monitored electrographic state during the delay period to confirm the patterns of activity present in the hippocampus. In order to instrument them, were anesthetized (at 4% induction, 1.5-2% maintenance) with isoflurane in 100% oxygen. Indications for adequate anesthesia were determined by assessing suppression of the withdrawal reflex and by the absence of changes in heart- and breathing- rates in response to toe pad pressure. Rats were then placed in a stereotaxic apparatus and body temperature was kept at 37^{o}C using a servo-controlled heating pad (55-7020 Homeothermic monitoring system, 12x20.5 cm, Harvard Apparatus, Holliston, MA) under a surgical field. A local anesthetic (Marcaine/Bupivacaine 0.5%) was administered topically by injection along the midsaggital plane of the scalp to alleviate potential pain from surgical cuts. Animals were implanted bilaterally with Teflon coated stainless streel wire (A-M systems, Sequim, WA) bipolar electrodes with a stagger of 0.5 mm-1 mm in the HPC (coordinates from bregma: anterio-posterior (AP), -3.3; mediolateral (ML), +2.2; dorsoventral (DV), -3.1mm) and neocortex (from bregma: AP, +0.3, ML, +1.0;

DV -1.5mm) (Wolansky et al., 2006). A screw placed over the cerebellum served as ground. A bipolar EMG electrode (miniature stainless-steel wires made of soft multi-strand, Teflon-coated, 0.013mm thick, A-M Systems) was implanted in the neck muscle to allow for characterization of phasic and tonic muscular activity. Wires and connectors were fixed to the skull using dental cement and anchored to self-threading screws fastened to the skull bone. The uninsulated ends of electrode wires were friction-fitted with male miniature connector pins (A-M systems)) in an 11-pin implantable electrical socket (Ginder Scientific, Nepean, ON). To mitigate post-operative pain, rats were given an i.p. injection of Rimadyl (0.1 ml) and were returned to their clean home cages, kept warm and observed until regaining sternal recumbency.

Recordings. During the delay period of the MWM, implanted rats were connected via the implanted Ginder plug to the amplification system via a multiwire tether that was positioned at the top and centre of a plexiglass cage 61 x 43 x 20 cm tall. Rats were encouraged to either forage/groom/or sleep as described previously. During the delay period, as before, rats were also monitored by video recordings that were synchronized to the acquisition of physiological signals (see below). Otherwise, the training and experimental procedures were identical as for phase 1. All local field potential recordings from brain were referenced in their bipolar electrode arrangements, amplified at a gain of 1000 and filtered between 0.1-500Hz using an AC amplifier (Model 1700, A-M Systems). Potentials from bipolar EMG electrodes were also referenced in their bipolar arrangements, amplified at a gain of 10,000, and bandpass filtered between 10 and 500 Hz, again using the same AC amplifier. All signals were digitized at 1000 Hz using anti-aliasing filtering through connections to a PowerLab 16/35 series Digitizer (AD Instruments, Colorado Springs, CO) and acquired though Labchart Pro Version 8 (AD Instruments) with simultaneous video recording (Logitech Webcam Pro C920, Silicon Valley, CA).

Histology. At the end of recording sessions, small lesions were made at the tips of active electrodes by passing 0.1–1 mA of DC current for 5 s using an isolated constant current pulse generator (model 2100; A-M Systems) allowing us to specify their location during histological procedures. Rats were deeply anesthetized with isoflurane (4%) in 100% oxygen and were then decapitated. Brains were extracted and placed in 30% sucrose in 4% paraformaldehyde for a period longer than 24 hours. The tissue was frozen with compressed CO_2 and sectioned at 60 µm using a microtome (1320 Microtome; Lecia, Vienna, Austria). Brain sections were then mounted on gel-coated slides and allowed to dry for a minimum of 24 hours. All slides were thionin-stained and sections were viewed under a microscope to confirm electrode placements. Microscopic inspection of stained slides were used to verify recording loci (Wolansky et al., 2006).

Analysis

Learning Criteria. We set a specific learning criterion for all rats in the MWM in order to ensure that they had sufficiently acquired the task in the limited number of trials used. In most cases, rats demonstrated learning in terms of faster latencies and more direct trajectories to the hidden platform location as a function of trial number (Figure 2B). As a criterion, we established a maximum latency of 13 seconds on trials 3 and 4. This cut-off was chosen by examining the distribution of all latencies during the training trials. It was clear from this distribution that latencies past 13 seconds were the outliers of this distribution on trials 3 and 4. Therefore, animals who were at or above this 13 second criteria for more than half of their total experimental days were eliminated from our analysis (Figure 2A). Upon review of maze videos, we also noted that rats that did not learn tended to engage in thigmotaxic behaviours for a longer period of time and reduced orientation responses when placed in the maze. This would suggest that non-learners adapted a different (or altogether lacked) learning strategy as compared to learners. The identification of a learning criteria was critical since we required rats to have sufficiently learned the task

during the four trials in order to assess their subsequent memory on the subsequent probe trial. In keeping with this, we also observed better probe trial performance on average once we eliminated data using this criterion (Figure 2B).

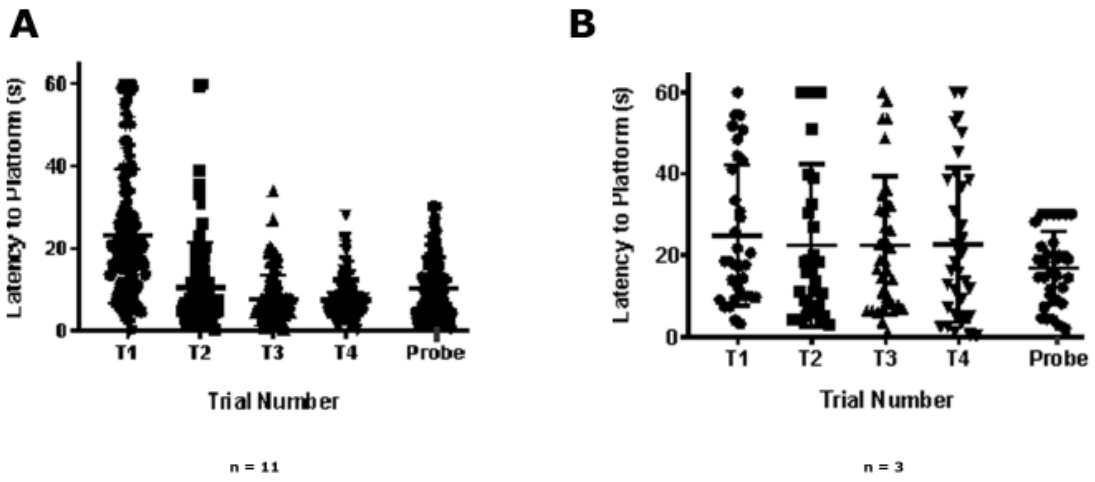

FIGURE 2: Water maze learning criteria implementation

Panel A: Plotted are the latencies (to a maximum of 60s) for rats to reach the hidden platform in the circular water maze. In this scatterplot, we show only those rats who performed according to our learning criteria. As shown, there is a marked tendency for latency values to decrease across trials on average and to cluster at values below about 20s. As well, there is a decreasing tendency for values to reach the maximum time allowed (60s) with increasing trials.

Panel B: Trail latencies for those rats who did not learn according to our learning criteria. Learning criteria was determined first by plotting the latency distributions for trials 3 and 4 of all rats. This distribution allowed us to determine that most values falls between <13s, thus, we re-categorized rats as non-learners who spent majority of their experimental days at or more than 13s on trials 3 and 4. In this case, there is little apparent systematic reduction of the overall latencies across trials. As well, there are many more instances of maximal time being observed across trials, including the probe trial.

Probe Trial Latency Criterion. As eluded to above, we were also concerned with removing instances in which the performance of the rat was not indicative of true memory. From a probabilistic analysis of the full dataset we determined that latencies of 1.5s and less were likely to be highly improbably chance occurrences (3.13%). Thus, we removed these from our dataset recollection.

Behavioural Video Coding. During the delay period, behaviour was manually coded and time-stamped by reviewing video recordings (as well as simultaneous electrographic traces in those cases when acquired). Exploration (theta) states were coded when a rat was walking, rearing, climbing, making head movements (including when the rest of the body was immobile), and any other open-ended motor behaviours of the limbs. Grooming (LIA) states were coded when a rat would engage in wet-dog shakes, face washing, digit licking, and flank grooming using either paws or the mouth. Chewing and food manipulation was also coded as an LIA state. Additionally, LIA was also coded when the rat exhibited standing immobility with eyes open, and the head up against gravity. Finally, sleep was defined as being immobile in a prone position with the head down. Although a one-to-one relationship between behavioural and hippocampal state has been previously shown (Vanderwolf, 1969), to ensure consistent classifications, we decided to classify behavioural epochs in 7 second bins. Classifying behaviour according to 7 second epochs allowed us to meaningfully categorize each behavioural state. Meaning, although we observed an immediate change in EEG upon change in behaviour, the beginning epochs of a "new" behaviour were still quite variable as the rat would still be adjusting and shifting back and forth between the previous behaviour they were engaged in. For instance: if a particular rat was grooming, then moved to exploring, they would often revert back to groom very transiently only to go back to exploring. Extending the behavioural epoch to 7 seconds ensured a clear distinction in both behaviour and HPC state and balanced out transitionary behaviours/states.

Memory Performance: In the MWM, the strength of spatial memory has been previously quantified using several different measurements: latency to platform, time spent in probe quadrant, and number of crosses in probe quadrant. While time spent in probe quadrant and number of crosses in probe quadrant are valuable measures as they provide information on how robust the memory has been encoded, we found that using our training paradigm of only four trials, latency to platform was the only consistent measure that we could extract from the probe trial.

EEG Processing and Analysis. In all cases, raw signals were first examined using LabChart (AD Instruments).

All statistical analyses were conducted in Excel or Graphpad

Results

Our study was created to evaluate what particular brain/behavioural state provided the most benefit to novel spatial memories using the one-hour delayed matching to sample (DMS) version of the circular swim maze in rats (Figure 1). Selecting our performance measure (latency) and establishing criteria for learning which ensured that our results were not confounded by non-learners or "lucky" results (Figure 2), we were then poised to investigate whether behavioural condition had any influence on episodic-like memory recollection.

As a first step, we made group-wise and within-group comparisons of these conditions. No significant differences were observed between (single factor ANOVA, $F(2,119)=2.36, p=0.09$; Figure 3A) or within conditions (repeated measures single factor ANOVA, $F(2,10)=2.24, p=0.06$; Figure 3B) for probe trial latencies. Although trends appeared to exist in the data suggesting that state might improve memory performance, this was statistically non-significant. We attributed this lack of effect due to the variation in the actual expression of behaviour during each condition.

Given that rats were unconstrained in terms of their emitted behaviour in all of our behavioural conditions administered during the delay period, we verified behavioural state based on the video recordings in order to separate proportions of time spent in each of the three states. Data for each behavioural condition is shown in Figure 4A-D. While increases in the encouraged behavioural condition was observed for all three conditions, the overlap in terms of proportions was substantial. This assessment also allowed us to ensure that encouragement of behaviour was not necessarily due to the situation or context they were placed in since the rats were able to freely behave in their cage regardless of the behavioural condition we encouraged.

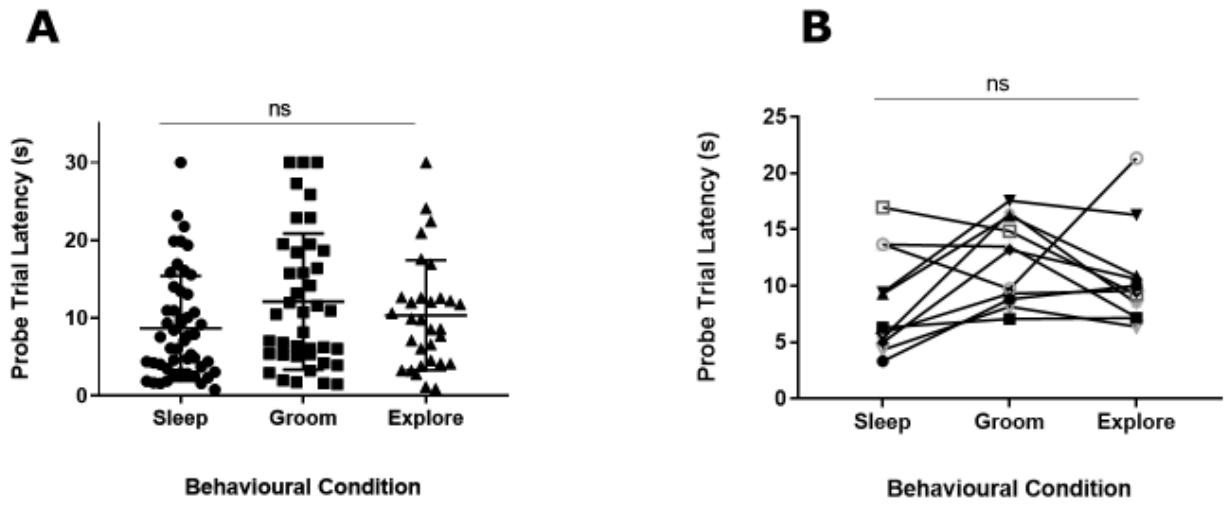

FIGURE 3: Placement in any particular behavioural condition following training did not produce any significant changes in spatial memory performance on the probe trial.

Probe trial data is plotted as a function of the behavioural condition for A) between- and B) within-groups comparisons. Although the sleep group showed a lower average latency than the other two groups (is this true for the overall averages for the within-groups comparison?), no significant differences were observed for either the between-groups comparison ($F(2,119)=2.36$, $p=0.09$) or for the within-groups comparison ($F(2,10)=2.24$, $p=0.06$) across behavioural conditions.

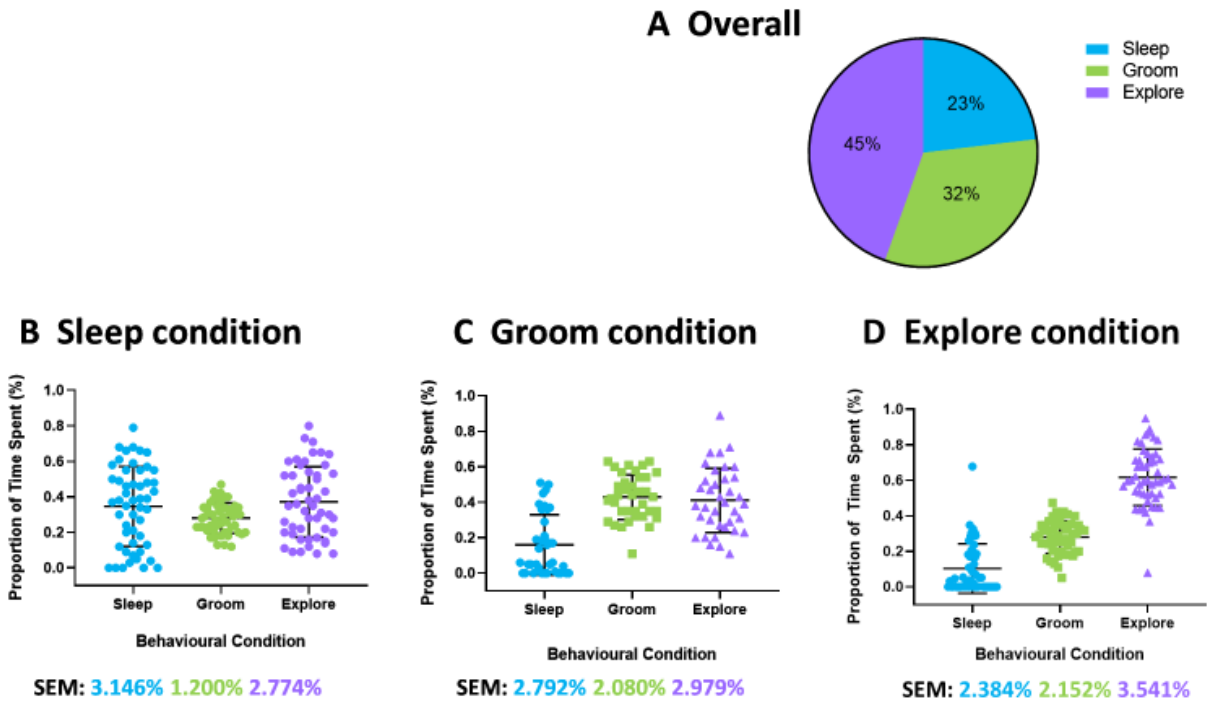

FIGURE 4: Distribution of time spent in each respective behaviour during the delay period overall and as function of behavioural condition.

The condition placement generally increased the encouraged behaviour during the delay period. **Panel A (pie chart):** Overall, the breakdown of video-coded behaviours showed a roughly equal proportion of the three behavioural classes with an ~10% advantage/disadvantage for exploring and sleeping, respectively. **Panels B-D:** During the sleep condition (B), there was a 19-26% increase in the average amount of sleep (as compared to the other conditions), whereas during the groom (C) and explore (D) conditions, there was a 12-17% and 21-25% increase in grooming and exploring, respectively. Regardless, exploring behaviour occupied the greatest proportion of each of the three conditions.

By treating the proportion of time spent in the three classes of behavioural state as a variable on which to regress memory performance, we collapsed our analysis over the conditions to examine if any one behavioural state tended to yield an advantage on probe trial latencies. A simple linear analysis showed that probe latency values significantly decreased as time spent in sleep increased (r=-0.204, p=0.04; Figure 5A). In contrast, latency values tended to increase with increases in exploration, although this relationship was not significant (r=0.17, p=0.08; Figure 5B). No significant relationship was observed between time spent in grooming (and other LIA behaviours) and probe trial latency (r=0.05, p=0.60; Figure 5C).

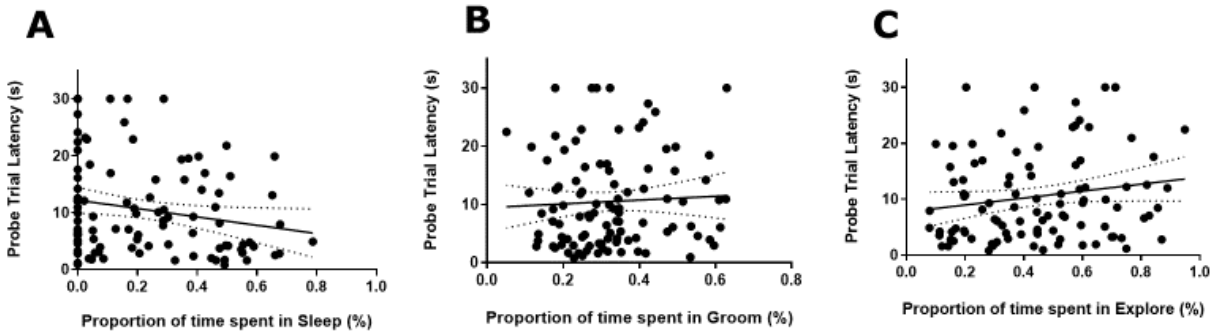

FIGURE 5: Correlation between time spent in the three main behaviours (sleeping, grooming, and exploring) during the one-hour delay following training, and memory performance on the probe trial.

Panel A: A significant negative correlation (r= -0.204, p value=0.04) between time spent in sleep during the one-hour delay and probe-trial latencies was observed, where increasing sleep corresponded to decreasing values of time to cross the platform location. This relationship was not observed for either grooming **(Panel B)** nor exploring **(Panel C).** Indeed, there was a marginal positive relationship between time spent exploring and our latency measure where greater time spent exploring led to increasing values of our latency measure. We suspect the neutral results found in grooming condition may be due to the theta-related interference during ongoing groom.

Given the significant relationship of sleep to memory performance, we created a criterion proportion to ensure that animals spent at least 40% (and thus, in most cases, a majority of the time) in any one of the three behavioural states during the delay period. In accordance with our assumptions, the distributions of time spent in each of the three behavioural conditions showed better separations for each of the states selected (ANOVA single factor repeated measures for sleep F(2,28)=72.28, p=<0.0001, groom F(2,18)=40.16, p=<0.0001, explore F(2,49)=178.5, p=<0.0001Figures 6A-C). In addition, when analysing the group-wise data for probe latencies in each state as separated by this criterion, we found significant differences between behavioural conditions (ANOVA single factor F(2,84)=3.64, p=0.03). Our Tukey's HSD post-hoc t-test identified significant differences between sleep and groom (t(28)=-2.52, p=0.04), with no significant relationship between sleep and explore (t(66)=-2.42, p=0.07), or explore and groom with no significant differences between groom and explore (t(32)=0.65, p=0.76). For the cases in which sufficient data was available across each behavioural state within animals following criterion selection, we also showed significant differences in memory performance across behavioural states (Repeated measures single factor ANOVA F(2,8)= 3.00, p=0.03; Figure 7A). Tukey's HSD post-hoc t test demonstrated significant differences between sleep and groom (t(14)=-2.83, p=0.03), and sleep and explore (t(16)=-2.52,p=0.02) conditions. No significant difference were found between groom and explore conditions (t(13)=0.92, p=0.32) Figure 7B. These results were not due to differences in swim speed as a function of behavioural condition as no significant correlation exists between our performance measure (and test of episodic-like memory, latency to platform) and swim speed (r=0.02, p=0.84) Figure 8.

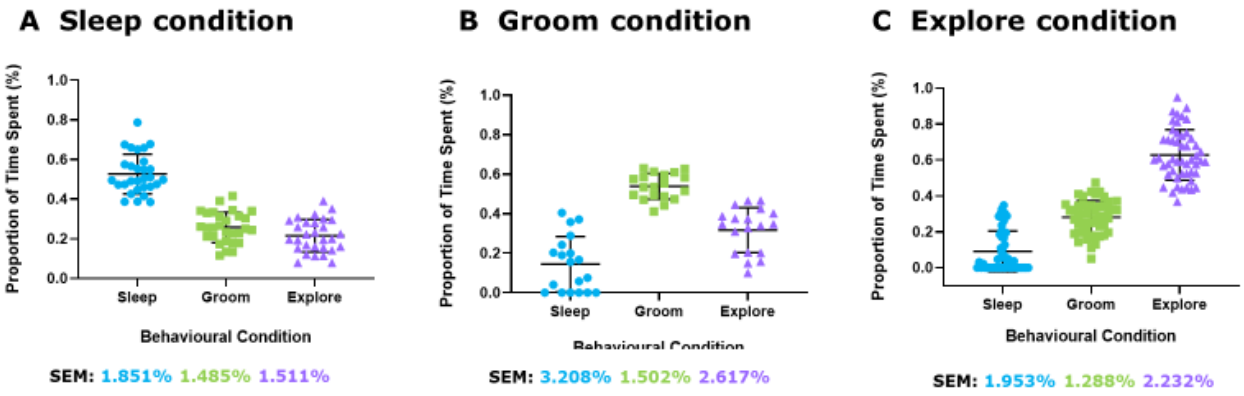

FIGURE 6: Distribution of time spent in each respective behaviour during the delay following minimum (>40%) selection criteria.

When data was selected based on a minimum of 40% for any behavioural condition, the average proportions were better distributed for the selected behaviour. In each condition, sleep **(Panel A)**, groom **(Panel B)**, or explore **(Panel C), the average proportion of time spent in those conditions actually formed the majority condition.**

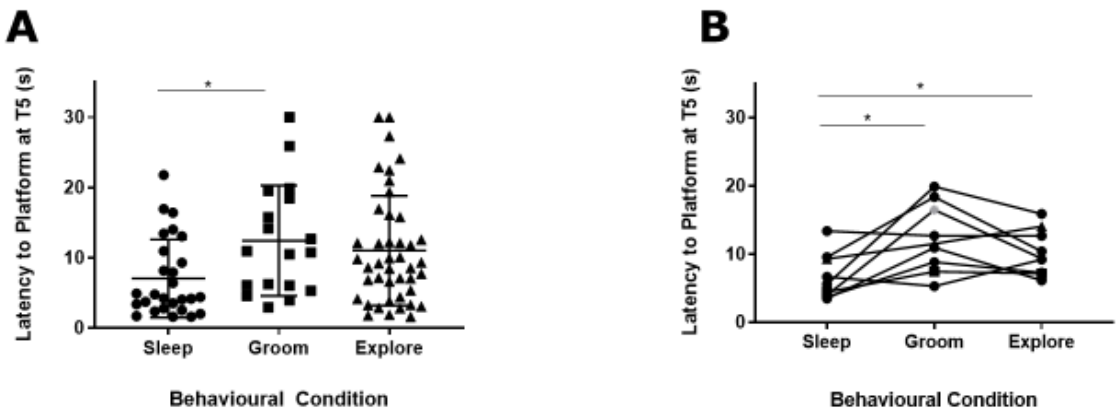

FIGURE 7: Memory performance differs across behavioural groups selected based on a minimum proportion (>40%) during delay periods.

Panel A: Probe trial latencies for each behavioural condition following minimum proportion selection. Significant differences are observed across groups (ANOVA single factor F*F*(2, 84)=3.64, *p*=0.03). Post-hoc t-test reveal significant differences between sleep and groom conditions (*t*(28)=-2.52, *p*=0.01) as well as sleep and explore conditions (t(66)=-2.42,*p*=0.02). No significant differences were found between t-test of groom and explore conditions (*t*(32)=0.65,*p*=0.52). Tukey's HSD post-hoc t test reveal significant differences between sleep and groom (*p*=0.04*)* while no significant differences were located between sleep and explore (*p*=0.07) and groom and explore (*p*=0.76)

Panel B: A within group analysis also shows a significant benefit of sleep on memory performance over both groom and explore conditions in our t-test (ANOVA single factor repeated measures *F*(2,8)=3.00 ,*p*=0.03**;** sleep versus groom: *t*(14)=-2.83,*p*=0.01; sleep versus explore: *t*(16)=-2.52,*p*=0.02) . No significant differences were observed between groom and explore conditions (*t*(13) stat=0.92,*p*=0.37) while Tukey's HSD post-hoc t-test reveals significant differences between sleep and groom (p=0.03), sleep and explore (p=0.02) and no significant differences between groom and explore (p=0.32)

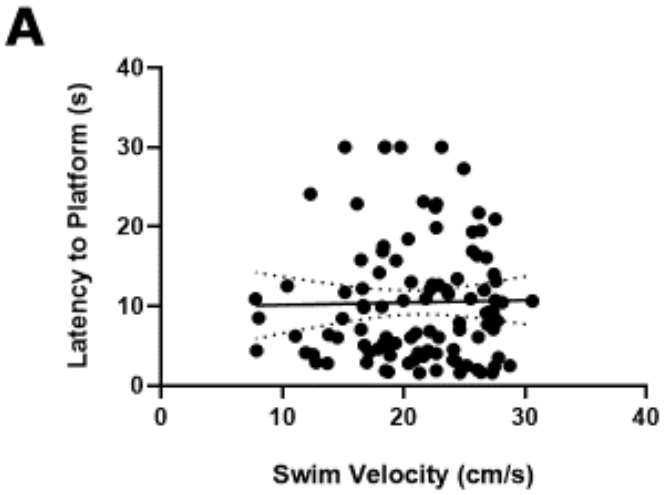

FIGURE 8: Correlation analysis between swim speed and our measure of episodic-like memory.

Panel A: To ensure that our results demonstrating sleep-related benefits to episodic-like memory was not attributed to our rats conserving energy during the one-hour delay as opposed to expending energy as observed during exploration or grooming, we ran a simple correlational analysis to determine whether any relationship existed between our performance measure, latency to platform, and swim speed. Our analysis does not show any such relationship (r= 0.02, p= 0.84). This tells us that regardless of rats expending energy through exploration or grooming or conserving energy when encouraged to sleep, has no influence on our test of episodic-like memory, and is therefore not a relevant factor.

To confirm the above findings, we performed an alternate analysis to determine how performance measures for the probe trial might predict behavioural state. We created a distribution of the entire dataset of latency values and computed the top, medium, and bottom ranges of performance by quartiles Figure 9A. Following this, we examined which behaviours showed maximal proportions during the delay period as a function of performance Figure 9B). While the numbers of rats showing higher sleep proportions tended to be evenly distributed among all levels of performance, the numbers for both groom and explore showed a systematic increase with decreasing memory performance in probe trials. These results align well with our previous level of analysis as they suggest that performance is best in those instances in which sleep dominated the delay period.

Based on these behavioural findings, we were interested in understanding what it was about sleep that appeared to provide an advantage in memory recollection of this episodic-like-like task. Though our behavioural results are compelling, we are only able to speculate about the actual state of activity of the hippocampus. Therefore, it was important that our next step allowed us to observe activity-dependent activity changes across behavioural conditions. This would allow us to determine the differences, if any, between electrophysiological states.

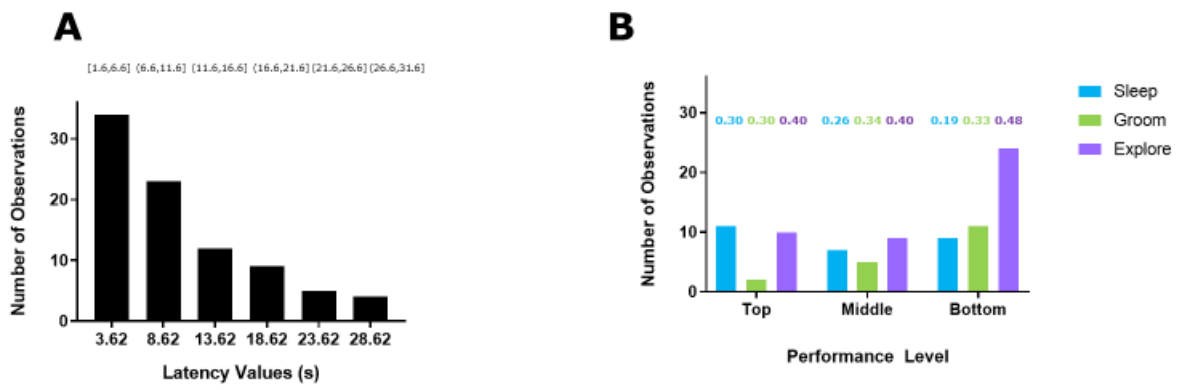

FIGURE 9: Sorting on memory performance shows that a majority of time spent sleeping, over both grooming and exploring, is associated with ameliorated performance scales.

Panel A: A histogram outlining the latency distribution, separated by 5s bins, of all rats during the probe trial reveals a Poisson shape. The skew as observed in this distribution suggests that rats who were included in the analysis had a greater tendency to fall within lower latency values at probe trials. This can be interpreted as a learning affect, since, rats who have been included in this analysis take less time to reach the location of the platform regardless of whether the platform is present or not. Therefore, any differences in performance could be attributed to behavioural condition during the one-hour delay. Panel A inset describes the range of values for each of the bins. **Panel B** sheds more light into whether there are any observable differences in latency distributions between groups. Data was organized by the top (1.6-4.08 s), medium (4.08-8.16), and bottom (8.16+) quartiles. Sleep and explore appear to be tied with respect to top performance level, however, a mirrored stepwise increase from top to bottom performance is observed in both groom and explore. This pattern is not observed in sleep. In fact, it would appear that sleep performance within the three levels of performance remains relatively consistent. Inset values signify proportion of time spent in each state as a function of performance level.

Discussion

What we have demonstrated in this study is that behavioural state, in the period directly following a novel spatial learning episode, appears to be an important component for the future retrieval of this information. We separated behaviours into three main categories according to the presumed electrographic state of the hippocampus, a structure upon which this type of memory is dependent: 1) ambulation, which is correlated with the production of theta rhythm, 2) grooming/immobility, which is correlated with the production of LIA and sharp-wave ripples, and finally 3) sleep, which is correlated with the production of slow oscillations (Buzsaki, 2015; Vanderwolf, 1969; Wolansky et al., 2006). We found that the proportion of time spent sleeping during the hour-long delay between training and testing correlated with enhanced probe trial performance and that the average performance of rats spending at least 40% of their time in sleep was the best of any group. In contrast, the proportion of time spent grooming seemed to have no benefit for spatial memory performance (and perhaps even shows a grooming-related disadvantage), and ambulation appeared to have a negative, albeit non-significant, influence on this measure (based on our regression analysis). This suggests that state, and perhaps slow oscillatory activity in the hippocampus may be an important aspect of activity-dependent solidification of novel episodic-like-like learning.

Sleep and memory benefit:

Sleep-derived benefits to the consolidation of hippocampal-dependent information has long been demonstrated by previous research. Studies comparing sleep vs wake groups have consistently identified benefit to memory after a bout of sleep (Rasch & Born, 2013). Alternatively, sleep deprivation studies have shown how a lack of sleep may disturb the solidification of information (Areal, Warby, & Mongrain, 2017). These and other studies demonstrating the influence sleep has on the process of memory solidification would

suggest that there is something particularly critical happening during sleep that provides these benefits. One such way could be due to the activity that occurs during each sleep stage. Another way could be due to the behaviour of sleep itself.

Differentiated effects on hippocampal-dependent memory consolidation based on sleep state:

Sleep is categorized between two overarching states: REM and NREM sleep. NREM sleep is further subdivided into 3 different stages, stage 3 often being referred to as slow wave sleep (SWS) (Stickgold, 2005). Studies have demonstrated differentiated affects to declarative memory based on these differing states.

REM sleep effects on HPC-dependent memory consolidation

Researchers have shown an increase in REM activity after learning non-declarative forms of information such as mirror-tracing in humans (Marshall & Born, 2007). REM sleep has also been shown to provide benefit to hippocampal-dependent forms of memory, yet with less consistency (Ackermann & Rasch, 2014). For instance: Boyce et al (2016) showed a REM-dependent effect of a novel object place recognition task and contextual fear conditioning. By optogenetically silencing REM activity during sleep behaviour in the mouse model, dysfunctional expressions of memory recollection on both hippocampal-dependent tasks were revealed suggesting REM related dependence on these tasks (Boyce et al., 2016). On the other hand, however, studies like Bunce et al (2004) tell us a different story. They show that intraseptal infusions of carbachol (which induces hippocampal theta) post-learning produces amnestic affects for retroactive, but not proactive, decisions in the delayed non-match to sample (DNMS) radial arm maze task (Bunce, Sabolek, & Chrobak, 2004). These findings are similar to what we have found in our study where we have demonstrated theta-related hindrance after acquisition in the DMS circular water maze task.

NREM effects on hippocampal-dependent memory consolidation

It has previously been shown that online learning experiences of explicit forms of information has been linked to increases in activity-dependent processes such as the slow oscillation and SPW-Rs during SWS. In addition, other studies have shown that age-related decline of SWS is correlated with reduced retention of word-pairs in older versus younger adults. A similar decline or interruption of REM sleep, interestingly, does not appear to reproduce such deficits (Backhaus et al., 2007). On the whole, when comparing the observed benefits that either REM vs NREM sleep stage provides to HPC-dependant types of information, NREM sleep has historically been more consistent in providing these benefits as opposed to REM. Add this to the possible explore/grooming (theta) -related hindrance we have observed in our own study, and we are inclined to suggest that the sleep-derived benefit we have reported has been due to NREM sleep (SO) as opposed to REM sleep. These benefits could be derived from the diverse range of neural activity that occurs in each stage of NREM sleep.

The slow oscillation during NREM Sleep

A possible candidate that may help explain the hippocampal dependent memory enhancement observed in post learning sleep could be due to the activity-dependent processes that occur during SWS specifically (Marshall & Born, 2007). These processes during sleep may be playing a role in the strengthening and subsequent solidification of information. For instance: induction of 1Hz frequency stimulation (activity that dominates SWS) in neocortical regions (Steriade et al., 1993b), has been shown to provide a boost in memory performance upon retesting in humans (Marshall et al., 2006). It is suggested that this specific slow activity temporally groups transient fast events such as thalamocortical spindles and hippocampal SPWRs (Marshall & Born, 2007), providing a basis unto which synaptic plasticity can occur. This grouping of fast activity , facilitated by the SO, could be

the mechanism unto which coordination between the neocortex and the HPC can redistribute information (Born et al., 2006). Further evidence pointing to the importance of slow activity to memory is described in the initial characterizations of the HPC SO by Wolansky et al (2006) who also suggest that the HPC SO may play a pivotal role in solidifying information between these two structures (Wolansky et al., 2006).

NREM sleep reactivations

Reactivations of online experiences during SWS have also been found to provide a benefit to memory. Studies have shown that navigating through a virtual town is assisted after a bout of SWS due to presumed reactivation of these experiences during offline behaviours (Peigneux et al., 2004). Reactivation both during SWS and immobility have been shown to contribute to memory consolidation (Buzsaki, 2015). Combined, sharp-waves and ripples, in addition to thalamocortical spindles, have received a significant amount of attention due to the content, or rather information, they seem to carry and the speculated role they play with respect to influencing synaptic efficacy (Foster, 2017; Kim, Pardilla-Delgado, & Alger, 2017). Studies have demonstrated that ripple events replay and preplay wake experience during periods of groom, rest, and sleep (Buzsaki, 1989; Vanderwolf, 1969). It has been shown that the probability of cell A, for instance, taking part in an SPW-R event increases if cell A was also active during wake experience (ie during ambulation) (Buzsaki et al., 1983; Buszaki 2015). SPW-Rs importance is further punctuated by studies demonstrating the affect on memory upon perturbation of these events suggesting that they have behavioural relevance (Girardeau, Benchenane, Wiener, Buzsaki, & Zugaro, 2009; Girardeau, Cei, & Zugaro, 2014; Tang, Shin, Frank, & Jadhav, 2017).

Although we have extensively discussed activity-dependent process during NREM sleep that we think may be helping with the solidification of information, some researchers suggest that the sleep-derived benefit that are so often observed in the literature are not necessarily

to do with the activity that occurs during NREM, but rather, has more to do with sleep behaviour itself.

Can the behaviour of sleep itself be contributing to the sleep-derived benefit to memory?

The interference theory is one theory, among several others, that are used to help explain the benefits to memory consolidation after a bout of sleep. This theory rests on the idea that the benefits of sleep to memory are largely due to the reduction, or altogether elimination of incoming sensory information. This reduction of interference allows for endogenous activity to solidify information of online experiences sans intrusion of external stimuli (Ezenwanne, 2011). The difficulty with this interpretation is that studies have shown that the fate of external information processing, especially during offline behaviour, is actually dependent on ongoing native brain activity, suggesting then, that that the presence of external stimuli is not exerting bottom-up control on brain activity, but rather, ongoing endogenous activity is influencing the processing of incoming information (Schabus et al., 2012). Thus, in this manner of speaking, internally generated activity is actually providing a template upon which a representation is being evaluated upon. If this is the case, then in a situation where both external and internal (i.e., rehearsing information) stimuli are limited during wake to match reduction of stimuli found during sleep, we would still find that sleep itself provides the benefit to memory comparatively. Indeed, it has been found that in cases where both external and internal stimuli are reduced in wake, quiescence, and sleep; sleep still provides the most benefit to memory as opposed to the other groups (Schönauer, Köck, Pawlizki, & Gais, 2014). Furthermore, the interference theory assumes brain activity to be in a passive state during sleep when several studies have shown that this is not the case (Rasch & Born, 2013). Parsing out behaviour from brain activity is an important endeavour, especially since previous literature, in addition to our study, has demonstrated a correlation between behaviour and brain state. Here, we have provided reasons as to why we believe sleep behaviour itself may not be the contributing factor in providing the benefit to memory,

however, we are tempted to ask whether the behavioural conditions we used to encourage specific HPC states in our study; somehow influenced or became a variable to consider in our study.

Investigating the influence of our behavioural conditions on the consolidation of declarative memory:

Given the correspondence of behaviour to brain state, especially in the hippocampus (Colgin, 2016; Foster & Wilson, 2006; Vanderwolf, Kramis, & Robinson, 1977) we set up our study to utilize behaviour as a way to manipulate changes in brain state during the delay period. Our assumption is that it is the brain state, and not the behavioural state that is important. Of course, we have no way of separating these two potential influences from each other. However, previous studies have demonstrated the concomitant activation of differentiated patterns of population activity upon initiation of specific behavioural classes: ambulation (theta activity), groom (LIA and SPWR), and SWS sleep (slow activity and SPWR) (Buzsaki et al., 1983; Vanderwolf, 1969). It has also been shown that these patterns of population activity facilitate the memory consolidation process. For instance, inactivating the ongoing theta rhythm during online behaviour results in retrieval deficits in episodic-like-like tasks (Eichenbaum et al., 1990; Hasselmo et al., 2002). Inhibiting SWP-Rs and slow activity have also demonstrated dysfunctional behavioural expressions of memory recollection after a delay (Girardeau et al., 2009). These studies, however, show how brain activity may contribute to memory solidification and not behaviour itself.

Parsing out behaviour from brain activity

One way to begin to think about differentiating the effects of behaviour itself onto brain activity is to think about ethological behaviour and a naturally ordered sequence of behaviour (i.e: food consumption followed by grooming followed by drinking water etc.). Since these species-specific action patterns, do not require overt planning *per se,* we can think about any associated brain activity arising from the initiation of these behaviours as facilitative to the ongoing sequence of behavioural elements (Nevin, 1973). Indeed, studies

have demonstrated a modulation of ongoing EEG activity during ethological behavioural transitions, which is the interval phase between two behaviours (preceding and following; forming a sequence of behaviours). For instance, theta activity appears to increase in power during a behavioural transition when a preceding behaviour is about to be followed by a type 1 theta behaviour(van Lier, Coenen, & Drinkenburg, 2003). Here again, however, we can argue that the ramping up in power suggests a level of top-down control in behavioural planning implicating influence based on activity and no so much behaviour.

A more concrete way to parse out the differences in contributions of behaviour to hippocampal-dependent memory would be to restrict overt behaviour of a rat in an open field while mimicking spontaneous brain activity that would otherwise naturally occur using stimulation techniques. This can then be followed by testing whether any cognitive deficits arise as a result of behaviour restriction. Our first step would be to gather baseline data of both behaviour and brain activity. This would consist of training on a hippocampal-dependent task followed by a delay which would then be followed by a probe/test phase. During this delay, a rat will be placed in an open field and be free to explore and engage in naturally-occurring behaviours. Our second step would then be to test specifically how behaviour itself may or may not contribute to memory recollection of episodic-like information. Testing in the hippocampal-dependent task as trained during our first step should be followed by a delay in the same open field environment as in step 1. However, this time, behaviour would be constricted during this delay. Instead, we would mimic brain activity observed during baseline using stimulation techniques. In the other words, while we constrain behaviour, we woulde mimick EEG activity observed during preliminary training (step one). Retesting on the memory task should then follow this delay. Any differences between control and experimental conditions can be attributed to the reduction or altogether elimination of overt behaviour. The influence on context can also be manipulated

to determine how behaviour, EEG, and context may play a role in encoding and subsequent recollection of information.

Caveats

In this study, we have demonstrated a sleep- (likely NREM) dependent memory enhancement of episodic-like-like information in the rat model. We suspect this benefit to be NREM-dependent because it has been shown that SWS dominates the first portion of a sleep period (Marshall & Born, 2007). Since our animals only had a one-hour delay and would often explore for the first 30 minutes, we presume that the rats in our study spent most of their time in SWS when they did eventually engage in sleep behaviours. Furthermore, the potential NREM-dependent memory enhancement that we observed in our study is further punctuated by the complete opposite affects we have observed related to ambulatory (theta) activity. We can therefore assume that theta activity during REM would likely not have contributed to the memory enhancement benefit.

Though we have observed a theta-related behavioural hinderance, it is important to acknowledge the many studies that have shown the importance of ongoing theta to online choice behaviours and working memory. (Belchior, Lopes-Dos-Santos, Tort, & Ribeiro, 2014; Beulen, 2011; Frank et al., 2000). We are inclined to wonder whether these inconsistent results may be due to the difference in the type of decisions being made for these tasks, or, perhaps a difference in the behaviour itself. Parsing out how and in what circumstances theta seems to help in this manner would be beneficial.

Speaking of behaviour, we have also noted that our initial hypothesis of separating behaviour by overt terms rather than by EEG activity may not have been appropriate since explore and grooming behaviour exhibited quite similar EEG patterns despite being defined in terms of different behavioural classes. This finding has fundamental implications to ours and other studies using behaviour as a proxy to represent differentiated patterns of neural activity and requires redefining behavioural classes. We are inclined to describe the poor

performance rates as theta-related for both explore and groom conditions because of the persistent presence of theta activity during sequences of grooming itself. We suspect that perhaps our groom behaviour regression analysis showed a non-consequential result because of this theta-interference during ongoing grooming behaviour.

Conclusion

Previous literature has demonstrated the importance of both time and activity in helping to consolidate memory. In this study, we have elaborated upon these requirements and are suggesting that, in addition to time and activity; brain and behavioural state also plays an important role in solidifying episodic-like information. We have demonstrated significant between and within group differences in episodic-like memory recollection as a function of hippocampal state/behavioural condition. Due to the results we have identified in our study, we are not only able to begin the task in parsing out the contributions of these distinct hippocampal states to episodic-like information but also recognize that brain state, at all levels of learning and memory, will have a critical impact in how information, and whether information, is acquired and subsequently solidified. The literature has demonstrated the unique neurochemical and EEG signatures that are associated with the HPC states we have investigated in this study (theta, LIA, and SO), some of which we have discussed above. Based on our results, it may be a fair statement to suggest that these unique environments that are associated with and make up these unique HPC states, can either hinder or facilitate the acquisition and storage of episodic-like information. Identifying the precise mechanisms nested within each of these states that dis/encourage weakening or strengthening of episodic-like information is fundamental to future studies related to the consolidation of episodic-like memory.

Chapter 3: Conclusion/Discussion

What is memory consolidation?

Memory consolidation is a broad term meant to describe and explain the cellular and systems level changes that take place shortly after a learning event. While cellular consolidation refers to the initial processes required to strengthen local synaptic networks over a period of hours to days, systems consolidation is thought to occur over weeks to even years and involves the stabilization and reorganization of information in a likely different form that is dependent on multiple structures (Rudy & Sutherland, 2008). The process of consolidation takes place in two steps: the first step involves learning information (acquisition) which was thought to be facilitated by the HPC. During this time, information is labile and subject to interference (specifically sensitive to damage to the HPC; cellular consolidation). The second step involves the reorganization of this information across the HPC to sites likely in the nCTX. Here, the information is thought to be less prone to interference and is more robust against hippocampal trauma (systems level consolidation) (Dudai, Karni, & Born, 2015).

Where does the term memory consolidation come from?

The concept of memory consolidation was initially brought to the forefront by Müller and Pilzecker who noted retroactive interference in humans while studying the behavioural concepts of learning and memory. It appeared that information was subject to interference for a period of time after learning suggesting that recently learned information is fragile (Lechner et al., 1999). This idea was already supported by the work of Théodule Ribot who observed that recent memories were more vulnerable to trauma than remote ones (retrograde amnesia), an insight he developed while working with memory-impaired patients (Ribot, 1882). In fact, much of what we know about memory has largely been due to studies investigating patients who suffered from amnesia. One such patient was H.M.

Insights from memory impaired patients: Patient H.M.

Patient H.M played a critical role in how we understand memory and memory consolidation today. His amnesia showed researchers the intricacies of what it meant to live with memory loss underpinned by not only a distinct temporal differentiation (Retrograde and Anterograde amnesia) of memory but also demonstrated how the loss of MTL structures would affect the storage of specific types of information along this temporal scale (Scoville & Milner, 1957). Researchers found that Patient H.M still had the ability to learn and store new non-declarative types of information in addition to retaining the capacity for semantic memory (Milner, Corkin, & Teuber, 1968). Therefore, upon closer inspection, it was determined that H.M did not necessarily suffer from a generalized loss of declarative memory, as initially thought, but rather, suffered from an extensive, temporally graded, episodic-like-specific deficiency (Corkin, 2002). This delineation between memory types observed with patient H.M was an important one as it provided researchers an indication as to where episodic-like information may reside.

Parsing out episodic-like memory from declarative memory

Episodic-like memory is often lumped into the general category of declarative types of information, however, its content is worthy of its own distinction. It is founded at the intersection of the spatial-temporal axis and allows us to generate experiences within the context of a whole event (Tulving, 2004). Loss of this function results in the elimination of recalling events in rich detail, significant or altogether loss in mental imagery/time travel, and reduced cognitive flexibility (i.e. loss of being able to navigate a new route in a familiar place) (Ferbinteanu, Kennedy, & Shapiro, 2006).

Rosenbaum et al (2004) show that amnesic patients with damage to their HPC are unable to imagine future scenarios; a key feature of episodic-like memory (R. Shayna Rosenbaum, McKinnon, Levine, & Moscovitch, 2004). They show that patients are unable to generate and

subsequently verbalize a whole event; they are only able to imagine fragments of it (R. S. Rosenbaum et al., 2005; R. Shayna Rosenbaum et al., 2004). Animal studies also reveal a similar pattern. Tasks that require relational contexts such as allocentric wayfinding appear to require an intact HPC. When rats are required to locate a platform in a circular water maze after experiencing an HPC lesion, they are unable to do so even if they adequately learned the task prior to the trauma (Eichenbaum, 2011; Nadel, Winocur, Ryan, Moscovitch, & Morris, 2007). Due to these results, it is suggested that the HPC may be providing the spatial context unto which these disparate elements can be bound together into a whole event (Hassabis, Kumaran, Vann, & Maguire, 2007). How precisely this is accomplished in the HPC is worth reviewing.

Activity mechanisms behind episodic-like memory consolidation:

Seminal studies conducted by Bliss and Lømo provided fundamental insight into how these disparate elements may be encoded in the first place. They were able to show, by building on the important work of Santiago Ramon Y Cajal and Charles Sherrington, that synaptic connections were able to strengthen under specific conditions of stimulation (tetanus; aka: long term potentiation, LTP) (Bliss & Lomo, 1973; Levine, 2007; Lopez-Munoz et al., 2006). The onset of modulating synaptic efficacy in this way is first marked by the activation of ionotropic receptor AMPA and occurs almost immediately (Mayer et al., 1984). The activation of AMPA receptors is critical for these initial stages unto which cellular consolidation can occur, and, perhaps, eventual systems consolidation (Kessels & Malinow, 2009).

LTP has been an important discovery to memory research as it provides a neural basis for how learning and memory might happen in the brain. Studies have shown how learning and LTP often affect and modulate one another, and at times, even compete for the same resources suggesting that the process of learning overlaps with LTP mechanisms, thereby

demonstrating similarity between the two constructs (Ishihara, Mitsuno, Ishikawa, & Sasa, 1997; Kentros et al., 1998; Morris, 2003). Furthermore, the properties upon which LTP seems to work within (specificity, cooperativity, and associativity) reflect the requirements needed in order to generate mnemonic memories; useful for the production of episodic-like information (Bliss & Lomo, 1973; Eichenbaum, 2011). The discovery of place cells in the dorsal CA1 region of the HPC provided a basis into how precisely single unit activity may be contributing to the formation and maintenance of episodic-like information with a spatial emphasis (O'Keefe, 1976; O'Keefe et al., 1998; O'Keefe & Dostrovsky, 1971).

Place cells are thought to be a critical building block in the formation of the cognitive map Edward Tolman initially envisioned. This cognitive map is thought to facilitate the ongoing, mnemonic navigation and planning of trajectories in the rat and perhaps the human model (O'Keefe & Nadel, 1978; Tolman, 1948). An elaboration on this research would reveal that not only are there place cells that fire action potentials based on self-location, but that there are also cells that show differentiated activation based on the episode/time of an event (Eichenbaum, 2014; Pastalkova et al., 2008; Salz et al., 2016). Interestingly, place cells have also been found to promote synaptic plasticity as their activity is often joined by an ongoing theta rhythm (Frank et al., 2000). Thus, associated mesoscopic events observed within the HPC are thought to facilitate the organization, de/potentiation, and solidification of this spatial and temporal information along varying time scales (Buzsaki, 2006; O'Keefe & Recce, 1993).

We have shown in this study, in addition to studies previous to ours, specific HPC states that can be encouraged vis a vis behaviour: theta activity appears while a rat is involved in active/goal-driven behaviours such as running, swimming, walking, jumping, rearing. LIA activity along with SPWRs dominate immobility, grooming, and drinking behaviours (Buzsaki et al., 1983). We also observe these differentiated patterns of neural activity during offline,

sleep behaviour (REM and NREM), with the added observation of the slow oscillation which occurs during SWS, a subset of NREM sleep (Wolansky et al., 2006).

As the current evidence points to the HPC playing a role in the binding of episodic-like elements into one cohesive hole, we were interested in determining how the overlapping presence of these HPC states may each be contributing to the consolidation of episodic-like memory.

Activity during sleep provides benefit to episodic-like memory:

We have demonstrated in our study that sleep provides the most benefit to the consolidation of episodic-like information in the rat model. We suspect that the benefit was SWS dominated as opposed to REM sleep for two reasons: 1) it has been shown that the first half of a sleep period is mostly dominated by SWS activity (Marshall & Born, 2007) and 2) we have identified, in our study, a theta-related hinderance to memory solidification of episodic-like information after a delay. Since REM sleep is mostly dominated by theta activity, we are therefore inclined to suggest that NREM state of sleep, as opposed to REM, contributed to the improved performance. Our sleep-related benefits to memory also aligns with previous research tying the importance of NREM sleep to the consolidation of episodic-like information. As we have discussed previously, NREM sleep has shown, more consistently, a benefit to declarative forms of information as compared to REM sleep (Rasch & Born, 2013) suggesting that different types of information may require specific types of brain activity and consequently, may undergo different consolidation processes (i.e. HPC-independent versus HPC-dependent).

Sleep and Memory:

Sleep-related benefits to memory have long been tied together. As discussed in a previous chapter, researchers have shown the detrimental affects on memory when there exists a lack of sleep. While on the other hand, the presence of sleep allows for the enhancement of

memory recollection (Marshall & Born, 2007). Dysfunctions of memory recollection in terms of particular memory types are also demonstrated upon disruption of specific activity during ongoing NREM sleep (Van Der Werf, Altena, Vis, Koene, & Van Someren, 2011). We suspect that the diverse range of activity observed during the three stages of NREM sleep may be facilitating the solidification of this episodic-like information (Naji, Krishnan, McDevitt, Bazhenov, & Mednick, 2018). It is suggested that the nCTX SO observed during NREM sleep, groups fast, transient activity such as thalamocortical spindles and HPC SPWR (Steriade, 1997). This endogenously generated activity is thought to originate in cortical structures but has also been meticulously characterized within the HPC by Wolansky et al, suggesting that this slow oscillatory activity holds functional importance, and perhaps, importance to the protection of episodic-like information across these two structures (Dickson, 2010; Wolansky et al., 2006). We suspect that, while ripple activity may provide the required recapitulation of activity to strengthen synaptic connections, as postulated by Hebb (Buzsaki, 2015; Hebb, 1949), both the HPC and nCTX SO may be providing the ideal temporal environment to facilitate the solidification of information across a longer time scale (Born & Feld, 2012).

While our study only allows us to speak in terms of correlation, we have been able to parse out activity within the HPC that may be of critical benefit to the solidification of episodic-like information within the framework of cellular consolidation. Here, we have used behaviour to encourage differentiated patterns of activity in the HPC in order to parse out the contributions of specific activity to the consolidation of episodic-like memory. We have shown that sleep behaviour appears to provide the most benefit to the solidification of episodic-like information in addition to a theta-related hinderance over a one-hour delay. It is important to note, however, that although we have identified a sleep-derived benefit to episodic-like memory solidification, it would be naive of us to credit only sleep behaviour for this improved performance. Theta activity must have been present during swimming

behaviour in the water maze, while grooming behaviour occurred during the inter-trial intervals possibly encouraging LIA and SPWRs. If these behaviours were then followed by sleep, specifically, SWS, we would observe a performance enhancement, according to the results of our study. Thus, the solidification of episodic-like information may be a consequence of combined behaviours/states working together in a specific sequential order as opposed to just one state. How precisely each of these states contribute to memory consolidation and whether a change in the sequence of behaviour would modify our results is beyond the scope of this work, however, would be an interesting topic for future research within our experimental paradigm. Another fruitful avenue would be to investigate state-dependent learning. We have described the implementation of a learning criteria to ensure that our final results did not contain any learning confounds. Due to this, we have collected EEG samples from rats who have both learned and not learned the task. This would be a fruitful opportunity to determine how (and if) there are any differences in hippocampal activity between learners and non-learners.

Final Summary/Conclusions

Early investigations into memory demonstrated that the stabilization of information appeared to rely on two major components: time and (repetitive) activity (Ebbinghaus, 2013; Lechner et al., 1999). Shortages of, or disturbances in, both time and brain activity resulted in deficient expressions of memory. A dysfunction in time appears to manifest itself into differentiated retention of information defined along a temporal spectrum (i.e., short term vs long-term memory) (Corkin, 2002; Squire, 2009). Other dysfunctional expressions of memory resulting from disruptions of repetitive activity result in reduced recollection of explicit forms of information (Girardeau et al., 2009). In light of the results of this study, we would also like to suggest that in addition to time and activity, state also plays an important role in the consolidation of episodic-like information. Because we were able to observe significant between group differences as a function of state/condition, we are inclined to

believe that the specific neurochemical and electrophysiological environment associated with each state can either encourage or discourage the solidification of episodic-like information. Since the functions of episodic-like memory allows us to recall rich details of events that are of significance to our lives, imagine futures and adapt to changing circumstances (cognitive flexibility), understanding how this information is solidified, and, more specifically, how it is influenced by HPC state, is of great benefit.

References

Ackermann, S., & Rasch, B. (2014). Differential effects of non-REM and REM sleep on memory consolidation? *Curr Neurol Neurosci Rep, 14*(2), 430. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24395522. doi:10.1007/s11910-013-0430-8

Algeo, Barnhart, & Steinmetz. (1989). *The Barnhart Dictionary of Etymology* (Vol. 65).

Areal, C. C., Warby, S. C., & Mongrain, V. (2017). Sleep loss and structural plasticity. *Curr Opin Neurobiol, 44*, 1-7. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28109973. doi:10.1016/j.conb.2016.12.010

Augustyn, Bauer, Duignan, Eldridge, Gregersen, Luebering, . . . Zelazko. (2017). In *Encyclopædia Britannica, inc*.

Backhaus, J., Born, J., Hoeckesfeld, R., Fokuhl, S., Hohagen, F., & Junghanns, K. (2007). Midlife decline in declarative memory consolidation is correlated with a decline in slow wave sleep. *Learning & Memory, 14*(5), 336-341. Retrieved from <Go to ISI>://WOS:000246148600003. doi:10.1101/lm.470507

Bartram, J., Kahn, M. C., Tuohy, S., Paulsen, O., Wilson, T., & Mann, E. O. (2017). Cortical Up states induce the selective weakening of subthreshold synaptic inputs. *Nat Commun, 8*(1), 665. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28939859. doi:10.1038/s41467-017-00748-5

Bear, M. F., & Abraham, W. C. (1996). Long-Term Depression in Hippocampus. *Annual Review of Neuroscience, 19*(1), 437-462. Retrieved from https://www.annualreviews.org/doi/abs/10.1146/annurev.ne.19.030196.002253. doi:10.1146/annurev.ne.19.030196.002253

Belchior, H., Lopes-Dos-Santos, V., Tort, A. B. L., & Ribeiro, S. (2014). Increase in hippocampal theta oscillations during spatial decision making. *Hippocampus, 24*(6), 693-702. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24520011

https://www.ncbi.nlm.nih.gov/pmc/PMC4229028/. doi:10.1002/hipo.22260

Beulen, M. (2011). *The role of theta oscillations in memory and decision making.*

Bi, G. Q., & Poo, M. M. (1998). Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J Neurosci, 18*(24), 10464-10472.

Bland, B. H. (1986). The physiology and pharmacology of hippocampal formation theta rhythms. *Prog Neurobiol, 26*(1), 1-54.

Bland, B. H., & Colom, L. V. (1993). Extrinsic and intrinsic properties underlying oscillation and synchrony in limbic cortex. *Prog Neurobiol, 41*(2), 157-208.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol, 232*(2), 331-356. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4727084.

Born, J., & Feld, G. B. (2012). Sleep to upscale, sleep to downscale: balancing homeostasis and plasticity. *Neuron, 75*(6), 933-935. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22998858. doi:10.1016/j.neuron.2012.09.007

Born, J., Rasch, B., & Gais, S. (2006). Sleep to remember. *Neuroscientist, 12*(5), 410-424. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16957003. doi:10.1177/1073858406292647

Boyce, R., Glasgow, S. D., Williams, S., & Adamantidis, A. (2016). Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science, 352*(6287), 812-816. doi:10.1126/science.aad5252

Braunewell, K. H., & Manahan-Vaughan, D. (2001). Long-term depression: a cellular basis for learning? *Rev Neurosci, 12*(2), 121-140.
Bunce, J. G., Sabolek, H. R., & Chrobak, J. J. (2004). Intraseptal infusion of the cholinergic agonist carbachol impairs delayed-non-match-to-sample radial arm maze performance in the rat. *Hippocampus, 14*(4), 450-459. doi:10.1002/hipo.10200
Buxton, Bolle, & Smith. (2017). In *Encyclopædia Britannica, inc*.
Buzsaki. (1989). Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience, 31*(3), 551-570.
Buzsaki. (2002). Theta oscillations in the hippocampus. *Neuron, 33*(3), 325-340.
Buzsaki. (2006). *Rhythms of the Brain*. New York: Oxfor University Press.
Buzsaki. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus, 25*(10), 1073-1188. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26135716. doi:10.1002/hipo.22488
Buzsaki, Leung, & Vanderwolf. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Res, 287*(2), 139-171.
Carr, M. F., Jadhav, S. P., & Frank, L. M. (2011). Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nat Neurosci, 14*(2), 147-153. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21270783. doi:10.1038/nn.2732
Colgin. (2016). Rhythms of the hippocampal network. *Nat Rev Neurosci, 17*(4), 239-249. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26961163. doi:10.1038/nrn.2016.21
Corkin, S. (2002). What's new with the amnesic patient H.M.? *Nat Rev Neurosci, 3*(2), 153-160. doi:10.1038/nrn726
D'Hooge, R., & De Deyn, P. P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Res Brain Res Rev, 36*(1), 60-90. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11516773.
Dickson. (2010). Ups and downs in the hippocampus: the influence of oscillatory sleep states on "neuroplasticity" at different time scales. *Behav Brain Res, 214*(1), 35-41. doi:10.1016/j.bbr.2010.04.002
Diekelmann, S., Biggel, S., Rasch, B., & Born, J. (2012). Offline consolidation of memory varies with time in slow wave sleep and can be accelerated by cuing memory reactivations. *Neurobiol Learn Mem, 98*(2), 103-111. doi:10.1016/j.nlm.2012.07.002
Dudai, Y., Karni, A., & Born, J. (2015). The Consolidation and Transformation of Memory. *Neuron, 88*(1), 20-32. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26447570. doi:10.1016/j.neuron.2015.09.004
Dudek, S. M., & Bear, M. F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *89*(10), 4363-4367. Retrieved from https://www.pnas.org/content/pnas/89/10/4363.full.pdf. doi:10.1073/pnas.89.10.4363 %J Proceedings of the National Academy of Sciences
Ebbinghaus. (1913). Memory: a contribution to experimental psychology. *Ann Neurosci, 20*(4), 155-156. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25206041. doi:10.5214/ans.0972.7531.200408
Ebbinghaus. (2013). Memory: a contribution to experimental psychology. *Annals of neurosciences, 20*(4), 155-156. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25206041

https://www.ncbi.nlm.nih.gov/pmc/PMC4117135/. doi:10.5214/ans.0972.7531.200408
Eichenbaum. (2011). *The Cognitive Neuroscience of Memory: an Introduction*: Oxford University Press.

Eichenbaum. (2014). Time cells in the hippocampus: a new dimension for mapping memories. *Nat Rev Neurosci, 15*(11), 732-744. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25269553. doi:10.1038/nrn3827

Eichenbaum, Stewart, & Morris. (1990). Hippocampal representation in place learning. *J Neurosci, 10*(11), 3531-3542.

Etkin, A., Alarcón, J. M., Weisberg, S. P., Touzani, K., Huang, Y. Y., Nordheim, A., & Kandel, E. R. (2006). A role in learning for SRF: deletion in the adult forebrain disrupts LTD and the formation of an immediate memory of a novel context. *Neuron, 50*(1), 127-143.

Ezenwanne, E. (2011). Current concepts in the neurophysiologic basis of sleep; a review. *Annals of medical and health sciences research, 1*(2), 173-179. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23209972

https://www.ncbi.nlm.nih.gov/pmc/PMC3507109/.

Feldman, D. E. (2012). The spike-timing dependence of plasticity. *Neuron, 75*(4), 556-571. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22920249

https://www.ncbi.nlm.nih.gov/pmc/PMC3431193/. doi:10.1016/j.neuron.2012.08.001

Ferbinteanu, J., Kennedy, P. J., & Shapiro, M. L. (2006). Episodic memory--from brain to mind. *Hippocampus, 16*(9), 691-703. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16906530. doi:10.1002/hipo.20204

Ferbinteanu, J., & Shapiro, M. L. (2003). Prospective and retrospective memory coding in the hippocampus. *Neuron, 40*(6), 1227-1239.

Foster. (2017). Replay Comes of Age. *Annu Rev Neurosci, 40*, 581-602. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28772098. doi:10.1146/annurev-neuro-072116-031538

Foster, & Wilson. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature, 440*, 680. Retrieved from https://doi.org/10.1038/nature04587. doi:10.1038/nature04587

https://www.nature.com/articles/nature04587#supplementary-information

Frank, L. M., Brown, E. N., & Wilson, M. (2000). Trajectory encoding in the hippocampus and entorhinal cortex. *Neuron, 27*(1), 169-178. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10939340.

Gerstner, W., Kempter, R., van Hemmen, J. L., & Wagner, H. (1996). A neuronal learning rule for sub-millisecond temporal coding. *Nature, 383*(6595), 76.

Girardeau, G., Benchenane, K., Wiener, S. I., Buzsaki, G., & Zugaro, M. B. (2009). Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci, 12*(10), 1222-1223. doi:10.1038/nn.2384

Gustafsson, B., Wigstrom, H., Abraham, W. C., & Huang, Y. Y. (1987). Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *J Neurosci, 7*(3), 774-780.

Hassabis, D., Kumaran, D., Vann, S. D., & Maguire, E. A. (2007). Patients with hippocampal amnesia cannot imagine new experiences. *104*(5), 1726-1731. Retrieved from https://www.pnas.org/content/pnas/104/5/1726.full.pdf. doi:10.1073/pnas.0610561104 %J Proceedings of the National Academy of Sciences

Hasselmo, Bodelon, & Wyble. (2002). A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. *Neural Comput, 14*(4), 793-817. doi:10.1162/089976602317318965

Hasselmo, M. E., Hinman, J. R., Dannenberg, H., & Stern, C. E. (2017). Models of spatial and temporal dimensions of memory. *Curr Opin Behav Sci, 17*, 27-33. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29130060. doi:10.1016/j.cobeha.2017.05.024

Hebb, D. O. (1949). *The Organizaton of Behaviour: A Neurpsycholoical Theory*. Mahwah, N.J: Psychology Press.
Huber, R., Tononi, G., & Cirelli, C. (2007). *Huber R, Tononi G, Cirelli C. Exploratory behavior, cortical BDNF expression, and sleep homeostasis. Sleep 30: 129-139* (Vol. 30).
Huerta, P. T., & Lisman, J. E. (1996). Synaptic plasticity during the cholinergic theta-frequency oscillation in vitro. *Hippocampus, 6*(1), 58-61. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8878743. doi:10.1002/(SICI)1098-1063(1996)6:1<58::AID-HIPO10>3.0.CO;2-J
Ishihara, K., Mitsuno, K., Ishikawa, M., & Sasa, M. (1997). Behavioral LTP during learning in rat hippocampal CA3. *Behav Brain Res, 83*(1-2), 235-238.
Julius, M. S., & Adi-Japha, E. (2016). A Developmental Perspective in Learning the Mirror-Drawing Task. *Front Hum Neurosci, 10*, 83. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26973498. doi:10.3389/fnhum.2016.00083
Kemp, A., & Manahan-Vaughan, D. (2004). Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. *Proceedings of the National Academy of Sciences, 101*(21), 8192-8197.
Kemp, A., & Manahan-Vaughan, D. (2007). Hippocampal long-term depression: master or minion in declarative memory processes? *Trends in Neurosciences, 30*(3), 111-118. Retrieved from http://www.sciencedirect.com/science/article/pii/S0166223607000033. doi:https://doi.org/10.1016/j.tins.2007.01.002
Kentros, Hargreaves, Hawkins, Kandel, Shapiro, & Muller. (1998). Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science, 280*(5372), 2121-2126.
Kessels, H. W., & Malinow, R. (2009). Synaptic AMPA receptor plasticity and behavior. *Neuron, 61*(3), 340-350. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19217372. doi:10.1016/j.neuron.2009.01.015
Kim, S. Y., Pardilla-Delgado, & Alger. (2017). Enhancing Memory Consolidation through Slow Oscillation and Spindle Synchronization. *37*(48), 11517-11519. Retrieved from http://www.jneurosci.org/content/jneuro/37/48/11517.full.pdf. doi:10.1523/JNEUROSCI.2512-17.2017 %J The Journal of Neuroscience
Kramis, R., Vanderwolf, C. H., & Bland, B. H. (1975). Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital. *Exp Neurol, 49*(1 Pt 1), 58-85. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1183532.
Kunec, S., Hasselmo, M. E., & Kopell, N. (2005). Encoding and retrieval in the CA3 region of the hippocampus: a model of theta-phase separation. *J Neurophysiol, 94*(1), 70-82. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15728768. doi:10.1152/jn.00731.2004
Latchoumane, Ngo, Born, & Shin. (2017). Thalamic Spindles Promote Memory Formation during Sleep through Triple Phase-Locking of Cortical, Thalamic, and Hippocampal Rhythms. *Neuron, 95*(2), 424-435 e426. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28689981. doi:10.1016/j.neuron.2017.06.025
Lechner, H. A., Squire, L. R., & Byrne, J. H. (1999). 100 years of consolidation--remembering Muller and Pilzecker. *Learn Mem, 6*(2), 77-87.
Levine, D. N. (2007). Sherrington's "The Integrative action of the nervous system": A centennial appraisal. *Journal of the Neurological Sciences, 253*(1), 1-6. Retrieved from http://www.sciencedirect.com/science/article/pii/S0022510X06005600. doi:https://doi.org/10.1016/j.jns.2006.12.002

Levy, W., & Steward, O. (1983). Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. *Neuroscience, 8*(4), 791-797.
Lopez-Munoz, F., Boya, J., & Alamo, C. (2006). Neuron theory, the cornerstone of neuroscience, on the centenary of the Nobel Prize award to Santiago Ramon y Cajal. *Brain Res Bull, 70*(4-6), 391-405. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17027775. doi:10.1016/j.brainresbull.2006.07.010
Luczak, A., Bartho, P., Marguet, S. L., Buzsaki, G., & Harris, K. D. (2007). Sequential structure of neocortical spontaneous activity in vivo. *Proc Natl Acad Sci U S A, 104*(1), 347-352. doi:10.1073/pnas.0605643104
Marozzi, E., & Jeffery, K. J. (2012). Place, space and memory cells. *Curr Biol, 22*(22), R939-942. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23174291. doi:10.1016/j.cub.2012.10.022
Marshall, L., & Born, J. (2007). The contribution of sleep to hippocampus-dependent memory consolidation. *Trends Cogn Sci, 11*(10), 442-450. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17905642. doi:10.1016/j.tics.2007.09.001
Marshall, L., Helgadottir, H., Molle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature, 444*(7119), 610-613. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17086200. doi:10.1038/nature05278
Mayer, M. L., Westbrook, G. L., & Guthrie, P. B. (1984). Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. *Nature, 309*(5965), 261-263.
Mehta, M. R., Quirk, M. C., & Wilson, M. A. (2000). Experience-dependent asymmetric shape of hippocampal receptive fields. *Neuron, 25*(3), 707-715.
Milner, B., Corkin, S., & Teuber, H.-L. J. N. (1968). Further analysis of the hippocampal amnesic syndrome: 14-year follow-up study of HM. *6*(3), 215-234.
Morris, R. G. (1989). Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. *J Neurosci, 9*(9), 3040-3057.
Morris, R. G. (2003). Long-term potentiation and memory. *Philos Trans R Soc Lond B Biol Sci, 358*(1432), 643-647. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12740109. doi:10.1098/rstb.2002.1230
Morris, R. G. (2006). Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. *Eur J Neurosci, 23*(11), 2829-2846. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16819972. doi:10.1111/j.1460-9568.2006.04888.x
Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*(5868), 681-683. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7088155.
Moser, E. I., Moser, M. B., & Andersen, P. (1994). Potentiation of dentate synapses initiated by exploratory learning in rats: dissociation from brain temperature, motor activity, and arousal. *Learn Mem, 1*(1), 55-73. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10467586.
Moser, E. I., Moser, M. B., & McNaughton, B. L. (2017). Spatial representation in the hippocampal formation: a history. *Nat Neurosci, 20*(11), 1448-1464. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29073644. doi:10.1038/nn.4653
Müller, G. E., & Pilzecker, A. (1900). *Experimentelle beiträge zur lehre vom gedächtniss* (Vol. 1): JA Barth.
Murre, J. M., Chessa, A. G., & Meeter, M. (2013). A mathematical model of forgetting and amnesia. *Front Psychol, 4*, 76. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23450438. doi:10.3389/fpsyg.2013.00076
Nadel, Winocur, Ryan, Moscovitch, & Morris. (2007). Systems consolidation and hippocampus: two views. *1*(2), 55-66. Retrieved from https://doi.org/10.1007/s11559-007-9003-9. doi:10.1007/s11559-007-9003-9

Naji, M., Krishnan, G. P., McDevitt, E. A., Bazhenov, M., & Mednick, S. C. (2018). Coupling of autonomic and central events during sleep benefits declarative memory consolidation. *Neurobiol Learn Mem, 157*, 139-150. doi:10.1016/j.nlm.2018.12.008
Neske, G. T. (2015). The Slow Oscillation in Cortical and Thalamic Networks: Mechanisms and Functions. *Front Neural Circuits, 9*, 88. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26834569. doi:10.3389/fncir.2015.00088
Nevin, J. A. (1973). *The Study of Behavior: Learning, Motivation, Emotion, and Instinct*: Scott, Foresman.
Nicoll, R. A. (2017). A Brief History of Long-Term Potentiation. *Neuron, 93*(2), 281-290. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28103477. doi:10.1016/j.neuron.2016.12.015
O'Keefe. (1976). Place units in the hippocampus of the freely moving rat. *Exp Neurol, 51*(1), 78-109.
O'Keefe, Burgess, Donnett, Jeffery, & Maguire. (1998). Place cells, navigational accuracy, and the human hippocampus. *Philos Trans R Soc Lond B Biol Sci, 353*(1373), 1333-1340. doi:10.1098/rstb.1998.0287
O'Keefe, & Dostrovsky. (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, & Nadel. (1978). *The Hippocampus as a Cognitive Map*: Oxford: Clarendon Press.
O'Keefe, & Recce. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus, 3*(3), 317-330. doi:10.1002/hipo.450030307
Okano, Hirano, & Balaban. (2000). Learning and memory. *Proceedings of the National Academy of Sciences, 97*(23), 12403-12404. Retrieved from http://www.pnas.org/content/pnas/97/23/12403.full.pdf. doi:10.1073/pnas.210381897
Pastalkova, E., Itskov, V., Amarasingham, A., & Buzsaki, G. (2008). Internally generated cell assembly sequences in the rat hippocampus. *Science, 321*(5894), 1322-1327. doi:10.1126/science.1159775
Peigneux, P., Laureys, S., Fuchs, S., Collette, F., Perrin, F., Reggers, J., . . . Maquet, P. (2004). Are spatial memories strengthened in the human hippocampus during slow wave sleep? *Neuron, 44*(3), 535-545. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15504332. doi:10.1016/j.neuron.2004.10.007
Quirk, G. J., & Stewart, M. (1988). Neurons in Entorhinal Cortex of Etherized Rats Exhibit Rhythmic Bursting Correlated with the Hippocampal EEG a. *Annals of the New York Academy of Sciences, 529*(1), 310-313.
Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiol Rev, 93*(2), 681-766. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23589831. doi:10.1152/physrev.00032.2012
Ribot, T. (1882). *Dieases of Memory: An essay in positive psychology* (Vol. 43): D. Appelton.
Rosenbaum, R. S., Kohler, S., Schacter, D. L., Moscovitch, M., Westmacott, R., Black, S. E., . . . Tulving, E. (2005). The case of K.C.: contributions of a memory-impaired person to memory theory. *Neuropsychologia, 43*(7), 989-1021. doi:10.1016/j.neuropsychologia.2004.10.007
Rosenbaum, R. S., McKinnon, M. C., Levine, B., & Moscovitch, M. (2004). Visual imagery deficits, impaired strategic retrieval, or memory loss: disentangling the nature of an amnesic person's autobiographical memory deficit. *Neuropsychologia, 42*(12), 1619-1635. Retrieved from http://www.sciencedirect.com/science/article/pii/S0028393204000879. doi:https://doi.org/10.1016/j.neuropsychologia.2004.04.010
Rosenzweig, E. S., & Barnes, C. A. (2003). Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. *Prog Neurobiol, 69*(3), 143-179.

Rudy. (2008). *The neurobiology of learning and memory* (Vol. Psychology).
Rudy, & Sutherland. (2008). Is it systems or cellular consolidation? Time will tell. An alternative interpretation of the Morris group's recent science paper. *Neurobiol Learn Mem, 89*(4), 366-369. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17977757. doi:10.1016/j.nlm.2007.07.017
Sainsbury, R. S., Heynen, A., & Montoya, C. P. (1987). Behavioral correlates of hippocampal type 2 theta in the rat. *Physiol Behav, 39*(4), 513-519. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3575499.
Salz, D. M., Tiganj, Z., Khasnabish, S., Kohley, A., Sheehan, D., Howard, M. W., & Eichenbaum, H. (2016). Time Cells in Hippocampal Area CA3. *J Neurosci, 36*(28), 7476-7484. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27413157. doi:10.1523/JNEUROSCI.0087-16.2016
Schabus, M., Dang-Vu, T. T., Heib, D. P., Boly, M., Desseilles, M., Vandewalle, G., . . . Maquet, P. (2012). The Fate of Incoming Stimuli during NREM Sleep is Determined by Spindles and the Phase of the Slow Oscillation. *Front Neurol, 3*, 40. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22493589. doi:10.3389/fneur.2012.00040
Schönauer, M., Köck, C., Pawlizki, A., & Gais, S. (2014). Exploring the Effect of Sleep and Reduced Interference on Different Forms of Declarative Memory. *Sleep, 37*(12), 1995-2007. Retrieved from https://dx.doi.org/10.5665/sleep.4258. doi:10.5665/sleep.4258 %J Sleep
Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry, 20*(1), 11-21.
Sereday, M. S., Marti, M. L., Damiano, M. M., & Moser, M. E. (1994). Establishment of a registry and incidence of IDDM in Avellaneda, Argentina. *Diabetes Care, 17*(9), 1022-1025. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7988300.
Sirota, A., & Buzsaki, G. (2005). Interaction between neocortical and hippocampal networks via slow oscillations. *Thalamus Relat Syst, 3*(4), 245-259. doi:10.1017/s1472928807000258
Sisti, Glass, & Shors. (2007). Neurogenesis and the spacing effect: learning over time enhances memory and the survival of new neurons. *Learn Mem, 14*(5), 368-375. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17522028. doi:10.1101/lm.488707
Skelin, I., Kilianski, S., & McNaughton, B. L. (2018). Hippocampal coupling with cortical and subcortical structures in the context of memory consolidation. *Neurobiol Learn Mem*. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29660400. doi:10.1016/j.nlm.2018.04.004
Spreng, Rossier, & Schenk. (2002). Spaced training facilitates long-term retention of place navigation in adult but not in adolescent rats. *Behav Brain Res, 128*(1), 103-108. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11755694.
Squire, L. R. (2009). The legacy of patient H.M. for neuroscience. *Neuron, 61*(1), 6-9. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19146808. doi:10.1016/j.neuron.2008.12.023
Steriade, M. (1997). Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. *Cereb Cortex, 7*(6), 583-604. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9276182.
Steriade, M., Nunez, A., & Amzica, F. (1993a). Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J Neurosci, 13*(8), 3266-3283.
Steriade, M., Nunez, A., & Amzica, F. (1993b). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci, 13*(8), 3252-3265.

Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature, 437*, 1272. Retrieved from https://doi.org/10.1038/nature04286. doi:10.1038/nature04286
Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T. J., & Steriade, M. (2000). Origin of slow cortical oscillations in deafferented cortical slabs. *Cereb Cortex, 10*(12), 1185-1199.
Tolman, E. C. (1948). Cognitive maps in rats and men. *Psychol Rev, 55*(4), 189-208. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18870876.
Tulving, E. (2004). [Episodic memory: from mind to brain]. *Rev Neurol (Paris), 160*(4 Pt 2), S9-23. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15118549.
Van Der Werf, Altena, Vis, Koene, & Van Someren. (2011). Reduction of nocturnal slow-wave activity affects daytime vigilance lapses and memory encoding but not reaction time or implicit learning. *Prog Brain Res, 193*, 245-255. doi:10.1016/b978-0-444-53839-0.00016-8
van Lier, H., Coenen, A. M. L., & Drinkenburg, W. H. I. M. (2003). Behavioral Transitions Modulate Hippocampal Electroencephalogram Correlates of Open Field Behavior in the Rat: Support for a Sensorimotor Function of Hippocampal Rhythmical Synchronous Activity. *23*(6), 2459-2465. Retrieved from http://www.jneurosci.org/content/jneuro/23/6/2459.full.pdf. doi:10.1523/JNEUROSCI.23-06-02459.2003 %J The Journal of Neuroscience
Vanderwolf. (1969). Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr Clin Neurophysiol, 26*(4), 407-418.
Vanderwolf, Kramis, & Robinson. (1977). Hippocampal electrical activity during waking behaviour and sleep: analyses using centrally acting drugs. *Ciba Foundation symposium*(58), 199-226. Retrieved from http://europepmc.org/abstract/MED/252441.
Vanderwolf, C. H. (1975). Neocortical and hippocampal activation relation to behavior: effects of atropine, eserine, phenothiazines, and amphetamine. *J Comp Physiol Psychol, 88*(1), 300-323. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/235571.
Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc, 1*(2), 848-858. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17406317. doi:10.1038/nprot.2006.116
Vorhees, C. V., & Williams, M. T. (2014). Assessing spatial learning and memory in rodents. *ILAR J, 55*(2), 310-332. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25225309. doi:10.1093/ilar/ilu013
Whishaw, I. Q., & Vanderwolf, C. H. (1973). Hippocampal EEG and behavior: changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. *Behav Biol, 8*(4), 461-484.
Winson. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science, 201*(4351), 160-163.
Wolansky, T., Clement, E. A., Peters, S. R., Palczak, M. A., & Dickson, C. T. (2006). Hippocampal slow oscillation: a novel EEG state and its coordination with ongoing neocortical activity. *J Neurosci, 26*(23), 6213-6229. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16763029. doi:10.1523/JNEUROSCI.5594-05.2006