PROBING HIPPOCAMPAL ACTIVITY IN SLEEP, SLEEP DEPRIVATION, AND RECOVERY SLEEP

by

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ABSTRACT

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Long-term memories are established over several hours of sleep following their initial acquisition. Large-scale recordings from the hippocampus have revealed that neurons reactivate or 'replay' wake-induced activity in subsequent sleep episodes, leading many to suggest replay as an underlying mechanism for sleep-mediated memory consolidation. In this dissertation, I describe experiments that examine the extent of hippocampal replay during sleep and how replay is affected in the absence of sleep. In Chapter 2, we found that the hippocampus reactivates neuronal patterns expressed during exploration of a novel environment for upto 10 hours in subsequent sleep with a half-maximum timescale of 6 h and is much longer than previously reported durations for hippocampal reactivation. On the contrary, reactivation lasted for less than 30 minutes after exploration of familiar environments. In Chapter 3, we carried out long-duration electrophysiology recordings to investigate how the hippocampal replay is affected when animals are subjected to prolonged wakefulness instead of sleep. We sleep deprived animals for 5 h following exploration of a novel environment, after which the animals were left undisturbed for 4 h to recover from extended waking. We observed that, compared to normal sleep, neuronal firing rates and the rate of sharp wave-ripples increased during sleep deprivation, indicating altered dynamics of the hippocampal network. Despite enhanced network activity, we found that the hippocampal replay was severely attenuated

by sleep loss. Interestingly, we observed a rebound in the replay when the animals entered recovery sleep, but its magnitude remained much lower compared to the levels seen during natural sleep. Overall, our findings provide a systems-level understanding of why sleep benefits, but sleep loss impairs, hippocampal memory.

To my parents,
Anjana and Prashant,
for their endless love and encouragement.

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1. Introduction

The hippocampus plays an essential role in learning and memory. Ever since hippocampal damage was reported to show severe memory deficits [Milner, 1965], much research has focused on understanding how the hippocampus contributes to proper memory functioning. Two main observations were made from these early clinical studies in human subjects. First, the hippocampus is necessary for encoding newer memories. Second, compared to recent memories, older ones were less vulnerable to hippocampal damage. What processes allow memories to become more resilient over time remains a fundamental question in modern neuroscience.

Episodic memory, a type of memory that binds the spatial and temporal aspects of a personal experience [Tulving and Markowitsch, 1998], depends critically on the hippocampus. These experiences are often stored for an extended period of time and require protection from any interference that may result in memory impairment. While encoding and retrieval of information is traditionally believed to occur at short time scales, storage, on the other hand, requires significantly longer duration, thereby making memory traces most vulnerable at this stage of memory formation. It is believed that this stabilization of labile memory traces into long-term memory, a process called *memory consolidation*, occurs primarily during off-line periods like sleep when the brain has lower encoding demands. Indeed, interrupting sleep following learning has been shown to be detrimental to memory consolidation [Havekes and Abel, 2017, Palchykova et al., 2009, Prince and Abel, 2013]. In particular, the first few hours of post-task sleep is absolutely necessary for sleep to benefit long-term memory. What underlying processes allow the hippocampus to transform new information into long-term memory? Electrophysiological recordings from the hippocampus have revealed that neurons repeat activity patterns expressed during active exploration in subsequent episodes of sleep [Wilson et al., 1994, Kudrimoti et al., 1999, Lee and Wilson, 2002]. This reactivation or 'replay' of wake-induced activity has been proposed as one of the mechanisms for sleep-

dependent memory consolidation [Born and Wilhelm, 2012]. However, previous studies have reported that hippocampal replay only lasts 10-30 minutes in sleep [Wilson et al., 1994, Kudrimoti et al., 1999, Tatsuno et al., 2006, Ji and Wilson, 2007], raising skepticism about the role of reactivation in supporting long-term memory [Sutherland et al., 2010, Tononi and Cirelli, 2014].

In this dissertation, our main aim is to re-examine the relationship between hippocampal replay and sleep, and how it may relate to memory consolidation. The first goal is to examine the extent of hippocampal reactivation in sleep following a novel experience. This is assessed using both pairwise and ensemble-based analyses. The second goal is to understand how the lack of sleep immediately after a novel experience impacts hippocampal reactivation and also other properties of the hippocampal network. In addition, this investigation will also allow us to understand why sleep loss is detrimental to long-term memory storage. Together, the findings of this thesis make a case for the integral role of hippocampal replay in memory consolidation.

1.1 Anatomy and the flow of information in the hippocampus

The hippocampus is a C-shaped structure located inside the medial temporal lobe of each hemisphere. Broadly, the structure and functions of the hippocampus have remained similar across many mammalian species [Manns and Eichenbaum, 2007, Strange et al., 2014]. Based on the cell layer organization, the hippocampus is divided into three main subregions, CA1, CA3, and the dentate gyrus (DG). Each of these subregions play different roles in memory processing [Knierim, 2015]. Another area within the temporal lobe, known as the entorhinal cortex (EC), serves as the main input and output structure of the hippocampus. The first projection occurs between axons of the EC and granule cells of the DG via perforant pathway. Cells in the granule layer then synapse on the dendrites of the CA3 pyramidal neurons

via mossy fibers. Neurons from CA3 then project to CA1 region via Schaffer collaterals. Collectively, these three synapses are referred to as the trisynaptic pathway. Initially, it was believed that the information through the hippocampus largely flows in one direction i.e, via the the trisynaptic loop [Yeckel and Berger, 1990]. However, since then, studies have shown far greater diversity in the types of connections involved in hippocampal processing. For example, there are direct projections from the entorhinal cortex to CA1 and CA3 [Naber et al., 2001, van Strien et al., 2009]. Then there are major inputs from other subcortical areas such as the medial septum, which is also responsible for pacing the hippocampal theta rhythm (discussed later). Even within the hippocampus, synaptic transmission is not unidirectional. There are back projections from the CA3 neurons to the dentate granule cells. The hippocampus also outputs to other subcortical structures such as the thalamus, amygdala, and basal forebrain. Combined, these connections present a far more complex picture for the hippocampus.

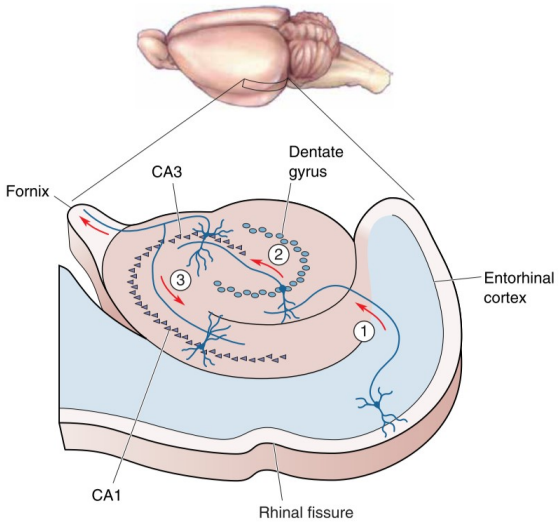

Figure 1.1: Hippocampal circuitry showing the information flow in the hippocampus. This figure highlights the trisynaptic pathway where inputs from the entorhinal cortex synapse on the dentate gyrus via the perforant path (PP). Mossy fibers emerging from the dentate gyrus form synaptic connection with pyramidal neurons of CA3 region. Axons from CA3 neurons synapse on CA1 pyramidal neurons via Schaffer Collaterals. Image borrowed from Bear et al. (2020).

1.2 Spatial memory and representations

Remembering an environment in which a particular event occurred is a critical cognitive ability and integral to the formation of episodic memory. This is also corroborated by the fact that spatial and episodic memory impairments are often the first symptoms observed in patients with damage to the hippocampus. For example, in clinical studies, navigating in virtual mazes and recalling the locations of recently seen objects were impaired in patients with hippocampal lesions [Milner, 1965, Corkin, 1965, Smith and Milner, 1981, Smith, 1988]. Similar impairments were also observed in non-human primates with lesions to the hippocampal structure for tasks evaluating spatial memory [Mahut, 1971, Mahut, 1972]. In rodents, this impairment was demonstrated by performing bilateral lesions of the fornix which caused increased errors in retrieving reward in a radial arm maze task[Olton et al., 1978].

The Hippocampus's role for storing spatial memory gained profound support post the discovery of 'place cells' in freely moving rats [O'Keefe and Dostrovsky, 1971]. These place cells, recorded from area CA1, fire at specific locations of an environment and are hypothesized as a mechanism of encoding spatial representations in the brain [O'Keefe et al., 1975, O'Keefe and Nadel, 1979]. Following this discovery, several studies investigated the role of intact hippocampus on spatial learning and memory. For example, place navigation in a water maze was heavily impaired in rats with total hippocampal lesions [Morris et al., 1982].

Place cells encoding a particular environment are also stable across days [Muller and Kubie, 1987, Ziv et al., 2013] and change their firing patterns in response to changes in surrounding environment. New place cells may appear, while existing ones can disappear or change locations. In addition, they can also alter their in-field firing rates. Even minor changes in sensory inputs can affect place cell activity. Removing visual cues affects place cell organization. Rotating or expanding an environment changes the location and size of the place fields. These substantial changes, called 'remapping', ensure that different

environments are uniquely represented and interference between overlapping information is minimized [Muller et al., 1991, Alme et al., 2014]. These evidences suggest visual sense of space are primary input for the formation of place cells. However, some studies have found that location specific firing is also observed in the absence of visual inputs. Animals that are navigating in darkness or congenitally blind also form place fields [Quirk et al., 1990, Save et al., 1998].

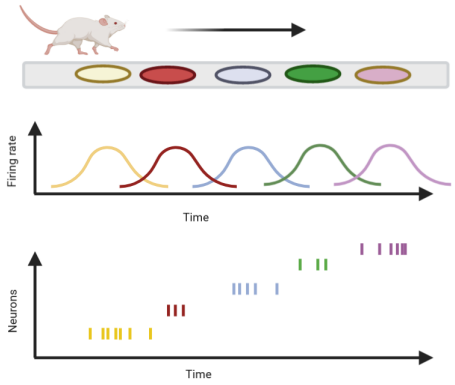

Figure 1.2: Schematic showing sequential activity of place cells during navigation.

Since place fields are localized in space, when an animal moves through an environment, place cells associated with animal's route get activated in a sequential manner (Fig. 1.2). Repeated firing in this sequential order has been hypothesized to strengthen connectivity between place cells that were active during exploration. In the following sections, we will learn how these unique firing patterns support plasticity and influence the activity of these neurons during offline states, such as sleep.

1.3 Memory Consolidation

Memories are initially vulnerable to interference (retroactive inhibition) and undergo a process of maturation and stabilization. The term 'consolidation' was coined by Muller and Pilzecker [Muller and Pilzecker, 1900] which emerged from the systematic study of the

Ebbinghaus Forgetting curve [Ebbinghaus (1885), 2013]. Interestingly, some of the insights related to memory existed before and came from clinical studies by Theodule Ribot. From his experience with clinical cases, he proposed that loss of memory followed a temporal gradient, which is now known as Ribot's law [Ribot, 1882]. According to this law, recent memories are more likely to be lost first, whereas older memories such as habits and emotional memories are much more resistant to disruptions. However, an indication of memory manifesting at two timescales for consolidation came from Burnham's work [Burnham, 1903]. This remained unexplored until 1949 when few studies showed retrograde amnesia in rodents induced via electroconvulsive shock [Duncan, 1949, Glickman, 1961]. Today, these timescales are formally studied under *synaptic* and *systems* consolidation [Dudai, 2004].

1.3.1 Systems consolidation

Examination of clinical cases had shown that memories can be vulnerable to manipulations. Later, Scoville and Milner [Scoville and Milner, 1957] provided more insight into understanding what regions of the brain might be important for consolidating memories. In that study, they found that lesions in the hippocampus and surrounding areas negatively affected normal memory functioning. All patients in that study had bilateral lesions and the severity of memory deficits was strongly correlated with the extent of damage to the hippocampus. In addition to memory loss for events of the recent past, patients also experienced difficulty in forming newer memories. Other studies also made similar observations, in which the extent of the hippocampal lesion was correlated with the severity of retrograde amnesia in patients, ranging from a few years to decades in the past [Manns et al., 2003, Bayley et al., 2006, Smith and Squire, 2009]. More evidence emerged from neuroimaging studies, where recollection of recent memories showed greater activity in the medial temporal lobe compared to remote memories [Smith and Squire, 2009, Haist et al., 2001]. Temporally graded retrograde amnesia has also been reported in nonhuman primates and rodents. For example, monkeys after the hippocampal lesion performed better in recalling tasks learned several weeks ago than

tasks that were learned just before the lesion [Zola-Morgan and Squire, 1990]. In contextual fear conditioning experiments, rodents showed reduced freezing for environments learned 1 day before the hippocampal lesion than for environments learned 28 days ago [Kim and Fanselow, 1992]. On the basis of this evidence, it is implied that storage of information requires the hippocampus initially, but over time they migrate to other brain regions and eventually become independent of the hippocampus, known as the Standard Model of Systems Consolidation. However, according to an alternative model, known as Multiple Trace Theory (MTT), retrieval of episodic memories depends on the hippocampus, regardless of how long ago the memories were stored [Nadel and Moscovitch, 1997]. In both models, episodic memories are encoded in hippocampal-cortical networks, but they disagree on the role of the hippocampus in retrieval of remote episodic memories. Nadel and Moscovitch (1997) observed that in many clinical studies the gradient of retrograde amnesia depended on the size of the lesion [Frankland and Bontempi, 2005]. In addition, studies using functional magnetic resonance imaging (fMRI) to probe brain activity during recent and remote memory recall observed that the hippocampus was equally active in both scenarios [Nadel et al., 2000]. In this thesis, systems consolidation generally means integration and reorganization of memory traces in the hippocampal-cortical network.

1.3.2 Synaptic consolidation

Synaptic consolidation occurs at the synapse level. These synapses undergo transformations which affect how neurons communicate with each other. However, these synaptic changes do not happen at random, rather are specific to neurons which fire together [Hebb, 1949]. The first preliminary evidence for modifiable synapses came from stimulation experiments in rabbit hippocampal slices [Bliss and Lømo, 1973]. Bliss and Lomo stimulated the perforant pathway and recorded synaptic activity in the dentate gyrus. They found that weaker stimuli caused a stronger response in the dentate gyrus if it was preceded by stronger stimuli. They named this phenomenon as Long-lasting potentiation but now more commonly known

as Long-term potentiation (LTP). This enhanced response lasted from minutes to hours (10 hours) and provided substantial evidence in support of the view that experience can modify synapses and can be a mechanism for storing memory. Later, Lynch and colleagues discovered that synapses can also get weakened after repeated stimulation, called as Long-term depression (LTD) [Lynch et al., 1977]. These results were further refined by later studies [Markram et al., 1997, Magee and Johnston, 1997, Froemke and Dan, 2002, Dan and Poo, 2004] which suggested that synaptic plasticity depends on the precise timing of spikes in pre- and post-synaptic neurons, defined as spike-timing dependent plasticity (STDP). With these important discoveries followed a series of studies investigating the molecular mechanisms underlying synaptic plasticity. It turns out a lot of molecular interactions contribute to LTP. Proteins, genes, receptors, and many signaling molecules engage in a chain reaction causing the synapses to modify their structure. Neurons communicate with each other using neurotransmitters such as glutamate and dopamine. An action potential in the presynaptic neuron causes the release of these neurotransmitters at the synapse of post synaptic neuron causing its thickening, called as post synaptic density (PSD). This thickening happens as a result of increased AMPA receptors and scaffolding proteins [Kim and Sheng, 2009]. The signaling cascade is initiated with the neurotransmitter binding to the receptors on the PSD and activating several molecules such as calcium, cyclic adenosine monophosphates (cAMP) and inositol triphosphate inside the cell which in turn activates proteins (kinases and phosphatases). While AMPA receptors have been the primary target of glutamate, later it was found that NMDA receptors also play a significant role in inducing LTP [Collingridge et al., 1983]. Collingridge and colleagues reported that applying amino-phosperic-valeri acid (APV, an NMDA receptor antagonist) before stimulation inhibited the induction of LTP. It is also interesting to note that LTP is an expensive process and requires molecular processes to support its expression at multiple stages. Frey and Morris [Frey and Morris, 1997] while investigating LTP on hippocampal slices reported that LTP exists in two phases (1) Early LTP (lasts for 2-3 hours) (2) Late LTP (lasts for $>$ 10 h). Based on these observations,

they hypothesized that the early phase of LTP occurs independently of protein but sets the stage for recruiting/generating proteins for inducing late LTP. This is now more commonly as the 'synaptic tagging and capturing theory'. LTP in its early stage is vulnerable and can be easily be reversed. If a strong stimulus is immediately followed by a stream of weaker stimuli, then the potentiated synapse no longer expresses LTP at the same level [Barrionuevo et al., 1980]. However, delaying weaker stimuli by 30 minutes or more had no observable effects on the already induced LTP [Larson et al., 1993, Stäubli and Chun, 1996]. These evidences suggest that synaptic plasticity is a key process for memory formation and retain their strength for hours to possibly support long term memory. Later we will discuss network properties that allow the hippocampus to support synaptic plasticity.

1.4 Sleep and memory

Sleep is mainly characterized by three characteristics: 1) increased sensory threshold 2) rebound sleep after prolonged periods of wakefulness and 3) a state of unconsciousness that is reversible. Over the years, studies have shown that sleep is essential for the optimal functioning of many physiological processes, such as cell healing and repair, regulating hunger, development, waste removal, and maintaining the balance of hormones such as ghrelin and leptin [Zielinski et al., 2016]. With all these functions supported by sleep, its role in reinforcing memory is substantial and has been demonstrated by numerous studies in various memory tasks. Interestingly, the effects of sleep are also time dependent, that is, sleep that occurs shortly after learning has greater benefits than sleep at a later time point [Benson and Feinberg, 1977, Gais et al., 2006, Talamini et al., 2008, Payne et al., 2012]. For example, participants who went to sleep within 3 h of a vocabulary learning task recalled more words compared to participants who slept after 10 hours [Gais et al., 2006]. Similarly, sleep immediately after learning was more beneficial in recalling word pairs than prolonged wakefulness [Payne et al., 2012]. Even relatively brief periods of sleep that occur shortly after the task

were beneficial to memory [Alger et al., 2012, Sheth et al., 2012]. The processes that occur during sleep that allow it to protect memory are not fully understood. Additionally, sleep is not uniform. It is composed of distinct stages that alternate for multiple cycles throughout the duration of sleep. Each stage is characterized by various rhythmic neural activities. Before discussing the underlying mechanisms, it is important to know about the sleep-wake architecture and neural activity patterns that occur during different stages of sleep.

1.4.1 Sleep-wake architecture

Seemingly mundane process of sleeping every day involves dramatic changes in brain activity. Traditionally, sleep has been measured using one or a combination of tools such as electroencephalography (EEG), electro-oculogram (EOG), and electromyography (EMG). EEG patterns are strikingly distinct between sleep and wake. While sleep primarily has large-amplitude irregular fluctuations in EEG, wake mainly comprises small-amplitude rhythmic oscillations. Sleep can be broadly subdivided into two stages; non-rapid eye movement (NREM) and rapid-eye movement(REM) [Stickgold, 1998]. Sleep alternates between these two substages, and each stage is characterized by distinct oscillations. Much of our understanding of sleep's role in memory has come from studying rodent models. It is important to note that rodents are nocturnal animals and their sleep cycle differs from that of humans. For example, a single cycle of NREM-REM lasts about 90 minutes in humans, and it is significantly shorter in rodents, lasting approximately 10 minutes. Compared to humans, sleep in rodents is also more fragmented. Although there are differences, basic physiological processes remain similar. From now on, the oscillatory patterns associated with sleep and wake that are discussed below will be specific to rodents.

During active exploration and REM sleep, hippocampal LFP in rodents is primarily dominated by theta oscillations (4-12 Hz). On the contrary, EEG activity during NREM sleep is dominated by large-amplitude slow (0.5-4 Hz) and spindle (8-16 Hz) oscillations. Hippocampal LFP during NREM and periods of immobility feature irregularly occurring

sharp wave-ripples, which are faster oscillations (140-250 Hz). Gamma oscillations (30-120 Hz) have mostly been observed during awake, when an animal is in an engaged state to perform certain tasks.

1.4.2 Role of oscillations in memory formation and consolidation

Brain oscillations have long been investigated for their role in orchestrating brain activity. These oscillations reflect and modulate communication within and between different brain areas. Multiple frequency bands have been associated with processes such as planning, encoding, and memory consolidation. Following few subsections provide background on some of the widely investigated LFP oscillations and their coupling.

Slow wave oscillations

Electrical activity during NREM sleep is dominated by large amplitude slow oscillations (0.5 - 4 Hz). Historically, < 1 Hz oscillations were designated as slow oscillation and $1-4$ Hz were called as delta waves. However, here I will collectively refer them as slow oscillations. These are global states that can be detected across multiple brain regions and have been described as traveling waves that originate locally in the prefrontal cortex and spread to other regions of the brain [Massimini et al., 2004]. The amplitude of slow wave is homeostatically regulated, where it is highest at the beginning of sleep onset and over sleep it decreases. This decrease in delta power has also been reported in infants [Fattinger et al., 2014], a finding that indicates slow oscillation may also support brain development. Since SWA is tightly coupled with sleep-wake history, wherein power in delta band increases monotonically with increasing wake duration [Dijk et al., 1987, Martinez-Gonzalez et al., 2008], quantitative models like the 'Process S' can accurately simulate a subject's sleep-wake distribution by only analyzing the delta-band dynamics [Borbély, 1982, Daan et al., 1984]. The decomposition of delta band activity into individual slow waves has revealed that the proportion of large amplitude slow waves decreases from early to late sleep, while the total number of slow waves largely

remains constant [Riedner et al., 2007]. Intracellular recordings from cortical, motor and visual areas have revealed that slow rhythmic ($< 1Hz$) oscillations of the membrane potential is the underlying cellular phenomenon for the slow oscillations [Steriade et al., 1993]. Slow oscillation comprises synchronized population activity, where neurons fire intensely ('UP' states) in the depolarized phase of the membrane potential and are almost silent ('DOWN' states) in the hyperpolarized phase. Slow oscillations have been suggested to mediate down-regulation of synaptic strengths, maintenance of cellular homeostasis, but more importantly the mediation of memory consolidation [Diekelmann and Born, 2010]. Some studies have reported a strong correlation between learning and changes in the slow oscillations during subsequent NREM sleep. For example, a strong increase in EEG coherence was reported following learning during NREM sleep [Mölle et al., 2004]. It is hypothesized that synchronous activity during the slow oscillations allows for more efficient communication between cortical and subcortical areas.

Theta activity

In rodents, theta oscillations (4-10 Hz) dominate local field potentials during active exploration. Interesting relationships have been observed between ongoing theta rhythm and place cells. When entering a place field, spikes from the associated place cell initially occur at late phases of theta cycle and then gradually shifts its firing to earlier phases on subsequent theta cycles, a phenomenon called *theta phase precession* [O'Keefe and Recce, 1993, Skaggs et al., 1996]. A consequence of this is that, as an animal moves through multiple place fields, place cells are organized at distinct phases of any given theta cycle, defined as *theta sequences* [Foster and Wilson, 2007]. Importantly, each theta cycle contains compressed representations of animal's environment. Furthermore, theta sequences are repeated over multiple cycles in which place cells fire within a few milliseconds of one another, a condition favorable for Hebbian synaptic plasticity (LTP and LTD) [Skaggs et al., 1996, Stuart, 2001, Wójtowicz and Mozrzymas, 2015]. Theta oscillations have also been suggested to coordinate transfer

of information between multiple brain regions. For example, in a spatial working memory task, neurons in the medial prefrontal cortex (mPFC) during correct-choice trials were more strongly phase-locked to hippocampal theta than during error trials [Jones and Wilson, 2005]. Similar observations were also made in a delayed nonmatch to position task, correct trials showed increased phase-locking of theta modulated mPFC neurons compared to incorrect trials [Hyman et al., 2010]. Other brain regions such as cingulate cortex [Colom et al., 1988], amygdala [Paré and Gaudreau, 1996], entorhinal cortex [Frank et al., 2001], and striatum [DeCoteau et al., 2007] have also been observed to be modulated by hippocampal theta.

Sleep spindles

Sleep spindles (8-16 Hz) identified during NREM sleep have long been hypothesized to play an important role in memory processing. This hypothesis was mostly based on It has often been observed that the frequency of spindle occurrence increases following learning [Schabus et al., 2004, Eschenko et al., 2006]. In humans, an increased spindle density during sleep strongly predicted subjects' performance in recalling word-pairs learned before sleep [Schabus et al., 2004]. Similarly, in rodents, post odor-reward association task, animals showed robust increase in spindle density [Eschenko et al., 2006]. Some recent studies even found that playing recently learned words during NREM sleep elicited more number of spindles compared to words that were not seen during the task [Cairney et al., 2018]. Spiking patterns during spindles may facilitate synaptic plasticity through long term potentiation [**?**]. Furthermore, low numbers of spindles during sleep has also been associated with age-related memory deficits [Fogel et al., 2017].

Gamma

Studies have also reported observing gamma oscillations(30-150 Hz) nested within theta oscillations at specific phases of theta cycle [Colgin et al., 2009, Belluscio et al., 2012]. Although both oscillations frequently occur together, theta and gamma are considered to be

independently generated [Leung, 1992]. Unlike theta, gamma oscillations occur in bouts, which has led many to suggest it serves the purpose of selecting cell assemblies during those timepoints [Jensen and Lisman, 2005, Jensen and Colgin, 2007, Senior et al., 2008]. It has also been suggested that gamma oscillations reflect the attentional selection processes in the hippocampus [Fries et al., 2001, Csicsvari et al., 2003, Fenton et al., 2010]. Gamma oscillations have also been reported to reflect cognitive demands of a task. For example, in a delayed spatial alternation task on a 8-shaped maze, increased gamma power is observed when animal is located in the central arm, suggesting a mechanistic role of gamma in facilitating decision making process [Yamamoto et al., 2014, López-Madrona et al., 2020]. Based on these evidences and operating at faster timescales, gamma oscillations have been suggested to support hippocampal processes like rapid selection of inputs, grouping neurons into ensembles during encoding, and retrieving memories to inform future decisions.

Sharp wave-ripples

During sleep and quiet wakefulness, hippocampal LFP shows brief periods of high-amplitude oscillations called sharp wave ripples (SWR) that occur in conjunction with synchronous depolarization of a large population of hippocampal neurons [Buzsáki et al., 1983, Vanderwolf, 1969]. SWRs are composed of a fast 'ripple' (120-250 Hz) like oscillation superimposed on a large amplitude 'sharp wave' (SPW, 5-16 Hz). Although both oscillations have been observed most often together, occasionally they also occur independently of each other [Buzsáki et al., 1983, Csicsvari et al., 2000]. Intermittent quiet wakefulness in between exploratory behavior often shows a burst of SWRs where 2-3 of these events occur within few milliseconds of each other. These short bursts (50-100 ms) of increased activity provide an optimal window for Hebbian plasticity, hypothesized to be an underlying mechanism for encoding, planning, and sleep-related memory benefits [Buzsáki, 1989, Buzsáki, 2015]. However, there is some evidence that suggests that SWRs play an important role in synaptic downscaling during sleep [Bukalo et al., 2013, Norimoto et al., 2018]. The characteristic oscillatory pattern of SWRs

is driven by CA3 and propagate throughout the entorhinal-hippocampal output pathway synchronizing the hippocampus to the cortical areas [Chrobak and Buzsáki, 1996, Gordon, 2011, Ólafsdóttir et al., 2016]. These events have been suggested to support various cognitive and memory functions [Buzsáki, 2015, Joo and Frank, 2018]. Rodents perform poorly in a spatial memory task if hippocampal activity is disrupted during immobility SWRs in the maze, indicating its role in planning and encoding [Girardeau et al., 2009, Ego-Stengel and Wilson, 2010]. On the other hand, disrupting SWRs in sleep has detrimental effects on memory. Studies have shown that disrupting SWR during sleep by suppressing CA3 output to CA1 or optogenetically altering the activity of other brain regions, such as the locus coeruleus or the median raphe nucleus, results in slower learning in hippocampus-dependent tasks [Nakashiba et al., 2009, Wang et al., 2015, Novitskaya et al., 2016]. Taken together, these studies suggest that SWRs play a crucial role in encoding and stabilizing new experiences.

Coupling between oscillations

Transfer of information across brain regions is enabled by precise coupling of several brain rhythms. While low frequency oscillations like theta are observed across multiple structures and are suggested to synchronize distant brain regions, faster oscillations like gamma are locally generated and reflect local computations. The interaction between global and local rhythms modulates the process of information transfer. For example, a widely studied coupling is between theta phase and the amplitude of gamma oscillations [Colgin, 2011, Hyafil, 2015]. This has been reported across several subcortical and neocortical brain regions [Chrobak and Buzsáki, 1998, Tort et al., 2008, Tort et al., 2009]. In situations of higher cognitive demand, increased theta-gamma modulation has been observed. Furthermore, different subbands of gamma, namely slow and fast gamma, are modulated at distinct phases of theta and have been suggested to facilitate memory encoding and retrieval. Theta-gamma coupling is also seen during REM sleep, where it is believed to consolidate the information

acquired during waking [Bandarabadi et al., 2020]. Another example of coupled rhythms is between slow oscillations and spindles. Younger adults show a more precise coupling compared to older people explaining poor memory performance [Muehlroth et al., 2019]. Another interesting coupling is observed between SWRs and slow oscillations. This coupling in particular has been suggested to favor hippocampal-cortical dialogue.

1.4.3 Mechanisms

Based on evidences discussed so far, multiple possible mechanisms for sleep-dependent memory benefits. While some studies suggest sleep primarily serves the function of enhancing previous learning [Fischer et al., 2002], others advocate sleep makes learning more resilient to subsequent interference [Ellenbogen et al., 2006, Sonni and Spencer, 2015]. Yet another theory, synaptic homeostasis hypothesis (SHY), claims that sleep benefits memory in a learning-independent manner [Tononi and Cirelli, 2014]. According to SHY, wakefulness causes a net increase in synaptic strength across all neurons, while during sleep all synapses are globally down-scaled. As a result of downscaling, weaker synapses get eliminated while the stronger ones survive, thus preserving memory traces that are represented by surviving synapses. There are also some studies, under the sequential hypothesis, which suggest that NREM and REM serve complimentary roles in supporting memory and is based on the cyclic succession of NREM and REM sleep. In this, it is argued that synapses are weakened during NREM as a result of slow oscillations and strengthened during REM sleep by theta oscillations [Giuditta et al., 1995, Ambrosini and Giuditta, 2001, Tamaki et al., 2020]. However, one of the most influential model is the two-stage model of memory formation.

Two-stage model of memory consolidation

In rodents, strong theta oscillations are observed in hippocampal LFP when an animal is actively exploring or performing certain task. During this engaged state, sensory inputs from cortical regions carry information about the surrounding environment to the hippocampus.

This information transfer is believed to be facilitated by theta oscillations causing structural and functional changes in the hippocampal neurons. Interestingly, during offline states such as resting or sleeping, neurons fire synchronously in burst like activity during sharp wave ripples (SWRs). Worthy to note that SWRs continue to occur over the entire duration sleep. Gyorgi Buzsaki proposed a two-stage model to explain the role these seemingly different oscillations (theta and SWRs) could play in supporting memory [Buzsáki, 1989]. The model states that the process of encoding and subsequently storing a new information occurs in two stages. In Stage I, theta activity forms a labile memory in CA3 via the perforant pathway, while in stage II, SWR-associated population bursts help strengthen this labile memory into a more permanent form. These oscillations provide favorable conditions for enhancing synaptic plasticity in both stages of memory storage. So basically, the hippocampus serves as a fast encoding temporary store and cortex functions as a slow learner for long term storage.

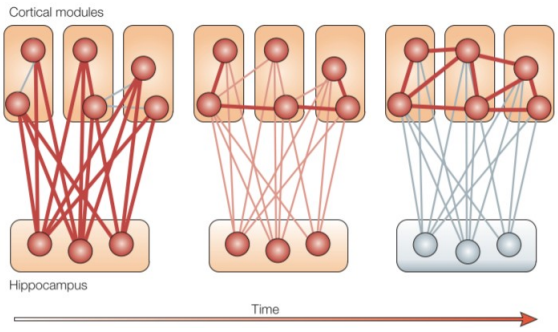

Figure 1.3: Two-stage memory model: Initially the hippocampus encodes information and over time through reactivation this information is re-distributed to cortical areas and becomes independent of the hippocampus. Borrowed from Frankland and Bontempi (2005).

Inherent in this model is a phenomenon called 'reactivation', where neurons repeat firing patterns formed during active exploration. Additionally, this model also provides a more physiological basis for the importance of sleep in supporting memory consolidation.

Memory consolidation by reactivation

We learned that neurons form sequential firing patterns that reflect animal's experience and are also tightly coupled to various brain rhythms like theta. These unique patterns recur

during offline periods such as sleep and are believed to mediate redistribution of temporarily stored representations to long-term storage. In addition to the two-stage model, reactivation also forms the basis for *active systems consolidation* hypothesis, where sleep plays an active role in integrating new information into preexisting memories instead of passively reducing interference from other inputs [Diekelmann and Born, 2010]. These repeated patterns occur primarily during NREM sleep and are driven by synchronized events like the SWRs. Signs of reactivation was first shown with an increased firing rate of hippocampal place cells during sleep periods immediately following exploration [Pavlides and Winson, 1989]. In this study, only those cells that were exposed to their place fields on the maze increased their firing rate in the following SWS sleep. Later, Wilson and McNaughton [Wilson and McNaughton, 1993] quantified the increased activity by using correlation measures. They found that cells with overlapping place fields on a maze showed an increased correlation in subsequent SWS from sleep before running on the maze. This suggested that experience on a maze can modify the structure of connectivity within the hippocampal neurons. Additionally, If two cells fire next to one another on the track, their temporal relationship during sleep was also found to biased in the same direction. They preserve the temporal order of firing on the maze reflecting animals' behavior [Skaggs and McNaughton, 1996]. This co-activity was later quantified using explained variance and reactivation was shown to last for 15-30 minutes post experience [Kudrimoti et al., 1999]. Subsequent analysis of activity during SWRs revealed that place cells within SWRs fire in temporally compressed sequences that resembles their activity during navigation. These ordered firing were termed as *replay*. The place cells firing can occur in both forward and reverse order to the direction of animal's trajectory. While sleep is mostly dominated by reverse replay, events of both forward and reverse order have been observed during quiet waking [Foster and Wilson, 2006, Diba and Buzsáki, 2007]. These evidences support that the hippocampus encodes experience in unique firing patterns and that these patterns actively repeated during sleep for supporting long term memory.

1.4.4 Synaptic Homeostasis Hypothesis

According to this model, the synapses throughout the brain are regulated by the sleep-wake cycle. During wake, there is a net increase in the total synaptic strength. This hypothesis proposes that synapses across brain regions are downscaled during NREM sleep. As a result of this downscaling, synapses avoid saturation and thereby allowing neurons to prepare for future learning [Tononi and Cirelli, 2014]. While being an influential model in the field, equally compelling evidence exist both in support and against the SHY hypothesis. In extracellular recordings, firing rates in neuronal population have often been used to indirectly measure synaptic strength. For example, an increase in overall firing rate may indicate increase in synaptic strength, while a decrease in firing rate will indicate weakening synapses. For example, Vyazovskiy et al. found that neurons in rat barrel cortex decreased their firing rate towards the end of the light cycle compared to the beginning of light cycle [Vyazovskiy et al., 2009].

1.5 Effects of sleep loss on memory

Sleep deprivation over the years has been utilized to test what physiological processes may explain the memory benefits of sleep[Alhola and Polo-Kantola, 2007]. Sleep loss causes severe memory impairments and is believed to interfere with the consolidation period, thus disrupting long-term memory formation. Interestingly, deficits were observed primarily for tasks dependent on the hippocampus, suggesting that sleep deprivation affects the hippocampus more than any other region of the brain [Smith and Rose, 1996, Graves et al., 2003]. For example, in a contextual fear conditioning paradigm, rodents showed reduced freezing to context but froze as much as control animals to tones post conditioning [Graves et al., 2003, Hagewoud et al., 2010b]. Likewise, memory deficits were observed only for spatial, but not non-spatial, versions of Morris water maze task [Smith and Rose, 1996, Hairston et al., 2005]. Similar dissociation between hippocampus-dependent and hippocampus-independent

tasks were also observed on Y-maze and T-maze, where animals switched from spatial to a response strategy after 5 hours of sleep deprivation following training [Hagewoud et al., 2010a]. In addition, rodent based studies also point to a specific time period within sleep that disproportionately affects memory. For example, in a contextual fear conditioning paradigm, rodents that were sleep-deprived in the first 5 hours of sleep following learning showed decreased freezing compared to animals who slept normally [Graves et al., 2003]. In the same study, delaying sleep deprivation by an additional 5 hours did not have an effect on animals' freezing behavior. Also, sleep deprivation immediately after training in dark cycle (active phase) had no significant effects on memory performance [Hagewoud et al., 2010b]. However, this difference may have been because the amount of sleep lost during active phase is lot less compared to resting phase. Importantly, this 'critical period' overlaps with time window when learning-induced synapses are stabilized (synaptic consolidation).

1.5.1 Effects on sleep states and brain oscillations

In addition to increase in delta power, sleep deprivation also causes changes in other sleep related variables. For example, the duration of NREM and REM episodes increase after prolonged wakefulness [Franken et al., 1991]. However, some studies have only observed changes in bout durations for chronic, but not acute, sleep deprivation [Kim et al., 2017]. In addition, an increased power in delta wave (0.5-4 Hz) is also observed during initial stage of recovery sleep [Borbély et al., 1981, Tobler and Borbély, 1990, Franken et al., 1991]. Some studies also report an increase in REM sleep duration during recovery sleep.

1.5.2 Cellular and molecular mechanisms

Over the years, many studies have investigated the cellular and molecular mechanisms that may be at the core of memory deficits associated with sleep deprivation. Hippocampal slices obtained from sleep-deprived animals have shown significant differences in cellular properties, such as dendritic spine density and membrane excitability [McDermott et al.,

2003, Havekes et al., 2016]. In the study by Havekes et al. (2016), forcing animals to stay awake for five hours caused a significant reduction in the number and length of CA1 dendritic spines. While the same manipulation did not have such an effect on CA3 dendritic structure, a follow-up study from the same group showed that granule cells in the dentate gryus show similar reduction in dendritic spine density [Raven et al., 2019]. This suggests that the input (DG) and output (CA1) regions of the hippocampus are more susceptible to sleep loss compared to CA3 region, possibly because of CA3's high degree of recurrent connectivity. However, when rats were exposed to chronic sleep deprivation (primarily REM) for more than 21 days, even CA3 neurons begin showing abnormalities such as increased oxidative stress and reduced dendritic arborization [Konakanchi et al., 2022]. Furthermore, McDermott et al. (2003) found that exposing rats to 72 h sleep deprivation (primarily REM) significantly reduced membrane excitability in CA1 neurons. However, in this study, the DG granule cells did not show a reduction in excitability. It is important to note that one study found that extended awakening led to an increase in spine density among CA1 neurons [Maria Spano et al., 2019]. However, this opposite finding may be due to the use of novel objects, instead of gentle handling, to keep animals awake. Synaptic potentiation (LTP) and depression (LTD) represent the efficacy of communication between neurons and are seen as cellular correlates of learning and memory. Both shorter (< 6 h) and longer durations (> 12 h) of sleep deprivation impair LTP in the CA1 area of the hippocampus [Campbell et al., 2002, Kopp et al., 2006, Tartar et al., 2006, Vecsey et al., 2009]. Interestingly, brief periods of sleep deprivation did not affect LTP induction, but only impaired LTP maintenance [Vecsey et al., 2009]. Sleep deprivation interferes with cellular and molecular processes in a way that inhibits LTP, necessary for proper memory consolidation. Total sleep deprivation as well as REM sleep deprivation impairs LTP in the hippocampus [Campbell et al., 2002, McDermott et al., 2003, Ravassard et al., 2009]. Further investigations have identified possible molecular markers which are responsible for LTP deficits. Studies have shown that sleep loss disrupts cAMP signaling which is required for LTP stabilization [Vecsey et al., 2009]. In the same

study, the authors were also able to rescue memory deficits by increasing cAMP signaling. Some other empirical studies have associated LTP deficits to changes in NMDA receptor composition [McDermott et al., 2006].

2. Hippocampal reactivation extends for several hours following novel experience

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2.1 Abstract

New memories are believed to be consolidated over several hours of post-task sleep. The reactivation or "replay" of hippocampal cell assemblies has been proposed to provide a key mechanism for this process. However, previous studies have indicated that such replay is restricted to the first 10-30 minutes of post-task sleep, suggesting it has a limited role in memory consolidation. We performed long-duration recordings in sleeping and behaving male rats and applied methods for evaluating the reactivation of neurons in pairs as well as in larger ensembles while controlling for the continued activation of ensembles already present during pre-task sleep ("preplay"). We found that cell assemblies reactivate for up to 10 hours, with a half-maximum timescale of 6 hours, in sleep following novel experience, even when corrected for preplay. We further confirmed similarly prolonged reactivation in post-task sleep of rats in other datasets that used behavior in novel environments. In contrast, we saw limited reactivation in sleep following behavior in familiar environments. Overall, our findings reconcile the duration of replay with the timescale attributed to cellular memory consolidation and provide strong support for an integral role of replay in memory.

2.2 Introduction

Sleep is necessary for memory consolidation [Rasch and Born, 2013]; disruption of sleep for 3-6 h following hippocampus-dependent tasks impairs the formation of long-term memories [Havekes and Abel, 2017]. One possible mechanism for memory consolidation in sleep is replay, the process by which neuronal assemblies active during tasks reactivate during subsequent sleep [Marr, 1971, Buzsáki, 2015]. However, previous accounts have indicated that replay is limited to the first 10-30 minutes of sleep following a task [Wilson et al., 1994, Kudrimoti et al., 1999, Tatsuno et al., 2006, Ji and Wilson, 2007]. This disparity between the short duration of replay and the long time course of sleep's effects on memory has presented

a major challenge for the field, leading many to argue that replay cannot effectively serve as a mechanism for memory consolidation [Sutherland et al., 2010, Tononi and Cirelli, 2014]. However, a careful inspection of previous studies reveals that replay was largely tested in well-trained animals following memory tasks or exploration in familiar environments and that most studies did not maintain unit recordings beyond the first hour of post-task sleep (POST) [Havekes and Abel, 2017]. Since novel experience is a powerful trigger for plasticity and learning in the hippocampus [van de Ven et al., 2016, Bittner et al., 2017], we re-examined the extent and duration of reactivation of rat hippocampal CA1 neurons in long-duration recordings following experiments in which both the task and the environment were designed to be novel experiences for the animal.

2.3 Materials and Methods

2.3.1 Surgery

Data from a total of ten Long Evans male rats weighing 250-400 g were analyzed in this study, and were previously used in other studies [Mizuseki et al., 2009, Grosmark and Buzsáki, 2016, Miyawaki and Diba, 2016]. We will hereby refer to these individually as the Miyawaki, Mizuseki and Grosmark datasets. Protocols originally used to obtain these data were approved by the Animal Care and Use Committees at the University of Wisconsin-Milwaukee, Rutgers University-Newark, and New York University, respectively.

All surgeries were performed in a stereotaxic frame under isoflurane anesthesia, with recording started only after full recovery ($>$ 5 days) from surgery. In all three datasets, recordings were performed using implanted 32-channel and 64-channel "Buzsaki" and "Buzsaki-sp" silicon probes (Neuronexus, MI). The Miyawaki dataset was obtained with a Neuralynx data-acquisition system (Neuralynx, MT) at 30 and 32 kHz sampling rate; the Mizuseki recordings used a DataMax system (RC Electronics, MT) at 20kHz sampling rate; Grosmark recordings were performed on Amplipex recording systems (Amplipex ltd., Hungary)

sampling at 20 kHz. All datasets were recorded from the CA1 layer of the hippocampus with two stainless steel screws implanted above the cerebellum used for referencing and grounding. Mizuseki also recorded simultaneously from the medial entorhinal cortex, which was not used in the present study. In addition, Miyawaki and Grosmark obtained the electromyogram (EMG) using wires placed in nuchal muscles. In all datasets, behavior was monitored and position of head-mounted LEDs was tracked using an overhead digital camera. In all datasets, spikes were detected by filtering and thresholding the wide-band signal using custom software in NDManager and its plugins [Hazan et al., 2006] (http://ndmanager.sourceforge.net) and automatically sorted with KlustaKwik [Harris et al., 2000] (http://klusta-team.github.io/klustakwik), followed by manual inspection and reclustering using the Klusters package [Hazan et al., 2006]. All data underwent additional manual inspection and reclustering to ensure consistent standards across datasets

All three datasets featured recordings sessions with sleep and rest before and after ("PRE" and "POST") a maze ("MAZE") epoch. Recordings were paused for between 3-45 min in between these epochs (to untangle recording cables, prepare setups, transfer animals, etc.). Additional criteria were implemented for all recordings to ensure unit stability across PRE and POST periods. Any units whose isolation distance changed by >50% across PRE-POST [Schmitzer-Torbert et al., 2005] were excluded from the analyses. Additionally, any units whose firing rates dropped below 30% of the mean firing rate for combined PRE and POST in any 1 h time window were also excluded. Units were categorized into pyramidal or interneurons based on their wave shape, refractory period, firing rates and burstiness [Csicsvari et al., 1998, Barthó et al., 2004].

2.3.2 Behavior

Three of the animals (rats R, T, and K) from the Miyawaki dataset, which provided initial data used to generate Figures 1, 2, and 4 (individual sessions shown in Figure 1-1), were trained to drink from water wells while water-restricted (30 mins ad libitum water per 24

h) but had not been placed on any track environments. Following recovery from surgery for electrode implantation, animals were again water-restricted to motivate running on the track. Recording in the home cage commenced 6 h into the dark cycle (PRE). Three hours before the start of light cycle, animals were transferred to the track (MAZE). Water rewards were supplied at either ends of the track to motivate running. For rats R and K, each extended track running session was composed of two consecutive blocks. In the first block, during the first 20 trials, the track was obstructed to confine the animal to the reward platform for 2 minutes after each trial. After 20 trials, the second block began, during which animals could freely explore the track and platforms for water rewards. For rat R, recordings were performed on this same track for two additional days. These additional track sessions were not considered novel experience and were not pooled with the other data. In the third animal, rat T, no obstruction block was performed and the rat ran freely for the entire session. Three recording sessions were performed with this animal with the shape of the track modified from I to L to U on consecutive days; all of these sessions were considered novel experience. The Miyawaki track sessions lasted 3 h. Following the track sessions, animals were returned to the home cage and recordings (POST) resumed during their sleep and waking rest. Animals had ad libitum access to food in the home cage. To compare correlations across entire light and dark cycles, we also identified and isolated among these data a total of twenty long-duration sessions from five Long Evans male rats spent entirely in the home cage; these sessions are individually depicted in (Fig. 2.9).

As described by Grosmark and Buzsaki (2016), the four animals from the Grosmark dataset "were pre-trained to search for water on a geometrically unrelated open-field 'cheese-board' maze for several day before novelty maze sessions. Once electrodes reached the CA1 pyramidal layer and the animals were well acclimatized to running for water reward as well as to the 'familiar' room as determined by the observation that the animals engaged in uninterrupted sleep in this room, a 'novelty' session was recorded. A novelty session consisted of a 'PRE' epoch in the familiar room, a novelty run in one of the three novel

rooms and 'POST' epoch back in the familiar room. Only one novelty room was used per novelty session. Note also that in the present study the animals had never been inside of the novel rooms, ensuring that the animals had no experience of the maze context, even fleeting ones during the plugging of the electrophysiological headstages, prior to novelty exposure." These animals received water rewards for completing runs across linear or circular mazes. "The RUN sessions were terminated once the animals were satiated and no longer ran for reward." [Grosmark and Buzsáki, 2016]. The recordings were all performed during the light cycle.

In the Mizuseki dataset, animals were water-restricted for 24 h prior to experiments and had been previously trained to alternate between two arms of a figure with additional running on a running wheel in a delay area [Pastalkova et al., 2008, Mizuseki et al., 2009]. One of these sessions was recorded on the first day of exposure (for 65 min) to the linear track ("novel"), though the animal had previously performed the alternation task and had multiple days of experience in the recording room. The other sessions were performed after at least 10 days of exposure to the linear track ("familiar") placed at varying orientations in the room. Sleep and rest before (PRE) and after (POST) the maze epochs were recorded. These recordings were all performed during the light cycle.

2.3.3 Sleep scoring

In the Miyawaki dataset, sleep scoring was performed using EMG, movement and theta-delta power. EMG was smoothed with a 1-s Gaussian filter and z-scored and a Schmitt-trigger threshold set at 0 and 0.5 was used to detect state transitions between low and high EMG power. Periods with low and high EMG power were labelled as sleep and wake respectively. The theta (5-10 Hz) over delta (1-4 Hz) plus (10-14 Hz) band ratio of the power spectral density was used to detect transitions between high theta and low theta, using custom MATLAB software, followed by visual inspection. Sleep states with high theta were classified as REM (rapid eye movement) and the remainder were classified as non-REM. Wake periods with

high theta were labeled as "active" and the remaining were labeled "quiet". In the Grosmark dataset [Grosmark and Buzsáki, 2016], "sleep scoring was performed using hippocampal LFP (theta/delta ratio), accelerometer (movement), and EMG data" and "all state scoring was performed using TheStateEditor developed by Andres Grosmark in the Buzsaki lab (https://github.com/buzsakilab/buzcoderough/tree/master/BehavioralStateDetection)". Active wake was "characterized by high theta-delta ratio and active movement/EMG-activity"; quiet wake (labeled "drowsy" by Grosmark) was "characterized by low overall spectral power, low movement/EMG-activity"; NREM was "characterized by high delta/theta ratio, and very low movement/EMG activity"; periods labeled as "intermediate" by Grosmark " which are short (30 second) states which occur at the NREM to REM transition and are characterized by highly-elevated pyramidal layer spindle (12 to 20Hz) power and low movement/EMG-activity" were considered as part of NREM here; REM was "characterized by high theta/delta ratio and very low movement/EMG activity occurring after NREM episodes" [Grosmark and Buzsáki, 2016].

In the Mizuseki dataset, sleep versus wake were scored by visual inspection of the animal and the local field potentials from CA1 and the entorhinal cortex while the animal was in its home cage. Quiet and active wake were not further separated. Theta (5-11 Hz) over delta (1-4 Hz) plus (12-14 Hz) band ratio of the power spectral density was used to detect transitions between high and low theta using custom MATLAB software. Sleep states with high theta were classified as REM (rapid eye movement) and the remainder were classified as non-REM. These periods were further "cross-validated with experimenter notes taken while observing theta activity on-line in sleep session and verifying that the rat was sleeping" [Mizuseki et al., 2011].

2.3.4 Experimental Design and Statistical Analysis

A total of sixteen sessions composed of PRE/MAZE/POST recordings from ten male Long Evans rats were used. Statistical comparisons between POST and PRE were performed in

each animal separately, as described in detail below, with the sample size depending on the number of isolated putative pyramidal neurons for each session. All individual sessions are shown in Figure 1-1 (available at https://doi.org/10.1523/JNEUROSCI.1950-18.2018.f1-1; including numbers of putative pyramidal neurons recorded in each session) to demonstrate consistency of findings across animals and datasets. Pooled results are shown in Fig. 2.1*b*, Fig. 2.2*c*, and Fig. 2.3*b*, with error bars indicating standard error of the mean pooled over sessions. Statistical analyses were performed using MATLAB. All correlations shown in figures were calculated using Pearson's r and significance level was set at $p = 0.001$. Exact p-values are provided in the Results section.

Explained Variance Measure

Spike times were binned into 250 ms time bins, creating an N x T matrix, where N is the number of neurons and T is the number of time bins. The time bin size was chosen based on a previous study [Tatsuno et al., 2006] and because it matches the timescales for 2 theta cycle oscillations and sharp-wave ripples. Pearson's correlations, R, were determined for spike counts from neuronal pairs recorded on different shanks in 15 mins sliding windows (window length 15 min, sliding 5 min steps) in PRE and POST and in the entire MAZE session to produce P, an M-dimensional vector, where M is the number of cell pairs. To assess similarity between P vectors from different windows, the Pearson correlation R of these vectors (i.e. the correlation between cell pair correlations) was determined (e.g. $R_{[PRE,POST]}$, $R_{[PRE,MAZE]}$ and $R_{[MAZE,POST]}$). The main element of interest here is $R_{[MAZE,POST]}$, but it may be contaminated by preexisting correlations before the animal has been exposed to a maze [Kudrimoti et al., 1999, Ribeiro et al., 2004, Tatsuno et al., 2006]. To address this issue, we calculated the explained variance for a window WIN, based on the square of partial correlation [Kudrimoti et al., 1999]:

$$EV(WIN) = \left(\frac{(R_{[MAZE,WIN]} - R_{[MAZE,PRE(k)]} \times R_{[PRE(k),WIN]}}{(\sqrt{(1 - R^2_{[MAZE,PRE(k)]}})\sqrt{(1 - R^2_{[PRE(k),WIN]})}} \right)^2 \tag{2.1}$$

averaged over all PRE(k) windows for which $WIN_{PRE(k)}$. To further assess how much of EV can be generated by chance, we also calculated the time-reversed explained variance (REV) for each window WIN, as proposed in a previous study [Tatsuno et al., 2006]:

$$REV(WIN) = \left(\frac{(R_{[MAZE,PRE(k)]} - R_{[MAZE,WIN]} \times R_{[PRE(k),WIN]}}{(\sqrt{(1 - R^2_{[MAZE,PRE(k)]})}\sqrt{(1 - R^2_{[PRE(k),WIN]})}} \right)^2 \tag{2.2}$$

averaged over all $PRE(k)$ windows for which $WIN \neq PRE(k)$. Error bars for EV and REV in Fig. 2.1*a*, Fig. 2.3*a*, Fig. 2.4*a*, Fig. 2.5, Fig. 2.6, Fig. 2.7 and Fig. 2.8 indicate standard deviation over PRE(k). For results pooled across animals and sessions, as shown in Fig. 2.1*b* and Fig. 2.3*b*, error bars indicate standard error of the mean pooled over sessions. For Fig. 2.1*c*-*e* analyses involving pooled pairwise relationships, NREM periods within PRE and POST were concatenated in each session and pairwise correlation vectors were generated. These vectors were pooled across sessions for PRE_{NREM}, $MAZE$ and $POST_{NREM}$. Residuals for $POST_{NREM}$ and $MAZE$ (Fig. 2.1*e*) were obtained from the respective regression lines with PRE_{NREM}. For Fig. 2.4 analyses on the amount of exposure required to produce significant reactivation, P of MAZE was calculated from spike taken from the first 1 min, 5 min, 15 min, 30 min or entire period of exploration in the MAZE session. To extract a timescale for reactivation from sessions which showed some evidence of decay in EV, we first measured the maximum EV across all 15 min (sliding) windows. We then determined the best-fit line through each window which was composed of at least 50% NREM sleep, starting up to 1 h before the maximum EV until the end of the session. The time point at which the EV dropped to half of the maximum EV was then obtained from the regression line (as shown in Fig. 2.8).

2.3.5 Temporal Bias Measure

The temporal bias was calculated from the cross correlogram for each cell pair (calculated with 1 ms bins) by taking the sum of the counts from +1 to +125 ms minus the counts from

-1 to -125 ms [Skaggs and McNaughton, 1996]. To compare the temporal order across all pairs in POST and PRE versus MAZE, we took the difference between the number of pairs in the upper-right and lower-left quadrants of the scatter plots (i.e. "positively related" in Fig. 2.1*f* scatter plots) and the number of pairs in the upper-left and lower-right quadrants (i.e. "negatively related"). This population temporal bias was greater in POST versus PRE. To determine the significance of this difference between POST and PRE (Fig. 2.1*g*), we compared the observed difference value against a null distribution obtained from 10000 independent shuffles of the cell pair identities in PRE and POST.

2.3.6 Cell Assembly Reactivation

To investigate cell assembly reactivation, we employed a recently developed technique [Lopes-dos-Santos et al., 2013, Trouche et al., 2016] based on independent component analysis (ICA) of spike trains projected onto bases obtained from principal component analysis (PCA). Briefly, a correlation matrix was generated using the N x T matrix of neuronal spike counts in 250 ms windows after it was z-scored. Principal components were calculated following eigenvalue decomposition. To eliminate spurious activity patterns, only principal components having eigenvalues above the Marcenko–Pastur distribution were retained. The z-scored activity matrix was then projected on the subspace spanned by these principal components. The resulting reduced activity matrix was then used to generate the ICA weight matrix. Each neuron received a weight for the corresponding independent component (assembly pattern). Given the random values of weights and their sign, each component was normalized to have sum 1. The dot product of each z-scored bin with the weight vector was then used to calculate the reactivation strength of the assembly pattern. An average reactivation strength was obtained by averaging over all of the independent components in a given time window.

2.4 Results

To examine reactivation following novel experience, we pre-trained rats to alternate drinking from two water wells in their home cages. On the first day of recording, in generating the "Miyawaki dataset", the rats were placed on a linear track with water wells on platforms at each end of the track. These animals had not previously experienced any linear tracks or other large mazes, which ensured that running for water across a track was a very novel experience on the first day. On subsequent days, the length and shape of the track were modified with new segments, but the task itself was not novel. The track sessions on the first day and on subsequent days showed similar results and were combined in these analyses. For all analyses, we included only putative pyramidal units that displayed stable and isolated clusters (see Materials and Methods). Each recording session was divided into a MAZE period (for the task itself) as well as PRE and POST periods (for sleep or rest in the home cage before and after the task).

Because co-firing is considered to bind neurons into ensembles and drives synaptic plasticity, we first examined co-activity in pairs of neurons (Fig. 2.1*a-b*); we employed a commonly-used "explained variance" (EV) measure [Kudrimoti et al., 1999, Tatsuno et al., 2006, Johnson et al., 2010] to test whether co-activity in 250 ms time windows during MAZE persists in POST beyond the levels present in PRE. This control was necessary because cell pairs showed significant correlations (Pearson's $r = 0.176$, $p = 5.386 \times 10^{-28}$, no. of pairs = 3809) between PRE and MAZE [Tatsuno et al., 2006, Dragoi and Tonegawa, 2011, Dragoi and Tonegawa, 2013] (Fig. 2.1*c*) and fairly strong correlations (Pearson's $r = 0.508$, $p = 7.482 \times 10^{-187}$, no. of pairs = 3809) between PRE and POST sleep [Tatsuno et al., 2006, Dragoi and Tonegawa, 2014] (Fig. 2.1*d*). However, after controlling for existing PRE correlations, regression analysis revealed a significant correlation (Pearson's $r = 0.448$, $p = 3.219 \times 10^{-187}$, no. of pairs= 3809) between MAZE activation and POST non-rapid eye movement (NREM) sleep patterns (Fig. 2.1*e*). The time window used in this measure was chosen to correspond to the

duration of typical hippocampal sharp-wave ripples and approximately 2 theta oscillation cycles; therefore, EV captures neuronal activation and reactivation compressed within theta sequences and sharp-wave ripple sequences. The mean reactivation was higher in NREM compared to quiet waking rest (EV(NREM) = 0.201±0.1464, EV(quiet wake)= 0.083±0.06, $p = 0.043$). In all sessions involving exploration of a novel track segment we observed elevated reactivation for the entire ∼3 h duration of POST (see individual sessions in Fig. 2.8), with surprisingly little evidence of decay over this time (Fig. 2.1*b*).

We and others previously reported that firing rates of hippocampal neurons increase after novel waking experience [Karlsson and Frank, 2008, Larkin et al., 2014, Miyawaki and Diba, 2016], with higher firing rates in POST compared to PRE [Miyawaki and Diba, 2016]. To rule out that these firing-rate differences account for the increased EV in POST compared to PRE, we repeated this analysis after sub-sampling to equalize the firing rate for each neuron in PRE and POST but found nearly identical results. In particular, the correlation between MAZE and POST NREM, partialed out for PRE NREM, remained significant (median Pearson's $r = 0.454$, median $p = 6.417 \times 10^{-197}$ for 100 different random subsamples equalizing PRE and POST spike counts of each cell; no. of pairs = 3809). We also redid the analyses with different size time bins, ranging from 50 ms to 500 ms, for calculating correlation. These also produced highly similar and consistent results (Pearson's $r_{50ms} = 0.507$, $p = 5.916 \times 10^{-248}$, $r_{100ms} = 0.505$, $p = 3.968 \times 10^{-245}$, $r_{500ms} = 0.3633$, $p = 3.420 \times 10^{-119}$; no. of pairs = 3809), confirming the robustness of the observation.

To examine whether the reactivation in POST is consistent with temporal sequences in the firing patterns of neurons [Skaggs and McNaughton, 1996, Lee and Wilson, 2002, Ji and Wilson, 2007], we employed methods introduced by Skaggs and McNaughton (1996) to evaluate temporally ordered replay. We measured the temporal bias in the spike times of neuronal pairs (the difference in the number of positive lag (+1 to +125 ms) and negative lag (-1 to -125 ms) spike counts in the cross correlogram) in PRE, POST and MAZE epochs (see Materials and Methods). The temporal bias in POST was significantly correlated to

the temporal bias in MAZE (Fig. 2.1*f*; Pearson's $r = 0.274$, $p = 1.1 \times 10^{-66}$, $n = 3809$ cell pairs). The temporal bias correlation between PRE and MAZE was weaker, yet also significant (Pearson's $r = 0.053$, $p = 0.0011$), consistent with previous reports [Dragoi and Tonegawa, 2011, Dragoi and Tonegawa, 2013]. Importantly, in POST compared to PRE, there was a greater concentration of cell pairs that showed a bias consistent with the MAZE patterns (number of points in upper-right and lower-left quadrants minus points in the upper-left and lower-right quadrants of the scatter plots in Fig. 2.1*f*; $p = 0.0010$ for POST — PRE compared to 10000 shuffles), indicating that the temporal spiking patterns in POST more strongly resemble the patterns observed during MAZE [Skaggs and McNaughton, 1996, Wikenheiser and Redish, 2013]. Our results therefore demonstrate that the neuronal firing patterns in post-task sleep are consistent with a temporal replay of the sequences experienced during behavior.

To extend our analyses to ensembles of neurons and track their reactivation at a finer temporal resolution, we employed a recent technique that identifies co-activate cell assemblies using independent component analysis (ICA) of neural population vectors [Lopes-dos-Santos et al., 2013, Trouche et al., 2016]. The resulting ICA weight vectors were extracted from the MAZE period and their reactivation (or pre-activation) was examined in both POST and PRE (Fig. 2.2*a*). In both the sample session and pooled sessions, cell assemblies showed greater activation in POST compared to PRE (Fig. 2.2*b,c*). As expected, these reactivations occurred in conjunction with sharp-wave ripple oscillations in the CA1 pyramidal layer [Buzsáki, 2015] (Fig. 2.2*b*). Importantly, the average of the POST sleep cell assembly reactivations lasted well beyond previously reported limits and well into the third hour of post-task sleep, in agreement with results from the EV analysis.

Because these sessions involved long ∼3 h durations of waking experience, we asked whether briefer exploration in a novel maze would lead to similarly prolonged replay. To answer this question, we extended these same analyses to the Grosmark dataset, generously provided by Grosmark et. al. (2016), with sessions composed of PRE and POST sleep before

and after ∼45 mins (range: 34-51 mins) of MAZE experience where both the track and the recording room were designed to be novel. Fig. **??***b* demonstrates a sample recording where POST replay is seen for > 4 h following MAZE. In the pooled Grosmark et. al. (2016) sessions as well, replay was prolonged for several hours in POST sleep in both EV and ICA measures (Fig. 2.3*b*). Based on the half-maxima of the EV in these individual sessions, reactivation persisted for a mean of 2.3 h (range = 1.9 – 2.85 h) following MAZE. Thus, ∼45 minutes of experience on a novel track was sufficient to promote long-lasting hippocampal replay, well beyond previously-reported limits.

During early exploration of a novel environment, neuronal ensembles composed of place-fields are still in the process of forming and stabilizing [Frank et al., 2004, Cheng and Frank, 2008, Feng et al., 2015]. We therefore asked whether even early MAZE experience could leave a lasting trace in POST sleep. To examine this question, we used only the first 1-min, 5-min, 15-min or 30-min initial segments of exploration to define the pairwise correlation structure of MAZE and examined whether we could detect significant reactivation of those early ensembles in POST. While POST reactivation more strongly resembles MAZE ensembles defined over longer periods (Fig. 2.4*a*-*b*), we found that the activation of cell pairs in just the first 5-min of MAZE was sufficient to produce significant reactivation in POST. This observation therefore indicates that ensembles that already emerge within the first few minutes of novel experience continue to reactivate during post-task sleep.

In previous reports, reactivation following experience in familiar environments was found only in the first 10-30 mins of sleep [Wilson et al., 1994, Kudrimoti et al., 1999, Tatsuno et al., 2006, Ji and Wilson, 2007]. To examine the role of novelty vs. familiarity, in one animal we recorded PRE, MAZE, and POST over three consecutive days of experience on the same linear track (Fig. 2.5). Replay was lower but still enhanced during POST after the second day of track running (Fig. 2.5, middle panel). However, by the third day on the track, the observed replay was essentially at the chance level (Fig. 2.5, right panel). To examine this question in additional animals, we analyzed another 4 sessions in 3 rats

from the Mizuseki dataset [Mizuseki et al., 2009]. In one of these sessions, the animal was newly introduced to the linear track, but had previously performed delayed alternation in a figure eight maze with a running wheel [Pastalkova et al., 2008, Mizuseki et al., 2009] in this recording room. Remarkably consistent with the Miyawaki sessions, replay persisted for over 3 h in POST sleep following this novel experience (Fig. 2.6; time of half-maximum = 2.22 h). In contrast, in 3 other sessions recorded from 2 animals after extensive exposure to the track (Fig. 2.6*b*), replay was much more limited and was significant for < 30 min (range of 15-30 min, mean =23.33 min, Student's t test one tailed paired $p < 0.001$) of POST NREM sleep. Similar results were seen when we assessed reactivation in the ICA-defined cell assemblies (Fig. 2.6*c*-*d*). In sum our observations indicate that the novelty of the environment experienced during spatial behavior is a key determinant of the duration of reactivation in the subsequent sleep.

To better determine the temporal extent of reactivation following novel experience in MAZE, in two sessions from the Miyawaki dataset we were able to extend our recordings for 10 h of POST sleep (Fig. 2.7*a*). In both these sessions, reactivation peaked at ∼3–5 h following MAZE, then showed progressive decrease, dropping past half-maximum at ~ 6.28 h into POST sleep. These sessions appeared to feature longer reactivation than the Grosmark sessions. Thus, it may be that the longer MAZE periods in our sessions led to a longer reactivation period. However, the longer replay we observed could also be attributed to the more continuous sleep we obtained in our light-cycle recordings compared with Grosmark et al. (2016) (e.g., compare fractions of sleep in Fig. 2.1*b*, and Fig. **??***b*), whereas fragmentation of sleep may limit the persistence of reactivation. Nevertheless, in all three datasets of recordings examined, we found that reactivation extended for several hours following novel experience.

We next examined the activation patterns of neurons over the course of sleep in the correlations between neuronal pairs across our long-duration recordings (15 min bins). Several interesting features can be gleaned from these figures (Fig. 2.7*b*). As noted previously, there are

large correlations between all recorded sleep periods, including those that extend from PRE to POST sleep. The MAZE patterns are largely divergent from those during sleep, though some correlations were still noted between PRE and MAZE (Fig. 2.1*c*). Thus, the MAZE experience lead to a correlation pattern that is largely unique. Although these patterns persist in POST sleep, compared with PRE, they occur alongside the stronger patterns that are in turn unique to sleep. As can also be seen in Fig. 2.7*b*, the correlation structure during POST shifts over time so that different neuronal groups are dominant at different times. For example, relatively weaker apparent correlations exist across than within the end and beginning of the Day 3 session recorded from Rat T, with the dominant patterns shifting over hours-long periods. Additional long-duration sessions (n = 20 long 6 –12 h light or dark cycle sessions across 5 animals; Fig. 2.9, available at https://doi.org/10.1523/JNEUROSCI.1950-18.2018.f7-1) further corroborate this viewpoint.

2.5 Discussion

These results demonstrate that cell assemblies associated during learning reactivate during subsequent sharp-wave ripples for a much longer timespan than has been generally appreciated. In particular, we found that cell assemblies in sleep reprise MAZE activity for up to 7 h following novel experience. Remarkably, similar timescales have been reported both for the post-task consolidation window during which sleep deprivation impairs memory formation in different hippocampus-dependent learning tasks [Havekes and Abel, 2017]. For example, deprivation of sleep in the 4–5 h after object-location exploration disrupted subsequent memory [Florian et al., 2011, Havekes et al., 2014, Prince et al., 2014]. Importantly, this memory deficit was observed even when the first hour of post-task sleep, and presumably concurrent replay, was left intact [Prince et al., 2014], indicating that processes beyond the first hour of sleep remain important for memory consolidation. Other studies investigating contextual fear conditioning [Graves et al., 2003, Vecsey et al., 2009] and object-recognition memory

[Palchykova et al., 2006] reported a similar 5 h post-task consolidation window, consistent with our report. Our results are also consistent with a recent report that enduring stability of neural ensemble patterns are supported by parvalbumin-positive interneurons [Ognjanovski et al., 2017] following contextual fear conditioning. The duration of replay we report here also closely matches the hours-long timescales reported for protein signaling following memory (often referred to as "cellular" or "synaptic" consolidation;[McGaugh, 2000, Dudai, 2004]). In particular, cAMP and protein kinase A signaling pathways, which regulate the cAMP response element binding protein, are critically involved in multiple forms of synaptic plasticity linked to memory, as well as in gene expression in the hours immediately following learning [Abel et al., 2013]. Importantly, these pathways are impaired by 5 h of sleep-deprivation [Bourtchouladze et al., 1998, Vecsey et al., 2009]. Protein synthesis through the mammalian target of rapamycin kinase complex, which is likewise implicated in both plasticity and memory formation [Hoeffer and Klann, 2010], is also reduced upon sleep-deprivation over a 5 h post-task window [Vecsey et al., 2012, Tudor et al., 2016]. Although more research is needed to understand the inter-relationship between reactivation and protein-signaling, these results help to reconcile previous discrepancies between the time windows considered for cellular/synaptic and systems consolidation of memory.

We used two different methods for evaluating and tracking reactivation in POST relative to PRE: the EV method based on pairwise correlations [Kudrimoti et al., 1999, Tatsuno et al., 2006] and an ICA method based on ensemble coactivity [Peyrache et al., 2009, Lopes-dos-Santos et al., 2011, Lopes-dos-Santos et al., 2013]. Although these methods are not designed to evaluate temporal sequences, we used a 250 ms time window to capture coactivation on the timescale of sharp-wave ripples during which replay sequences are observed [Nádasdy et al., 1999, Buzsáki, 2015]. Furthermore, we examined the temporal structure of spike times within this window using a third method, by comparing the temporal bias of spike times in pairs of neurons, and found that POST firing sequences showed significant fidelity to MAZE sequences, and greater temporal fidelity compared with PRE sequences, consistent with

previous accounts of temporal replay [Skaggs and McNaughton, 1996, Lee and Wilson, 2002, Wikenheiser and Redish, 2013]. Preliminary analysis of our data with additional methods that are sensitive to sequential structure at the population level [Chen et al., 2016, Maboudi et al., 2018] have also produced consistent results to what we report here [Maboudi et al., 2018], but further work is needed to evaluate and differentiate temporal relationships across PRE, MAZE, and POST.

Importantly, studies by different investigators, some using methods identical to ours, had previously observed much shorter durations for replay than we report here [Wilson et al., 1994, Kudrimoti et al., 1999, Tatsuno et al., 2006, Ji and Wilson, 2007]. This discrepancy may arise from two reasons. First, with the exception of Tatsuno et al. (2006), these studies did not examine reactivation beyond the first 1h of post-task sleep as we have, potentially missing re-emergent reactivation in subsequent sleep. Second, the MAZE experience in these studies involved exploration of environments that were highly familiar to the animal, whereas we observed that the time course of reactivation depended on the novelty of the task environment. Interestingly, Tatsuno et al. (2006) introduced novel objects to animals in a familiar environment but failed to find evidence of protracted replay. This was in contrast to Ribeiro et al. (2004) who performed similar experiments that appeared to show reactivation lasting for days but did not control for extant correlation in PRE. Our analysis (Fig. 2.1*c,d*) demonstrates the importance of these controls, in agreement with Tatsuno et al. (2006), which may otherwise give an illusion of replay. Overall, our findings indicate that prolonged reactivation in post-task sleep is contingent upon exposure to a novel environment, which induces a new hippocampal map (or "global remapping"), rather than the experience of novel objects or events in a familiar environment, for which the hippocampus makes use of the same spatial map (or "rate remapping"; [Muller and Kubie, 1987, Leutgeb et al., 2005]. An intriguing possibility is that other experiences, such as a shock [Moita et al., 2004] or exposure to a predator's odor [Wang et al., 2012], which produce global remapping can generate a similarly prolonged reactivation in subsequent sleep.

The long duration of replay demonstrated in our study counters the notion that the bulk of neuronal patterns in sleep following learning is random or noisy, and strengthens the argument that memory can be consolidated during sleep through a systems level process involving hippocampal replay [Buzsáki, 1989, Diekelmann and Born, 2010]. We propose that novelty increases the firing rates of those hippocampal neurons activated in the environment [Hirase et al., 2001] through increased membrane excitability alongside potentiation of synapses that bind the activated cell assemblies [Takeuchi et al., 2014]. Some of these processes are likely enhanced by activation of membrane dopaminergic receptors [Takeuchi et al., 2016], which promotes the reactivation of these assemblies in post-task sleep. Over the course of sleep, reactivation during sharp-wave ripples promotes the consolidation of memories [Maingret et al., 2016]. But as memories are consolidated, hippocampal firing rates then decrease [Miyawaki and Diba, 2016], likely as a consequence of synaptic downscaling also triggered by sharp-wave ripples [Tononi and Cirelli, 2014, Miyawaki and Diba, 2016, Norimoto et al., 2018]. It is interesting and worthwhile to note that while extended replay is clearly a significant phenomenon, even in POST sleep the MAZE patterns are not the most dominant activation patterns. Additionally, a small but significant correlation exists between PRE and MAZE periods, consistent with the notion of preplay [Dragoi and Tonegawa, 2011, Dragoi and Tonegawa, 2013]. Thus, replay must contend with activity patterns that are unique to sleep, including those that carry onto subsequent experience [Dragoi and Tonegawa, 2014, Grosmark and Buzsáki, 2016]. However, the function performed by these other (non-replay) activities within sleep still remains elusive. Because network, cellular, and synaptic processes are intimately intertwined, additional research is needed to better understand whether and how reactivation and non-reactivation activities during sleep contribute to consolidation and other sleep functions by engaging proteins and plasticity processes to strengthen and weaken specific synaptic connections.

2.6 Acknowledgements

We are grateful to Asohan Amarasingham, Laura Ball, Caleb Kemere, Andrew P. Maurer, and Brendon Watson for helpful comments, and to Andres Grosmark, John Long and Gyorgy Buzsaki for publicly sharing their valuable data. This project was funded by the National Institute of Mental Health (NIMH) R01MH109170 and R01MH117964 to K.D., with additional support to S.C. from the German BMBF 01GQ1506 and to K.M. from the Toray Science Foundation.

2.7 Data Sharing

Data and analyses code will be made available to readers upon reasonable request.

2.8 Figures

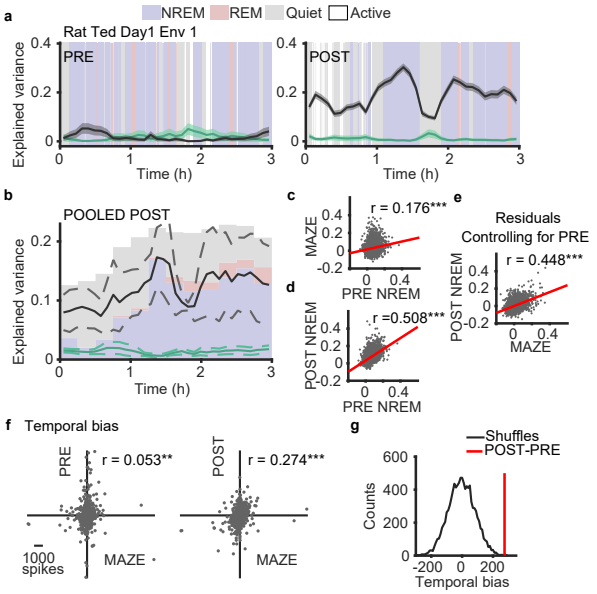


Figure 2.1: Neuronal reactivation persists for hours following novel experience. a, Reactivation as assessed by EV (black; mean ± SD in 15 min bins, sliding in 5-min steps) was significantly enhanced in POST sleep following novel MAZE in a sample recording (37 cells, 497 pairs), relative to the chance level (green; mean ± SD) assessed by reversed EV (REV ;[Tatsuno et al., 2006]; *see Materials and Methods*) (REV; see Materials and Methods). Detected brain states are indicated in the background. b) Results pooled across 5 POST sessions from 3 animals (Miyawaki dataset; mean ± SEM for n = 5 sessions, 205 cells and 3809 pairs; see Figure 1-1 for individual sessions). The total ratio of time in each sleep/wake state is shown in the background for each bin as a proportion of the y-axis limit. c) Neuronal pairwise correlations persist from PRE to MAZE. d) Much stronger correlations are observed across PRE and POST NREM sl'eep. These relationships are controlled for in the EV measure [Kudrimoti et al., 1999, Tatsuno et al., 2006]. e) After regressing out for the correlations with PRE, strong partial correlations are evident between POST NREM and MAZE periods. f) Scatter plots of the temporal bias (total counts in the cross-correlogram from +1 ms to +125 ms minus counts from -1 ms to -125 ms [Skaggs and McNaughton, 1996]) of cell pairs (3809 pairs from 5 sessions) on MAZE versus PRE and POST (same axes scales for both plots). g) The population temporal bias (number of scatter plot points in upper-right and lower-left quadrants minus those in upper-left and lower-right quadrants) was significantly greater in POST compared to PRE ($p = 0.001$, permutation test of observed value of POST – PRE compared against 10000 shuffles of cell pair identity). ** indicates $p < .01$ and *** indicates $p < .001$.

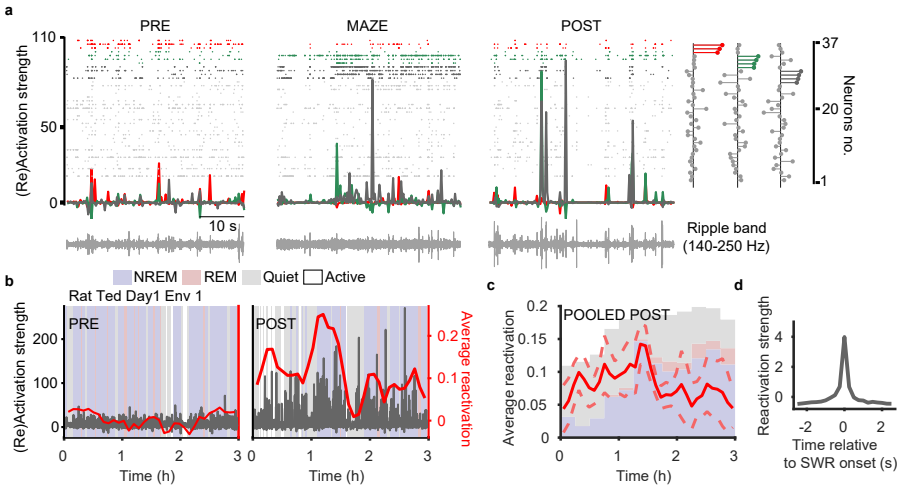


Figure 2.2: Assembly reactivation persists for hours following novel experience. a) Replay was assessed using independent component analysis (ICA) of cell assemblies [Lopes-dos-Santos et al., 2013]. In a sample recording showing rasters for 37 neurons (right axis) spanning PRE, MAZE, and POST periods, ICA was used to define cell assemblies from the MAZE period (3 out of 10 identified assemblies shown on far right). (Re)Activation strength of these three assemblies (left panel) is shown in corresponding colors. Ripple band local-field potential is shown below each panel. b) (Re)Activation of the third assembly from panel a is shown in PRE (left) and POST (right) panels for the same recording as Fig. 1a, with sleep/wake states shown in the background. The average (re)activation strength of all 10 cell assemblies for this recording is overlaid in red (mean in 15 min windows sliding in 5-min steps; right axis). c) Mean ($\pm$ SEM) cell assembly reactivation strength pooled across 5 sessions from 3 animals (num. of assemblies = 54; Miyawaki dataset), with the total ratio of time in each sleep/wake state in the background for each bin. Assembly reactivation strength remained significantly above zero throughout 3 hours of POST. d) Assembly reactivation was coincident with hippocampal sharp-wave ripple events.

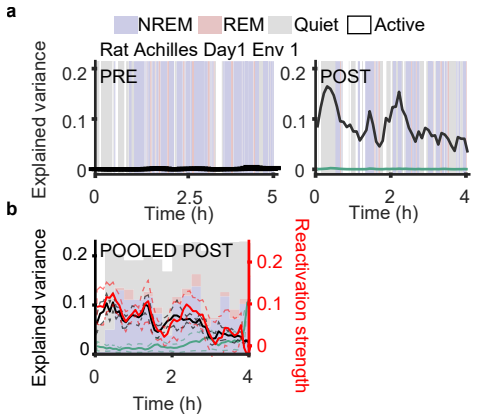


Figure 2.3: Replay following brief novel experience. a) Reactivation (EV; mean ± SD from 108 cells, 4984 pairs) in POST following brief (45 mins) novel track experience in a sample session from the Grosmark dataset (Grosmark et al., 2016). Sleep/wake states are indicated in the background. b) Reactivation analysis results pooled across 5 POST sessions from 4 animals (total 278 cells, 8810 pairs; see Figure 1-1 for individual Grosmark sessions) shows extended replay for 3 h following novel experience (mean ± SEM for EV, black, left axis and Assembly reactivation strength, red, right axis). Note that these animals demonstrated less NREM sleep after this point, making it difficult to assess the continuation of replay. The total ratio of time in each sleep/wake state is shown in the background for each bin as a proportion of the y-axis limit. Since individual sessions varied in duration, N varies from 4 to 5 in different bins.

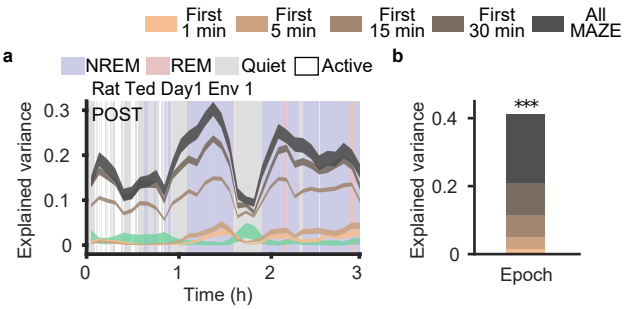


Figure 2.4: Reactivation of the onset of MAZE experience. a) We iteratively used the first 1 min, 5 min, 15 min, 30 min or entirety of the behavior session to define the MAZE pairwise correlation template and calculated reactivation (EV; mean ± SD) in POST accordingly (sleep/wake states indicated in background). The strength of reactivation increased with the length of the initial period used to define the template in this sample session, but some reactivation could be seen of even the first 1-min of MAZE. b) Pooled results from EV in NREM in POST (n = 5 sessions from the Miyawaki dataset, with 205 cells and 3809 pairs) for these incremental durations of MAZE experiences. All durations produced significant Pearson partial correlation coefficients (1-min $p = 1.82 \times 10^{-13}$; 5-min $p = 4.835 \times 10^{-32}$; 15-min $p = 3.647 \times 10^{-57}$; 30-min $p = 3.917 \times 10^{-84}$; All MAZE $p = 3.219 \times 10^{-187}$).

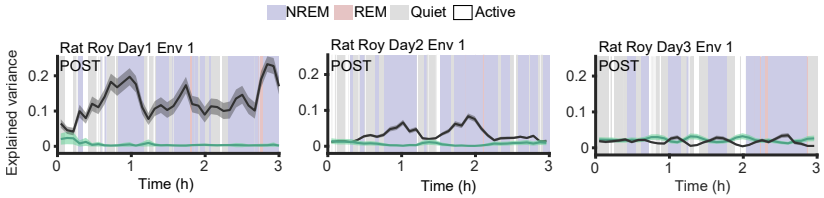


Figure 2.5: Reactivation decreases following repeated exposure to an environment. Reactivation (EV; mean ± SD) in POST following repeated exposure of an animal to same environment (rat R, Env 1), on Day 1 (58 cells, 1332; left panel), Day 2 (86 cells, 2966 pairs; middle panel) and Day 3 (83 cells, 2717 pairs; right panel). The strength of reactivation shows a progressive decrease over days of exposure to the environment. Sleep/wake states are indicated in the background.

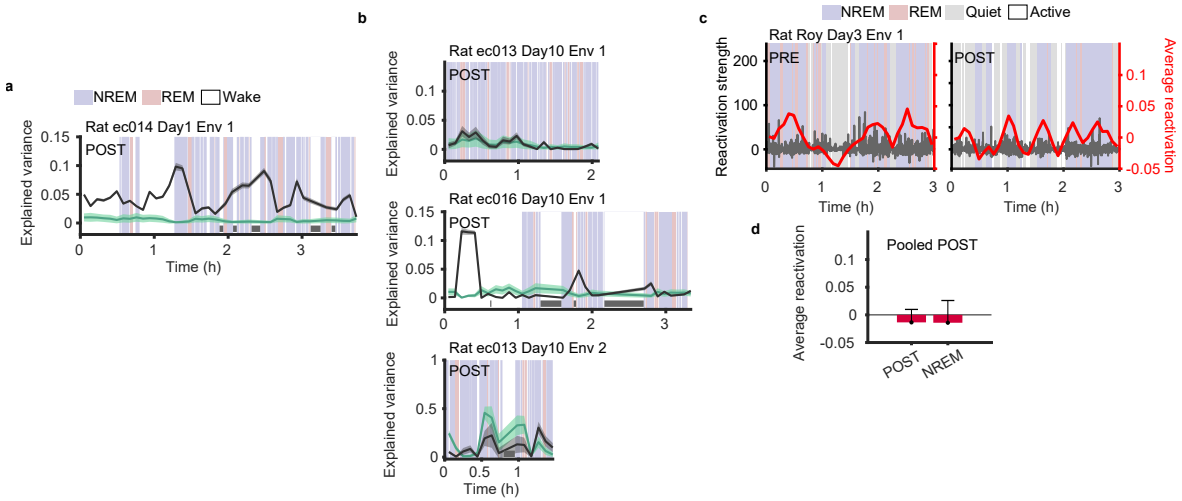


Figure 2.6: Limited reactivation in sleep following behavior in a familiar environment. a) Extended reactivation (EV; mean ± SD from 42 cells, 567 pairs) is observed in POST following a 65-min session on a novel track from the Mizuseki dataset. Sleep/wake states are indicated in the background. b) In contrast, very limited reactivation (EV; mean ± SD from 3 sessions from the Mizuseki dataset; 44 cells, 248 pairs) is observed in POST following behavior on a highly familiar track (> 10 exposures). Horizontal gray lines along the x-axis indicate intervals during which recording was paused by the experimenter. c) (Re)Activation of a sample ICA assembly (gray) is shown in PRE (left) and POST (right) panels for the Roy Day 3 Env 1 session, with sleep/wake states shown in the background. The average (re)activation strength of all 20 cell assemblies for this recording session is overlaid in red (mean in 15 min windows sliding in 5-min steps; right axis). d) Reactivation strength pooled across 4 POST sessions following MAZE in a familiar environment show evidence for little or no reactivation ($p = 0.6$ for all POST; $p = 0.74$ for NREM only; one-tailed one-sample t-test against mean 0). Error bars denote $\pm SEM$.

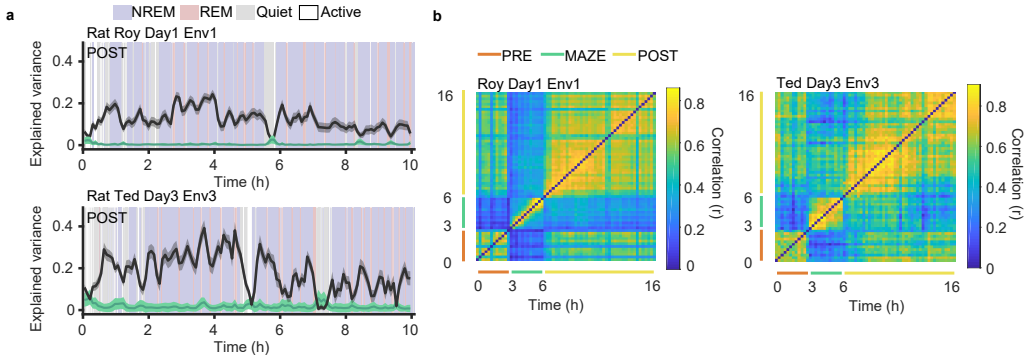


Figure 2.7: Reactivation in long-duration sleep recordings. In long-duration recordings from two sessions from two different animals, a) replay (EV, black, mean ± SD versus REV, green, mean ± SD) persisted for the duration of the recording (timestamp corresponding to half-maximum = 6.55 h for rat R, 58 cells, 1332 pairs and = 6.01 h for rat T, 17 cells, 102 pairs). b) The vector of pairwise correlations was compared across the recordings (from PRE to MAZE to POST in 15-min bins). As noted, strong correlations exist within and across PRE and POST sleep, though correlations with MAZE are stronger in POST for several hours. As sleep progresses, different pairs dominate, with weaker correlations across, rather than within, late sleep and early sleep. See Fig. 2.9 for additional sleep sessions across entire light/dark cycles.

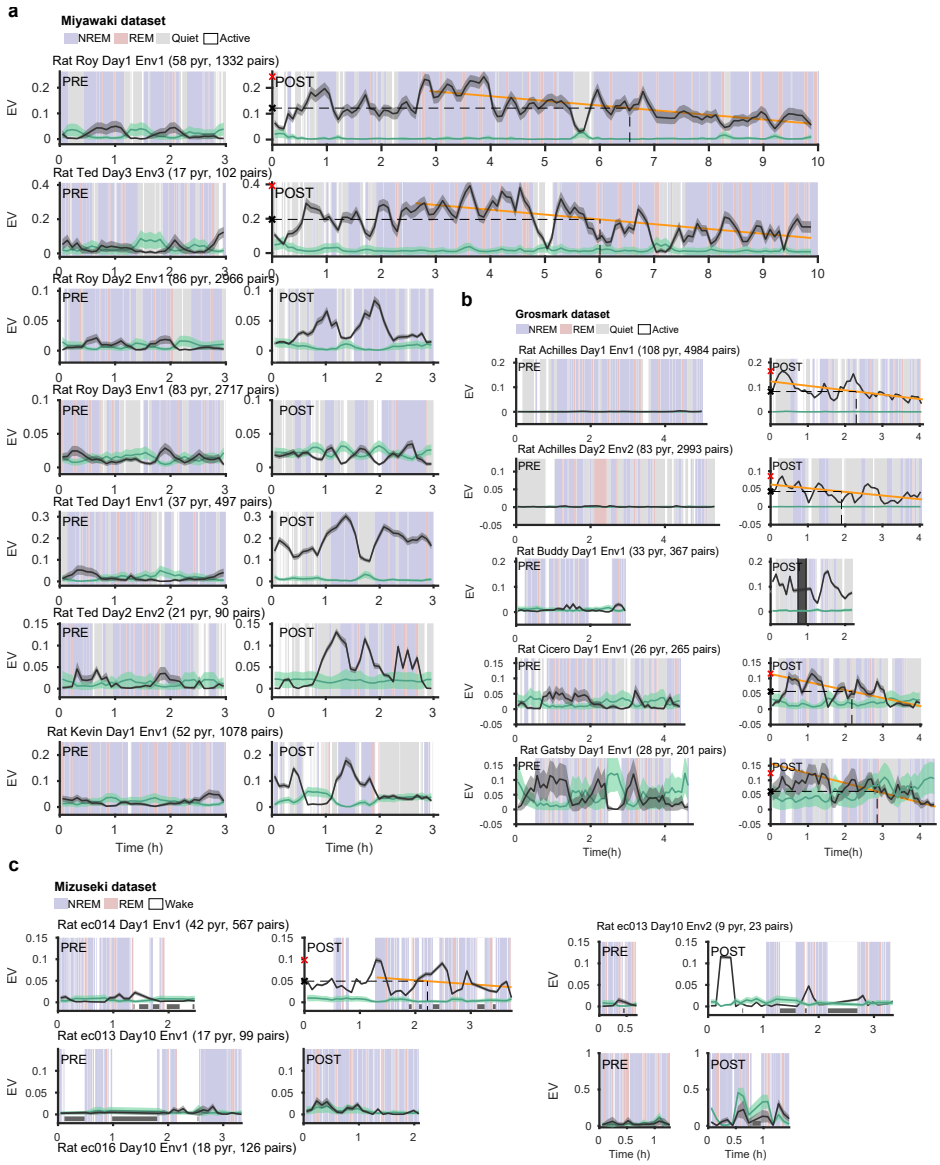


Figure 2.8: (Extended data related to Fig. 2.1). POST reactivation for each session shown separately. a) Explained variance (EV, black; mean ± SD) and time reversed EV (REV, green; mean ± SD) measured in 7 sessions from 3 animals in the Miyawaki dataset. Animal name, recording day, environment label (e.g, Env1) and number of putative pyramidal cells (e.g, 58 pyr) used in the analysis are indicated on top of each panel. Detected brain states are shown in background. Note that all sessions were performed in novel environments (numbered Env1 to Env3), except for RoyDay2Env1 and RoyDay3Env1, which featured repeated exposures to the same maze as RoyDay1Env1 and were therefore not pooled with the others for the novel MAZE analyses in (Fig. 2.1), (Fig. 2.2), and (Fig. 2.4). b) Similar to (a) shown for the Grosmark dataset (Grosmark et al., 2016). Blackened background in BuddyDay1Env1 indicates a period that contained excessive high-frequency noise that temporarily prevented reliable unit identification. c) Similar to (a) shown for sessions from the Mizuseki dataset. Horizontal gray lines along the x-axis indicate intervals during which the recording was interrupted by the experimenter. In sessions in which reactivation appeared to decay over time, we determined the maximum EV (red circle on y axis), then found the best-fit line (shown in orange) for the EV for windows with ¿ 50 % NREM sleep from 1 h before the max EV time point to the end of the session (see Materials and Methods). We then used the best-fit line to determine the time point (time constant) corresponding to the half maximum of the EV.

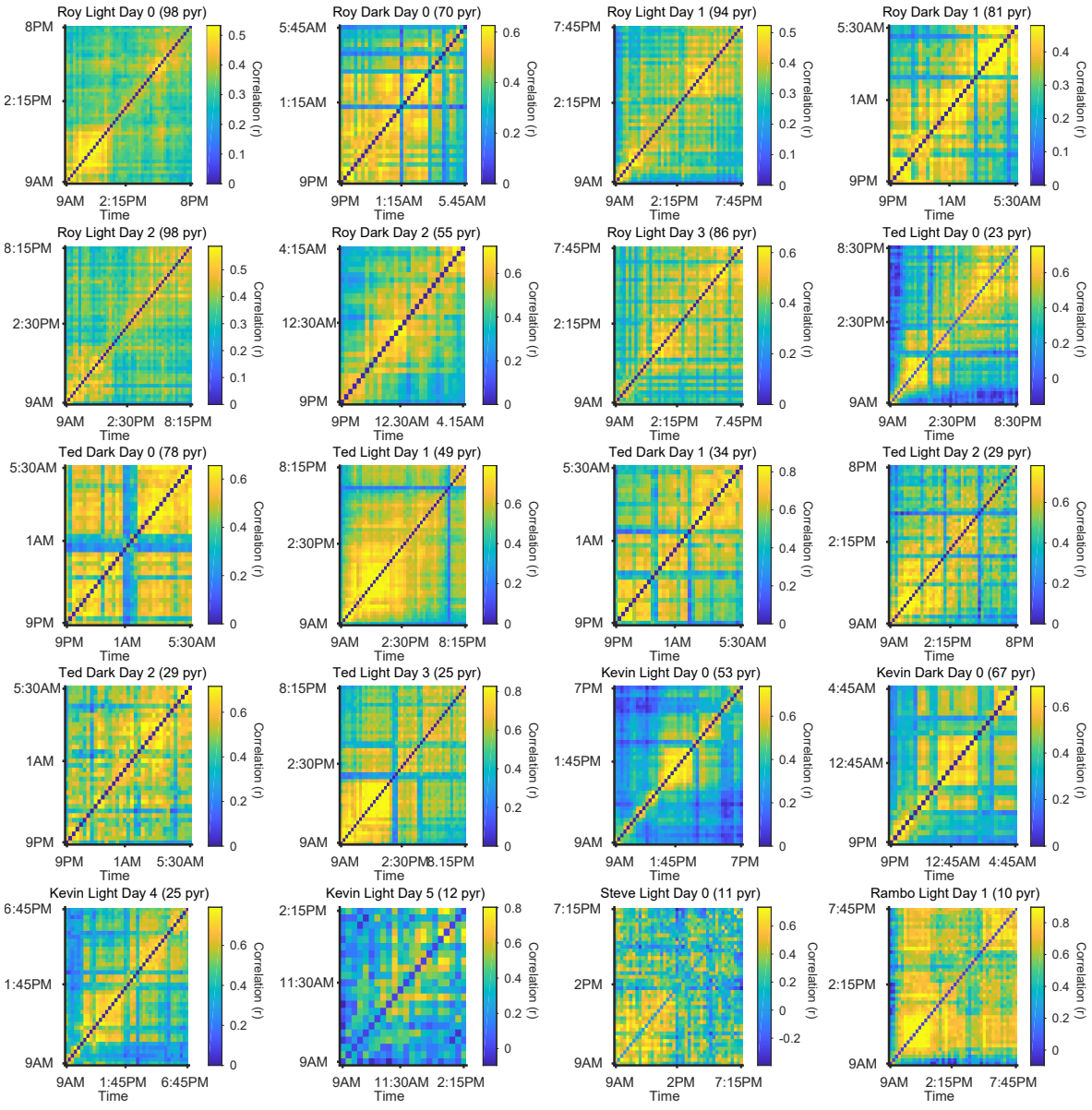


Figure 2.9: (Extended data related to Fig. 2.7). Co-activation persists for several hours during light and dark cycles. Twenty sessions from five different animals freely sleeping/waking in the home cage during light/dark cycles. Animal names, cycle, day of recording and of putative neurons are provided on top of each correlation matrix. Some of these sessions overlapped with the Miyawaki sessions used in the PRE/POST analyses but were reclustered to track units over the periods shown. Methods were otherwise identical to those used in the Miyawaki dataset as described. Matrices show pairwise correlations of each time window compared across the entire sleep session (15 min bins). In each 15-min bin, a vector of all neuronal pairwise correlations was computed; the correlation coefficients of these vectors were then calculated to determine similarity across time. While there was variability across sessions, many sessions feature similar pairwise correlations that persist for several hours that are then replaced by other dominant pairs.

3. Sleep facilitates, but sleep deprivation impedes, long-lasting hippocampal replay following novel experience

Chapter 3 is draft manuscript for journal submission:

Authors: Bapun Giri, Utku Kaya, Kourosh Maboudi, Ted Abel, Kamran Diba

3.1 Abstract

Memories are considered to improve or consolidate over the course of sleep, and studies using sleep deprivation indicate that the first 5 hours immediately following learning are critical for memory consolidation. One of the key mechanisms suggested for sleep-mediated memory consolidation is hippocampal replay during sleep, where neuronal patterns expressed during waking experience are reactivated during sharp-wave ripples (SWRs), brief periods of high frequency (125-250 Hz) oscillations. In support of this conjecture, our previous work demonstrated that hippocampal reactivation continues for several hours ($\sim$6h) in SWRs during sleep following novel experience. We next asked how hippocampal reactivations are affected when animals are subjected to prolonged wakefulness instead of sleep. To investigate this, we carried out long term recordings (>14h) from high density silicon probes implanted in CA1 area of the rat hippocampus. After $\sim$3h of baseline activity, animals were put in a novel environment and then for 5h were either sleep deprived by gentle handling or left undisturbed in their homecage. We observed that the rate of SWRs showed no decay during sleep deprivation as opposed to its gradual decay during regular and recovery sleep. Also, internal frequencies of SWRs during sleep deprivation were significantly higher compared to SWRs of regular sleep. Despite abundant SWR activity, standard methods using explained variance and Bayesian decoding revealed that reactivation and replay are significantly lower, and decay more quickly in sleep deprived animals compared to control animals. Additionally, even in recovery sleep following sleep deprivation we failed to observe reactivation, indicating that reactivations do not recover in "recovery sleep". Combined, our results suggest that poor memory reactivation may underlie sleep deprivation-associated memory deficits.

3.2 Introduction

Memories are consolidated over many hours of sleep [Rasch and Born, 2013], but disrupted by few hours of sleep deprivation [Havekes and Abel, 2017]. Previous studies have shown that memories are vulnerable to sleep deprivation when performed immediately, not hours, after the initial task acquisition [Palchykova et al., 2006, Smith and Rose, 1996, Smith and Rose, 1997], suggesting sleep right after learning is crucial for long-term memory formation. Specifically, researchers have indicated a five-hour time window from the onset of the light cycle to be critical for memory consolidation processes. In addition, studies have also observed that hippocampal memories are more susceptible to sleep interruption compared to memories that do not require the hippocampus for long-term storage [Graves et al., 2003, Smith and Rose, 1996, Smith and Rose, 1997]. Experimenters investigating unit activity in the hippocampus have observed that hippocampal neurons reactivate or 'replay' wake-induced neuronal patterns during subsequent offline periods such as sleep. This has led many to propose replay to be one of the key mechanisms for sleep-mediated memory consolidation [Buzsáki et al., 1983, Marr, 1971]. In support of this hypothesis, our previous work demonstrated that hippocampal replay continues for hours in the sleep [Giri et al., 2019], which has been corroborated by few other studies [Gridchyn et al., 2020]. However, it is not well understood how sleep deprivation in the first five hours of the light cycle affects long-lasting hippocampal reactivation. Using long-duration and high-density electrophysiology recordings from the dorsal CA1 area of the hippocampus, we compared hippocampal activity, in particular the replay, between undisturbed and sleep-deprived animals following exploration in a novel environment.

3.3 Materirals and Methods

3.3.1 Animals and surgical procedures

Three male and three female Long-Evans rats weighing (300-500 grams) were used in this study. All surgeries were performed on isoflurane anesthetized (1.2 %) animals head fixed on a stereotaxic frame. After removing hair from the head, incision area was cleaned using alcohol and betadine. Next, incision was made to expose the skull underneath. Skull was cleaned of tissues and blood, after which hydrogen peroxide was applied. Coordinates for probe implantation were marked above the dorsal hippocampus (AP:-3.36 , ML:$\pm$2.2) following measurement of bregma and lambda. Craniotomies were drilled at the marked location. Using a blunt needle, dura was removed carefully to expose the brain surface. Post cessation of bleeding, probe implant was performed next. Animals were implanted with 64 channel (8 shanks, buzsaki probe) or 128 channel (8 shanks, diagnostic biochips) silicon probe. Ground and reference screws were fixed over the cerebellum. Craniotomy was covered with dowsil-silicone and wax. A copper mesh was built around the probe for protection and electrical shielding.

3.3.2 Behavior

Prior to probe implant surgery animals were habituated to the experimenter for 40 minutes for 5 days. Following habituation animals were water restricted and trained on associating water with plastic water wells. During post-implant recovery period (7 days) animals were brought to the recording room for monitoring electrophysiology signals and probes were slowly lowered to the dorsal CA1 region of the hippocampus. In addition, animals were also habituated to sleep box for ¿1 hour every day. Following this, animals were water restricted for at least 24 hours before experiments. Experiment session began by transferring animals to their sleep box $\sim$4 hours before the onset of light cycle. After 3 hours of recording in

the homecage, animals were transferred to a novel track. Animals alternated for $\sim$ 1 hour between two water wells fixed at either ends of the track to retrieve water rewards. Following exploration, animals were transferred to their sleep box and the recording continued for 10 hours. Animals had access to ad libitum food.

3.3.3 Sleep deprivation protocol

Animals were sleep deprived in their homecage using 'gentle handling' [Colavito et al., 2013, Prince and Abel, 2013, Havekes and Abel, 2017]. This procedure was followed to minimize the effects of stress. Animals were extensively habituated to experimenters conducting the sleep deprivation. During initial hours of sleep deprivation, animals were kept awake by making mild noises, taping or gentle shaking of the cage when any signs of sleepiness appeared. In addition, other techniques such as gently stroking animal's body with soft brush or disturbing bedding were employed to increase the efficacy of sleep deprivation. As sleep pressure is enormous towards the end of 5h sleep deprivation, animals were occasionally handled to ensure they stay awake. Sleep deprivation starts at the onset of light cycle and post sleep deprivation animals are allowed to continue with their regular sleep-wake cycle.

3.3.4 Data acquisition

Electrophysiology data was acquired using OpenEphys board (Siegle et al., 2017)or Intan recording controller sampled at 30 kHz. For analysis of lower frequency activity (0.5-600 Hz), signals were downsampled to 1250 Hz. Animal's position on track was obtained using Optritrack, which uses infrared to locate a 3d markers that were clipped to animal's crown. Position data was sampled at either 60 Hz or 120 Hz and later interpolated for aligning with ephys data. Water rewards during alternation on track were delivered via water pumps interfaced with custom built hardware using Arduino. The timestamps for water delivery were recorded via TTL pulse through OpenEphys GUI.

3.3.5 Spikesorting, classification, and stability

All datasets went through filtering, thresholding and automatically sorting using Spykingcicus [Yger et al., 2018], followed by manual inspection and reclustering using the Phy package (https://github.com/cortex-lab/phy/. Only well isolated units were included for analysis. Putative neurons were classified into pyramidal and interneurons based on peak waveform shape, firing rate, and interspike-interval. To ensure that a given neuron is reliably tracked across the recording duration, we divided each session into 5 equally sized bins ($\sim$2.5 h) and excluded any unit that fired below 25% of its overall mean in any given time bin. All LFP and unit analyses were performed using custom codes written in PYTHON and are available in our lab's GitHub repository (https://github.com/diba-lab/NeuroPy).

3.3.6 Sleep scoring

Sleep scoring was performed using correlation EMG, theta, and delta power. Correlation EMG was estimated by summing pairwise correlations across all channels calculated in 10 s time windows with a 1 s step. For theta power, channel having highest mean power in theta band was selected. Following theta channel selection, Fourier power in 5-16 Hz was estimated. Transitions between high and low values in each parameter were estimated using Hidden Markov model. Periods with low and high EMG power were labeled as sleep and wake, respectively. The theta (5–10 Hz) over delta (1– 4 Hz) plus (10 –14 Hz) band ratio of the power spectral density was used to detect transitions between high theta and low theta, using custom python program, followed by visual inspection. Sleep states with high theta were classified as rapid eye movement (REM) and the remainder were classified as NREM. Wake periods with high theta were labeled as "active" and the remaining were labeled "quiet". All detected states went through additional visual inspection and were occasionally corrected to fix any misclassification.

3.3.7 Sharp wave ripple detection and estimating related properties

Ripples were detected using an automated algorithm. Multiple channels were used for detecting ripples. Best channels from each shank were selected based on highest mean power in the ripple band (125-250 Hz). Hilbert amplitude was averaged across all selected channels, then smoothed using a gaussian kernel (=12.5 ms) and then zscored normalized. Putative ripple epochs were identified as timepoints exceeding 2.5 s.d. and start/stop were extended to 0.5 s.d.. Epochs with duration below 50 ms and greater than 450 ms were excluded from further analyses. Maximum zscore value and the corresponding time within a ripple epoch were taken as that epoch's peak power and peak time respectively. Sharp wave amplitude was estimated using bandpass (2-30 Hz) filtered LFP as the difference between maximum and minimum value across all recorded channels within a given ripple epoch. Peak frequency within a ripple epoch was estimated using complex wavelet transform. LFP was first high-pass filtered above 100 Hz. This filtered signal was then convoluted with complex Morlet wavelets with central frequencies selected from linearly spaced frequencies in the band of interest (100 to 250 Hz). Within a ripple, frequency with maximum absolute wavelet power was designated as peak ripple frequency.

3.3.8 Population burst events

Multiunit activity (MUA) was used to detect PBEs. Within a session, all putative spikes from all clusters were binned in 1 ms time bin and smoothed using a Gaussian kernel of 20 ms. A PBE event was defined if peak MUA activity exceeded a threshold of 3 std with start and stop times were extended to values above mean. Events occurring within 10 ms of each other were merged. Events with duration below 80 ms and above 500 ms were discarded.

3.3.9 Explained variance analysis

Explained variance measure Explained variance was calculated using method described previously (Giri et al., 2019; Kudrimoti et al., 1999; Tatsuno et al., 2006). Briefly, spike times were binned into 250 ms time bins, creating an N X T matrix, where N is the number of neurons and T is the number of time bins. Pearson's correlations, R, were determined for spike counts from neuronal pairs in 15 min sliding windows (window length 15 min, sliding 5 min steps) in PRE and POST and in the entire MAZE session to produce P, an M-dimensional vector, where M is the number of cell pairs. To reduce spurious correlations arising from cross contamination between units detected within a given shank, only pairs whose waveform similarity was below 0.8 were included. Next, to assess similarity between P vectors from different windows, the Pearson correlation R of these vectors (i.e., the correlation between cell pair correlations) was determined (e.g., R[PRE, POST], R[PRE, MAZE] and R[MAZE, POST]). Controlling for preexisting correlations in PRE, the explained variance for a 15 min window (WIN) is calculated as following:

$$EV(WIN) = \left(\frac{(R_{[MAZE,WIN]} - R_{[MAZE,PRE(k)]} \times R_{[PRE(k),WIN]}}{(\sqrt{(1 - R^2_{[MAZE,PRE(k)]})}\sqrt{(1 - R^2_{[PRE(k),WIN]})}} \right)^2 \tag{3.1}$$

averaged over all PRE(k) windows for which $WIN_{PRE(k)}$. To further assess how much of EV can be generated by chance, we also calculated the time-reversed explained variance (REV) for each window WIN, as proposed in a previous study (Tatsuno et al., 2006):

$$REV(WIN) = \left(\frac{(R_{[MAZE,PRE(k)]} - R_{[MAZE,WIN]} \times R_{[PRE(k),WIN]}}{(\sqrt{(1 - R^2_{[MAZE,PRE(k)]})}\sqrt{(1 - R^2_{[PRE(k),WIN]})}} \right)^2 \tag{3.2}$$

To extract a timescale for reactivation from each session, we first measured the maximum EV across all 15 min (sliding) windows within the first 5 hours of POST. We then determined the best-fit line starting from the maximum EV until the end of the 5-hour window (ZT 5). The time point at which the EV dropped to half of the maximum EV was then obtained

from the regression line. If this half-maximum time point fell before the starting time of POST, we adjusted it to the time point of maximum EV.

3.3.10 Place fields calculation

Prior to calculating place fields, animal's 2D positions were linearized using ISOMAP (Tenenbaum et al., 2000). All linearized positions were manually inspected to ensure its accuracy. Occupancy within 2 cm spatial bins using times when animal's speed exceeded 8 cm/s were calculated and then this occupancy map was smoothed with a gaussian kernel (sigma = 4 cm). For each neuron spike counts within each spatial bin were calculated and smoothed with a gaussian kernel (sigma = 4 cm). Then each neuron's firing rate maps were generated by dividing smoothed spike counts by smoothed occupancy map. Neurons with peak firing rate below 0.5 Hz were excluded from further analysis. For MAZE alternation two firing rate maps were generated corresponding to each running direction.

3.3.11 Sequence replay analysis

Decoding and sequence selection Before decoding, candidate PBE events were chosen if they satisfied three criteria, minimum number of active neurons (5 units), should occur when animal is not moving (speed $< 8cm/s$), and peak ripple power (averaged over CA1 channels across all shanks) within PBE should exceed 1 standard deviation. To improve upon decoding, we also included noisy stable clusters, which has previously been shown to reduce decoding error (van der Meer et al., 2017). Sequence decoding was carried out on PBE events as described previously. the number of neurons participating in each PBE should exceed Probabilities for positions $(x_1, x_2, \ldots x_P)$ on the track within each time bin was decoded using the following:

$$P(x_p|n_t) = K_t\left(\prod_{i=1}^{N} \lambda_i[x_p]^{n_{i,t}}\right)e^{-\tau\sum_{i=1}^{N}\lambda_i[x_p]} \tag{3.3}$$

where τ is the duration of the time window (20 ms) of observation, ${}_i[x_p]$ is the firing rate of $i-th$ neuron at $p-th$ position on the track, K_t is a normalization constant such that sum of probabilities equals to 1 in each time bin, nt is the number of spikes fired by each neuron in that time window. To detect candidate sequences from posterior probability matrix of PBEs, we applied procedure similar to previous studies [Carey et al., 2019, Pfeiffer and Foster, 2013]. A candidate PBE event was considered 'replay' if it depicted continuous trajectory across space and time i.e., the distance between decoded locations in two adjacent bins should be below 40cm and the total length of the sequence should be greater than or equal to 60ms.

3.4 Results

We analyzed single unit recordings obtained from silicon probes implanted in the CA1 subregion of the hippocampus. Each recording session was carried out over a period of ∼13 hours beginning ∼ 3.5 h before the onset of light cycle in the homecage. After ∼ 2.5 h (PRE) of rest in the homecage, animals were placed on a novel environment where they alternated for ∼ 1 h (MAZE) between two ends of a track for water reward. Following MAZE, the animals were returned to their homecage where recording continued for an additional ∼ 9 h (POST). During POST, animals were either sleep deprived for first ∼5 h (SD session) or were left undisturbed (NSD session) for ad lib sleep (Fig. 3.1*A*). Both (NSD and SD) sessions were carried out at least once in each animal in a pseudo-random order.

3.4.1 Sleep deprivation alters E/I balance

To assess the effects of sleep deprivation on hippocampal activity, we first quantified the firing dynamics of hippocampal neurons throughout the duration of the recording. All putative units were classified using standard techniques and only units that met a strict stability criterion were included in subsequent analyses that resulted in 754 pyramidal neurons (PN) and 96 interneurons (IN) (see Methods). Consistent with previous studies (Miyawaki Diba,

2016), in both NSD and SD sessions pyramidal and interneurons significantly increased their firing rate during MAZE (PN firing rate = $233 \pm 35.71\%$, $p = 1 \times 10^{-4}$; IN firing rate = $127 \pm 5.43\%$, $p = 1.36 \times 10^{-6}$). However, both groups showed different dynamics during the light cycle (Fig. 3.1*B-D*). For easy comparison, we quantified firing rates in ∼2.5 h time windows within a session that are labeled as: PRE, 0-2.5, 2.5-5 and 5-7.5 (Fig. 3.1*C-D*). During regular sleep, PNs gradually decrease their firing rates from early to late hours of the light cycle, where the firing rates in 5-7.5 are significantly below PRE levels (0-2.5 vs 5-7.5: $p = 7.76 \times 10^{-6}$, PRE vs 5-7.5: $p = 1.46 \times 10^{-3}$). In contrast, during sleep deprivation (0-2.5 and 2.5-5), PNs have significantly increased their firing rates compared to PRE (PRE vs 0-2.5: $p = 4.55 \times 10^{-3}$). However, during recovery sleep (5-7.5) PN firing rates decreased rapidly to below PRE levels (PRE vs 5-7.5, $p = 4.03 \times 10^{-4}$). Furthermore, comparing the most dense periods of sleep in either condition, we observed that recovery sleep firing rates were significantly lower compared to ZT0-2.5 of regular sleep (NSD 0-2.5 vs SD 5-7.5, $p = 3.20 \times 10^{-3}$). Next, we examine activity in fast-spiking interneurons. Like pyramidal neurons, INs during regular sleep decreased their firing rates over the course of light cycle and were significantly below PRE levels (PRE vs 0-2.5: $p = 3.93 \times 10^{-2}$, PRE vs 5-7.5: $p = 4.5 \times 10^{-3}$). Like pyramidal neurons, Ins had significantly higher firing rates during extended waking compared to PRE and decreased to below PRE levels during recovery sleep (PRE vs 0-2.5: $p = 1.78 \times 10^{-2}$, PRE vs 5-7.5: $p = 7.52 \times 10^{-5}$). Furthermore, interneuron firing rates were slightly lower in the recovery sleep compared to similar time window of the regular sleep (NSD 5-7.5 vs SD 5-7.5: $p = 4.95 \times 10^{-2}$). Given different levels of activity between the NSD and SD sessions, we calculated E/I balance to quantify network activity in both conditions (Fig. 3.1*D*). The E/I balance within each session was estimated by dividing mean PN firing rate from mean IN firing rate and normalizing by PRE. While the E/I balance decreased during normal sleep, it had an opposite trend for SD sessions. These results show that extended waking increases hippocampal activity across both the pyramidal and the interneurons. Furthermore, the hippocampus showed less activity during the recovery period

compared to the early hours of regular sleep, suggesting an overcompensation to account for the higher activity during enforced waking.

3.4.2 Effects on hippocampal ripples

Given the increased activity for both excitatory and inhibitory neurons during sleep deprivation, we asked how it affects hippocampal sharp wave-ripples. We leveraged high-density electrode layout of our recordings to estimate sharp wave amplitude, ripple power, and peak ripple frequency and examined their dynamics in both conditions (Fig. 3.2*A,B*). While the SWR associated features changed systematically from early to late hours of sleep in the NSD session, this gradual change was absent during extended waking (Fig. 3.2*A*). In NSD, following novel exploration, the sharp wave amplitude increased significantly relative to PRE and gradually decreased toward the end of the sleep (Fig. 3.2*B, leftmost panel*). However, the sharp wave amplitude during forceful waking was significantly lower compared to corresponding blocks of the NSD sessions. During the recovery period, the SWRs had much higher sharp wave magnitude compared to the SWRs from sleep deprivation and the corresponding block from NSD sessions. Next, we compared ripple power across conditions (Fig. 3.2*B, middle panel*). In agreement with previous studies, in the first block of regular sleep, SWRs had significantly higher ripple power relative to PRE (PRE: 1 ± 0.44 ; 0-2.5: 1.12 ± 0.51; $p = 2.58 \times 10^{-278}$). During normal sleep, the ripple power gradually decreased from early to late hours of the light cycle (NSD 0-2.5: 1 ± 0.44 ; NSD 5-7.5: 1.07 ± 0.49; $p = 8.31 \times 10^{-43}$). On the other hand, during sleep deprivation, the ripples had significantly lower power compared to the ripples that occurred in the corresponding blocks of the NSD condition (SD 0-2.5: 1.01 ± 0.39 , $p < 1 \times 10^{-200}$; SD 2.5-5: 1.05 ± 0.43 , $p = 5.0 \times 10^{-70}$). However, the ripple power during recovery sleep was significantly higher compared to corresponding block of NSD (NSD 5-7.5: 1.07 ± 0.49 ; $p = 1 \times 10^{-200}$; SD 5-7.5: 1.15 ± 0.54 , $p = 2.98 \times 10^{-75}$). On the other hand, ripple frequency showed the opposite trend (Fig. 3.2*B, rightmost panel*). Following MAZE, the ripple frequencies decreased significantly from early

to later hours of regular sleep. In contrast, ripples during sleep deprivation had significantly higher frequencies and only showed a marginal decrease towards the end of sleep deprivation (NSD vs SD: $p < 1 \times 10^{-105}$). During the sleep-dense recovery period, the ripple frequencies slowed down by 20 Hz and were in a range similar to the corresponding time block of the NSD session. Next, we assessed if sleep deprivation also affects the rate of ripple occurrence (Fig. 3.2*C*). Consistent with previous reports [Miyawaki and Diba, 2016, Petersen et al., 2022], the ripple rate decreased from early to late sleep in NSD sessions (NSD 0-2.5 vs 2.5-5: $p = 1.86 \times 10^{-3}$). However, this decreasing trend was not observed during sleep deprivation, where ripples occurred at a similar rate in the first and second blocks of the light cycle (NSD 0-2.5 vs 2.5-5: $p = 0.11$). Like ripples, we also made similar observations for population burst events (Fig. 3.2*D*). In summary, these results suggest that despite significant differences in SWR-associated parameters, there is abundant amounts of SWR activity during extended waking periods.

3.4.3 Sleep deprivation impedes reactivation

In our previous work we demonstrated that the hippocampus reactivates waking neuronal patterns for hours in the subsequent sleep. Here, we examined how absence of sleep immediately following novel exploration affects the hippocampal reactivation. To do so we calculated explained variance (EV), which captures similarity of pairwise correlations between MAZE and POST while controlling for pre-existing correlations in PRE [Giri et al., 2019, Kudrimoti et al., 1999, Tatsuno et al., 2006]. Like in our previous work, pairwise correlations were estimated by binning spiketrains in 250 ms time windows. We also calculated reversed-explained variance (REV), which estimates how much of EV can be generated by chance. Explained variance during POST for few individual sessions from both the conditions are shown in Fig. 3.3*A*. Consistent with our previous work, we observed long-lasting reactivation in NSD sessions where animals slept uninterrupted (Fig. 3.3*A*, left column). However, for sessions in which the animals were sleep deprived, we observed some degree of

variability in both the magnitude and the temporal extent of reactivation. In some sessions, EV was initially high but decayed quickly to chance level (Fig. 3.3*A*, RatS and RatV). While in others, EV was barely above chance levels in the entire 5 h period of sleep deprivation (Fig. 3.3*A*, RatN and RatV). Interestingly, during recovery sleep, the explained variance increased slightly above the chance levels in most of our sessions. To compare reactivation between conditions and between time windows, we calculated the difference between EV and REV for individual sessions in 0-2.5, 2.5-5 and 5-7.5 time windows (Fig. 3.3*B*). Similar to the observations made for individual sessions, reactivation in the first block of the light cycle for NSD sessions is significantly higher compared to reactivation during recovery sleep (NSD 0-2.5 vs SD 5-7.5: $p = 0.022$). However, reactivation was marginally rescued by recovery sleep as it increased significantly from the second block of sleep deprivation and was also significantly above chance levels (NSD 0-2.5 vs SD 5-7.5: $p = 0.03$; SD 5-7.5 vs 0: $p = 0.00028$). To provide an estimate for timescale of reactivation, we calculated the half-maximum decay constant, which is the time it takes for the explained variance to go below half of its peak value [Giri et al., 2019]. As expected, reactivation during regular sleep took much longer to reach half-maximum compared to sessions where animals were sleep deprived ($p = 4.22 \times 10^{-3}$)(Fig. 3.3*C*). Together, these results suggest that sleep loss is detrimental to hippocampal reactivation and highlights the importance of uninterrupted sleep immediately following learning for memory-related processes.

3.4.4 Sleep deprivation impairs sequential structure of place cell activity

Hippocampal activity during sharp-wave ripples and population burst events often exhibit temporally compressed sequences of place cells that mirror their order of activity from a recent exploration (Fig. 3.4*A*). Investigations of sequential replay has primarily been limited to brief periods of immobility occurring within a task or an hour or so of subsequent sleep. Taking advantage of our long duration recordings, we investigated how sequential replay

unfolds during sleep and how it is affected by sleep deprivation. To do so, we decoded firing patterns within PBEs using Bayesian decoding (Fig. 3.4*B*). Only PBEs that met certain criteria were included for analyses (see Methods). Since the number of neurons recorded can vary from session to session, comparing traditional measures such as radon scores and weighted correlations between conditions can be misleading. To overcome this, we evaluated the sequential structure using the distance between decoded locations in adjacent time steps (jump distance). Since good replay events typically depict continuous movement through space, we defined detected PBE events as replay if the jump distance in at least three consecutive time steps was below 40 cm. To compare the amount of replay between both conditions, we examined the proportion of PBE events that depicted this continuous trajectories or replay. As expected, the proportion of PBE events containing replay trajectory increased significantly on the MAZE and were similar between both the conditions. However, during POST, proportion of replay events in SD sessions were significantly lower compared to NSD sessions in the last two blocks of POST (NSD 0-2.5 vs SD 5-7.5: $p = 0.02$,NSD 2.5-5 vs SD 2.5-5: $p = 0.03$; NSD 5-7.5 vs SD 5-7.5: $p = 0.02$) (Fig. 3.4*C*). These results suggest that absence of sleep immediately following novel experience negatively impacts the replay of place cell activity during prolonged waking and is not recovered even during recovery sleep.

3.5 Discussion

We examined single unit activity and LFP in the hippocampus to assess the effects of sleep deprivation on hippocampal network activity. We present four main findings: (1) extended wakefulness alters E/I balance in the hippocampal network with increased activity during sleep deprivation and decreased activity during recovery sleep in both pyramidal cells and interneurons; (2) Compared to regular sleep, SWRs during sleep deprivation showed higher ripple frequency but lower ripple power and sharp wave amplitude, while recovery sleep had higher ripple power and higher sharp wave amplitude; (3) Reactivation during sleep

deprivation decays quickly and is not rescued by recovery sleep; (4) Proportion of replay sequences exhibiting continuous trajectory were significantly lower during sleep deprivation and recovery period compared to regular sleep. Taken together, these results provide evidence that hippocampal network is less efficient in replaying previous experience after acute sleep deprivation despite abundant SWR activity, which may underlie memory impairments associated with sleep deprivation.

We suggest that these findings are in line with the observations made from sleep deprivation studies, where sleep loss in the first 5 hours was detrimental to sleep-mediated memory consolidation [Havekes and Abel, 2017]. Sleep deprivation is known to disrupt molecular processes such as cAMP-PKA signaling, which are involved in stabilizing recently potentiated synapses [Vecsey et al., 2009]. In fact, even a few hours of sleep loss have been shown to impair LTP induction in the hippocampus. Reactivation has long been hypothesized to be a mechanism for maintaining potentiated synapses during offline periods. This hypothesis has also gained support from modeling work, where self-sustaining reactivation within the network or dictated by external variables can help maintain long-term LTP [Fauth and van Rossum, 2019]. Interestingly, a recent study showed that memory for a specific environment can be impaired by disrupting reactivations related to that environment, while preserving memory for another [Gridchyn et al., 2020]. In this study, we sleep-deprived animals for 5 h immediately after exploration of a novel environment and observed significantly less reactivation compared to animals that were left undisturbed in their homecage. Specifically, the magnitude and duration of reactivation were much lower in sleep-deprived animals. Although the exact relationship between LTP and reactivation has not yet been established, lack of reactivation during sleep deprivation can accelerate the degradation of LTP. However, more research is needed to understand the interaction between LTP and reactivation. Nonetheless, our results provide a system-level understanding of why sleep facilitates, but sleep deprivation impairs memory consolidation.

Our results also highlight why reactivation during sleep as opposed to in wakefulness

may be essential for long-term memory storage. As we showed in Fig. 3.3, some of our sleep deprivation sessions showed high reactivation in the first block of the light cycle when the animals are awake. However, we suggest that this awake reactivation may not be sufficient to support long-term memory. It is hypothesized that communication between the subcortical and cortical structures is essential for memory consolidation, and sleep provides an ideal time when the hippocampal-cortical dialogue can occur without interference [Marr, 1971, Buzsáki, 1989]. And some studies have linked the coordination between the two structures with the coupling of various oscillations, such as between the cortical delta waves and the hippocampal SWRs [Battaglia et al., Dec, Sirota et al., 2003]. In fact, a recent study demonstrated a causal role for delta waves and SWRs, where they improved memory recall by enhancing the temporal coupling between both oscillations [Maingret et al., 2016]. However, in our experiments, by the time the animal falls asleep during the recovery period, when the slow oscillations are at their peak, the hippocampal network was hardly reactivating MAZE neuronal patterns. This further suggests that hippocampal reactivation must occur during sleep to benefit long-term memory storage.

According to the Hebbian mechanism for plasticity, neurons that fire within a few milliseconds of each other are likely to strengthen their connections. Based on this assumption, hippocampal SWRs are considered a likely candidate to mediate plasticity among neurons [Ego-Stengel and Wilson, 2010, Girardeau et al., 2009, van de Ven et al., 2016, Sadowski et al., 2016]. However, some studies have suggested that SWRs may promote synaptic depression [Norimoto et al., 2018, Miyawaki and Diba, 2016]. In our study, SWRs during sleep deprivation had significantly lower ripple power but higher ripple frequency compared to regular sleep. The sharp waves associated with these ripples were significantly lower in amplitude, suggesting a weaker drive from the CA3 region. We also observed a higher rate of ripples during sleep deprivation. As a result, SWRs can accelerate the down-regulation of synapses in sleep-deprived animals, causing a rapid decay in reactivation.

The synaptic homeostasis hypothesis (SHY) proposes that wakefulness leads to an overall

increase in synaptic strength, while sleep renormalizes the network by causing net synaptic weakening [Tononi and Cirelli, 2014, Vyazovskiy and Harris, 2013]. Assuming that the firing rates are indicative of synaptic strengths, some studies have reported that prolonged wakefulness led to increased firing rates in cortical neurons [Vyazovskiy et al., 2009, Torrado Pacheco et al., 2021]. Consistent with this finding, we also observed significantly higher firing rates for hippocampal neurons during sleep deprivation and lower firing rates during recovery sleep. However, a direct measure of synaptic strength after extended periods of waking revealed an increased area for the axonal-spine interface [de Vivo et al., 2017]. Based on this observation, one may expect the potentiated synapses to strengthen further or stay at similar levels if an animal continues to stay awake. In other words, we would have expected a much higher reactivation of MAZE patterns during sleep deprivation compared to sleep. On the contrary, our explained variance analysis revealed a sharp decline in reactivation in sleep-deprived animals.

In general, our findings imply that sleep promotes hippocampal reactivation, while acute sleep loss in the first five hours immediately following novelty exposure disrupts long-lasting hippocampal reactivation. However, more research is needed to causally link the lack of reactivation with sleep-deprivation-induced memory impairments.

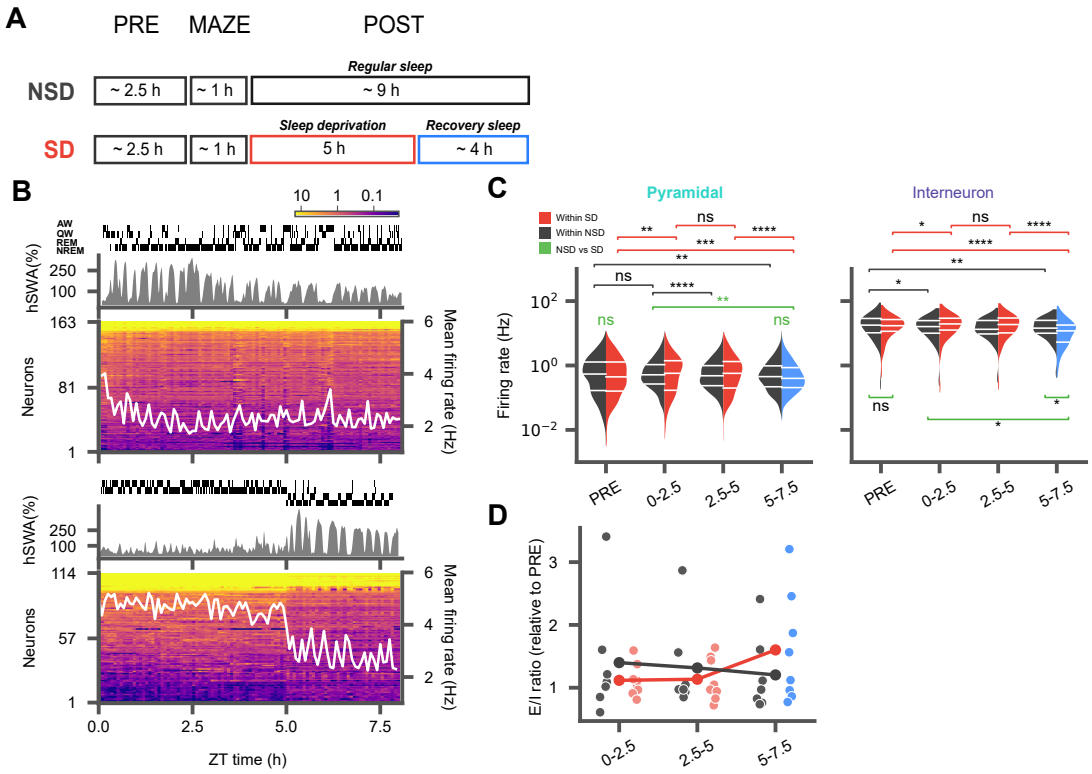


Figure 3.1: Firing-rate of hippocampal neurons across session. (A) Experimental paradigm. Following ∼2.5 h of homecage recording (PRE), animals were placed on a novel track (MAZE), after which they were returned to their homecage where they were either sleep deprived for first 5 h followed by a recovery period or left undisturbed for regular sleep. (B) Example NSD (top) and SD (bottom) sessions from one animal. Three rows in each subpanel depict hypnogram, slow wave amplitude (measured as a percentage of session mean and averaged in 1 min bins) and hippocampal unit activity (each row represents mean firing rate in 5 min bins). (C) Split violin plots depicting firing rate distributions across all pyramidal (left panel) and interneurons (right panel) estimated at various epochs (PRE, ZT 0-2.5, ZT 2.5-5, and ZT 5-7.5) for NSD (left violins, n= 7 sessions, 6 animals) and SD (right violins, n= 8 sessions, 7 animals) sessions. (D) E/I ratio across NSD (black) and SD (red) calculated by dividing mean firing rate of PN and IN neurons. Each marker represents one session. ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$)

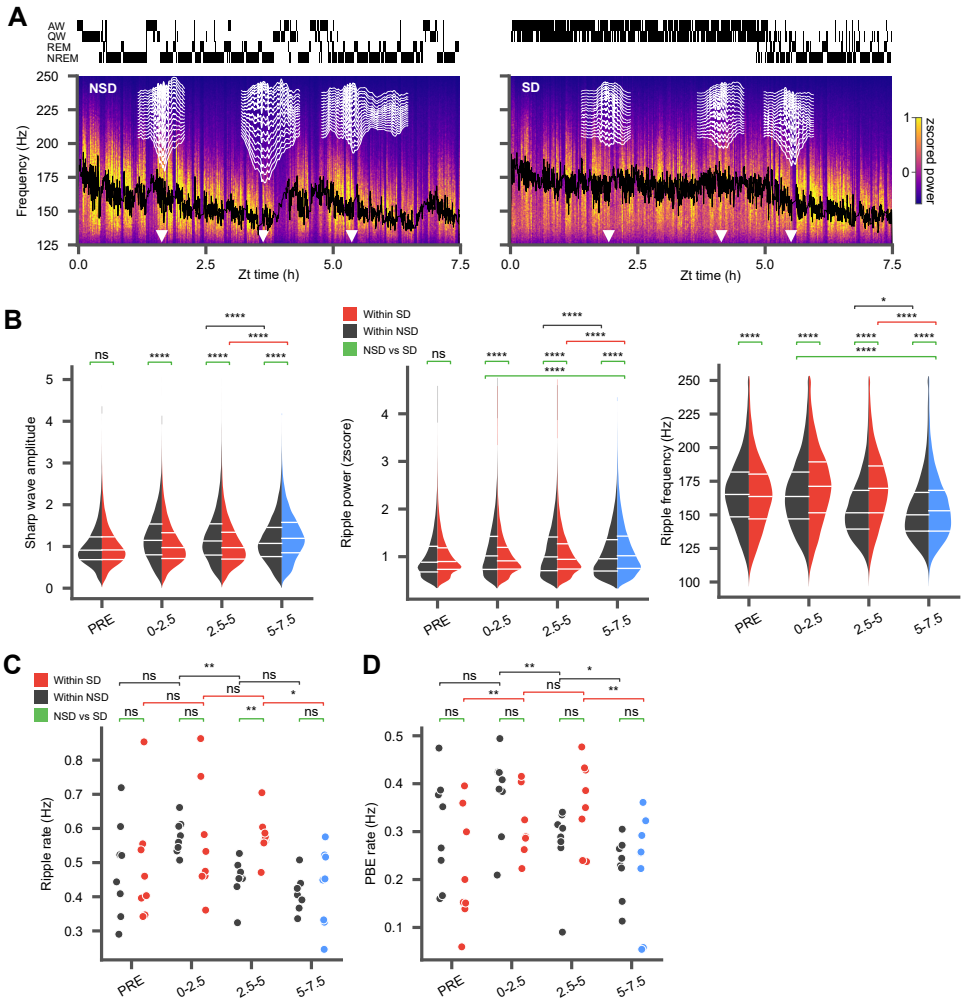


Figure 3.2: Ripple and PBEs compared between SD and NSD sessions. (A) NSD (left) and SD (right) session from one rat showing hypnogram (top) and spectrogram (bottom) of band-pass filtered (125-250 Hz) LFP from a channel in the CA1 layer. Black trace superimposed on the spectrogram depict moving average of ripple frequency. Three example SWRs (white traces) superimposed on the spectrograms showing LFP activity across multiple channels in a 16-electrode shank. White arrow heads on the x-axis depict the corresponding time for SWRs. (B) Split violin plots showing distribution of SWR-associated features at various epochs for NSD (left violins) and SD (right violins) sessions. Sub-panels from left to right represent, sharp wave amplitude, normalized ripple power, and ripple frequency, respectively. (C) Rate of ripples at various epochs compared between NSD and SD sessions. Each marker represents rate from an individual session. (D) Same as (C) but showing rate of population burst events (PBEs) at various epochs. (SD: animals = 7, sessions = 8; NSD: animals = 7, sessions = 8). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

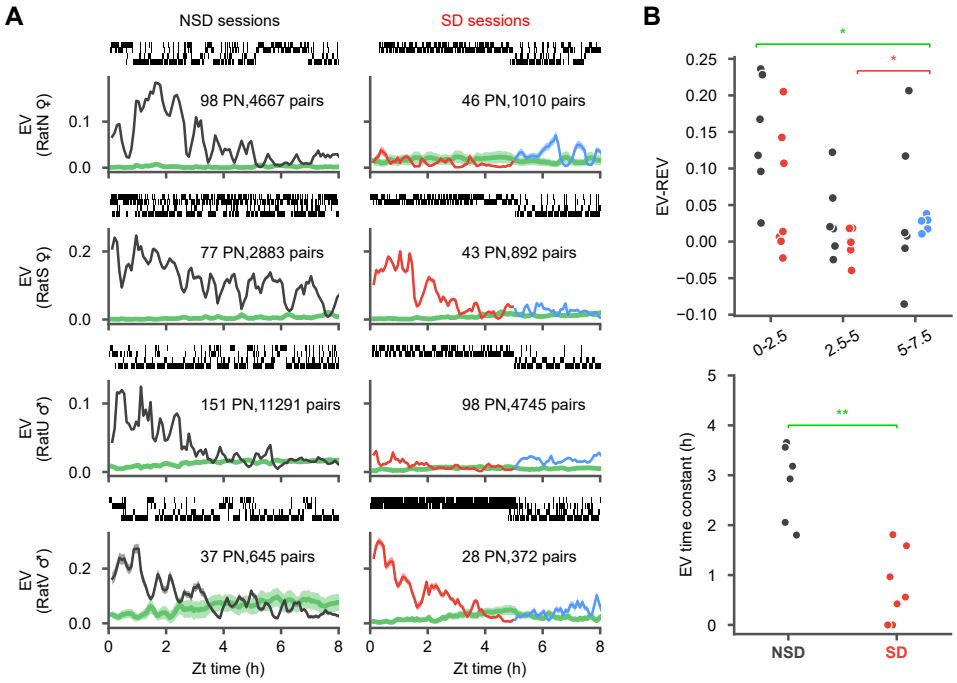


Figure 3.3: (A) EV (NSD, black; SD, red) and REV (green) during POST in NSD (left column) and SD (right column) sessions from 4 animals. Error bars indicate ± SD. (B) Difference of EV and REV calculated at ZT 0-2.5, ZT 2.5-5 and ZT 5-7.5 and compared between NSD and SD sessions. Each marker within any epoch represents one individual session. (C) Half-maximum decay constant for explained variance calculated within first 5 hours of the light cycle for NSD and SD sessions. (NSD: animals = 5, sessions = 6; SD: animals = 6, sessions = 7). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

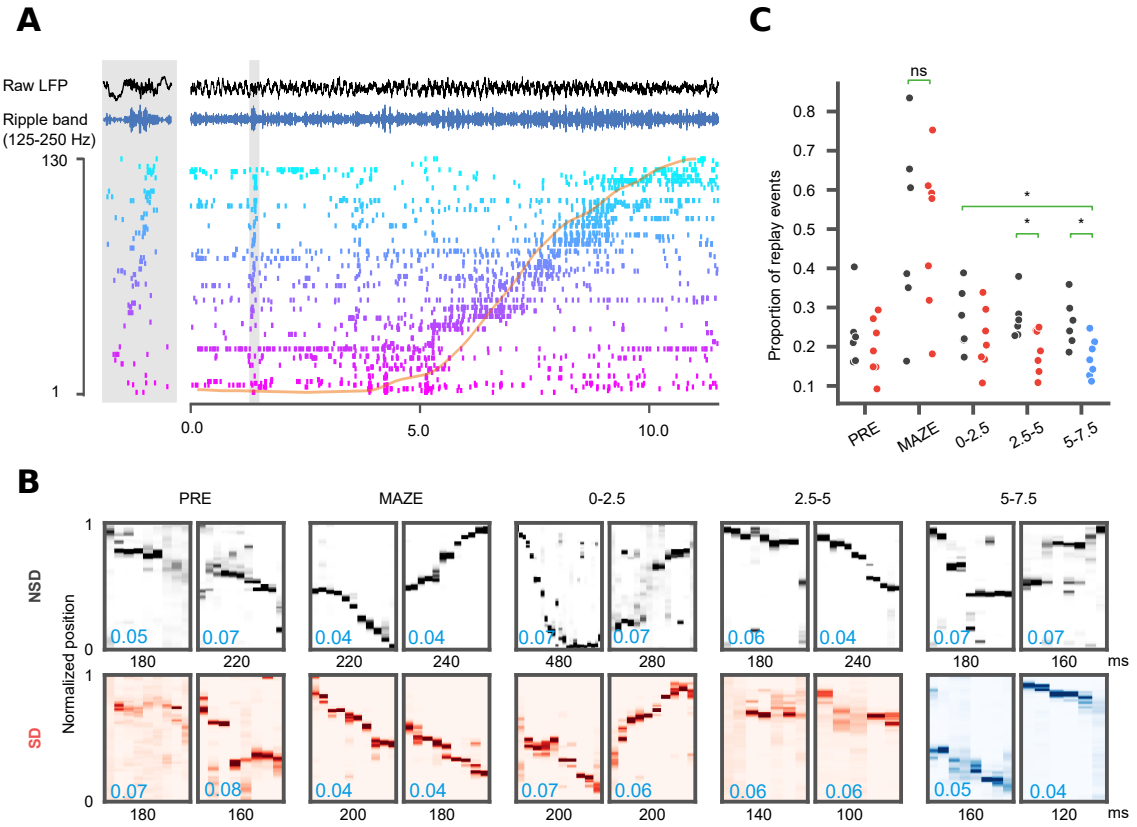


Figure 3.4: Sequential replay is deteriorated during sleep deprivation and recovery sleep.: Hippocampal activity during a sample run (orange line) on the track. Each row represents a single neuron and are ordered by their place field location on the track. Black and blue lines represent Raw LFP and ripple-band filtered traces from one electrode. Grey box represents a replay sequence. (B) Two example replay sequences shown for each of the PRE, MAZE, 0-2.5, 2.5-5, and 5-7.5 epochs. In each epoch, selected events had maximum traversed distance and mean jump distance falling in bottom 10 percent. (C) Proportion of replay sequences in each epoch. (NSD: animals $= 5$, sessions $= 6$; SD: animals $= 6$, sessions $= 7$). ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$)

4. General discussions and future directions

This dissertation adds insights into the existing hypotheses [Buzsáki, 2015, Paller and Voss, 2004] about the role of hippocampal replay during sleep in supporting learning and memory. I used previously collected datasets [Miyawaki and Diba, 2016, Grosmark and Buzsáki, 2016, Mizuseki et al., 2009] and long-duration recordings from my experiments to explore the hippocampal activity following exposure to new and familiar environments. In this discussion, I will summarize the main experimental findings from Chapter 2 and Chapter 3. Additionally, I will discuss open questions that can be investigated in future experiments to advance our understanding about sleep and memory consolidation.

In Chapter 2, we investigated the extent of hippocampal replay in sleep following exposure to a novel environment. The hippocampus is essential for transforming new information into memory traces that can be accessed for long periods of time. In parallel, studies have also shown that sleep is crucial for maintaining long-term memory. This has led many researchers to investigate how hippocampal activity during sleep may explain the underlying mechanisms of memory consolidation. In line with the Hebbian mechanism for plasticity, previous studies examining single-unit activity from the hippocampus observed that neurons co-active during exploration were more likely to fire together in subsequent episodes of sleep [Wilson et al., 1994, Kudrimoti et al., 1999]. However, reactivation of wake-induced coactivity was reported to decline rapidly within the first few minutes of sleep [Wilson et al., 1994, Kudrimoti et al., 1999, Tatsuno et al., 2006, Ji and Wilson, 2007], leading some to argue that reactivation could not possibly support memory consolidation [Sutherland et al., 2010, Tononi and Cirelli, 2014]. However, we pointed out several drawbacks in previous studies such as the usage of highly trained animals, exposure to novel objects in an otherwise familiar environment, and relatively short duration of post-task sleep recordings. We improved upon these limitations and examined the extent of reactivation in long-duration recordings in naive animals following exploration of a novel environment. We observed that the hippocampus

reactivates wake-induced neuronal patterns for up to 10 hours in sleep and decays with a half-maximum timescale of 6 hours, which is in stark contrast to previously published reports. Furthermore, periods of high reactivation were more likely to co-occur with sharp-wave ripples. The core finding of our study becomes even more prominent when we realize that sleep deprivation studies have also indicated that uninterrupted sleep in the first five hours of the light cycle is critical for memory-related processes [Havekes and Abel, 2017]. Together, our study provided important evidence for reactivation-mediated memory consolidation and highlights that reactivation may be one of the reasons why sleep is beneficial for long-term memory.

Findings from the Chapter 2 also open avenues to interesting questions that can be investigated in future experiments. The core claim of Chapter 2 was the enhanced replay occurring after novelty exposure but not following exploration of a familiar environment. However, it is not well understood what underlying mechanisms allow such long-lasting reactivation. According to synaptic tagging and capture hypothesis, potentiated synapses need to capture plasticity-related proteins for their stabilization. Dopamine has been indicated to play an important role in tagging synapses for later strengthening [Navakkode et al., 2007]. One study even observed enhanced reactivation and improved memory performance for goal location following photostimulation of dopaminergic neurons [McNamara et al., 2014]. However, the dynamics of dopamine in the hippocampus during sleep after a novel experience has not been investigated. With recent improvements in dopamine sensors [Elizarova et al., 2022, Patel et al., 2020], which are capable of measuring dopamine levels at sub-millisecond precision may help us understand the co-dynamics of reactivation and dopamine in the hippocampus. In our experiments, the animals were exposed to only one piece of novel information in the form of a maze. However, in our daily activities, we often encounter multiple pieces of novel information which may or may not be related with each other. How does the hippocampus consolidate two distinct pieces of information that are presented in succession ? Experiments that have investigated hippocampal activity in

multiple novel environments have found that place cells can encode up to 11 new environments when presented in succession [Alme et al., 2014]. However, it is not understood how memories associated with different environments are consolidated during sleep ? To do so, a future experiment can sequentially expose animals to more than one novel environment and examine how the reactivation of patterns specific to each environment evolves in sleep. Another interesting hypothesis is that hippocampal reactivation supports the strengthening of memory representations in the neocortex [Diekelmann and Born, 2010, Klinzing et al., 2019]. Traditionally, the hippocampus is seen primarily as a fast learner, while the neocortex learns at a much slower pace [Rasch and Born, 2013]. However, it is unknown how reactivation in the hippocampus influences reactivation in a cortical region like the prefrontal cortex over several hours of post-task sleep.

In Chapter 3, we examine how hippocampal replay is affected when sleep is interrupted in the first five hours of the light cycle following novel exploration. This chapter further extends the claims made in Chapter 2. Previous studies investigating the effects of sleep deprivation on hippocampal functioning have highlighted a specific time window during which sleep interruption is most detrimental to memory consolidation. In Smith and Rose (1997), post-training sleep deprivation of 4 hours or more impaired memory in the Morris water maze paradigm. Even delaying sleep deprivation by 1 h resulted in memory deficits for a fear conditioning paradigm [Prince et al., 2014]. However, animals did not show memory impairments when sleep deprivation was started much later (>ZT5) in the light cycle [Graves et al., 2003, Smith and Rose, 1996]. Based on these findings, uninterrupted sleep in ZT 0-5 was suggested to be crucial for memory consolidation. Since hippocampal replay has been suggested to support memory consolidation, it is less understood how replay is affected by sleep deprivation. We first started by exploring the effects of sleep loss on hippocampal firing rate. We observed that both pyramidal cells and interneurons increased their firing rate relative to their baseline activity. Moreover, ripples during sleep deprivation had higher frequency but lower ripple power and sharp wave amplitude. That in the absence of sleep,

hippocampal reactivation was significantly suppressed despite sufficient sharp wave ripple activity. In addition to poor reactivation, hippocampal sharp wave-ripples and population burst events were less accurate in representing MAZE activity in sleep-deprived animals compared to animals that slept ad libitum. Thus, this study provides evidence for poor hippocampal replay as an underlying mechanism for memory impairments associated with sleep deprivation.

On the basis of Chapter 3's findings, important questions remain that can be investigated in future experiments to gain more insight into sleep loss and memory. As noted earlier, the timing of sleep deprivation is important to observe impairments in long-term memory. Associated with this, some studies have reported that interfering with sleep during the dark phase of the rodent's circadian clock has no detrimental effect on fear and novel object recognition memory [Hagewoud et al., 2010b, Palchykova et al., 2009]. Although this difference may have been due to a small loss in the amount of sleep in the dark phase compared to the light cycle, it will be interesting to observe the extent of hippocampal replay during the dark phase. Schaffer collaterals arising from the CA3 directly project on CA1 area. Previous studies have shown that sleep loss affects hippocampal subregions differently, where dendritic structure in CA1 and DG neurons are negatively affected, but have no such effect on CA3 neurons [Havekes et al., 2016, Raven et al., 2019, McDermott et al., 2003]. It is unknown how CA3 activity differs from the CA1 area during the process of sleep deprivation. Some prior reports have found CA3 plays a crucial role in ripple-associated reactivation in CA1 region [Nakashiba et al., 2009]. With the advent of high density silicon probes, it is possible to record simultaneously from both CA1 and CA3 subregions, and examine reactivation of wake-induced activity during sleep and sleep deprivation. Combined, these new experiments will provide better understanding about the underlying mechanisms of sleep-deprivation associated memory impairments.

This thesis provided the first evidence that reactivation of a novel experience continues for several hours during sleep, supporting the view that repeating neuronal patterns dur-

ing offline periods may underlie memory consolidation. In addition, we demonstrated that interrupting regular sleep disrupts hippocampal reactivation, which may explain memory impairments related to sleep loss. However, future experiments probing the causal link between replay for memory tasks will provide a more holistic understanding of replay's role in memory consolidation.

A. Appendix

A.1 NeuroPy: A python library for electrophysiology analysis

Modern electrophysiology experiments often involves recording neuronal activity using a large of number of electrodes and occasionally collecting data for very long durations (>10 h). For example, one of my 16 h recordings using 256 electrodes generated 800 GB of data. This enormous data generated from each session requires preprocessing, managing, and carrying out complex analyses. To do so, I developed NeuroPy, a python library for analyzing electrophysiology data.

The notebook below provides a tutorial on how to use NeuroPy and also best practices one may follow to manage huge datasets like ours. More detailed examples can be found in the our lab's github repository (https://github.com/diba-lab/NeuroPy).

Loading recording information from Neuroscope's .xml file

In [ ]:
```
%matplotlib inline
import numpy as np
import pandas as pd
from neuropy.io import NeuroscopeIO
from pathlib import Path

xml_file = Path(
    "/data/Clustering/sessions/neuropy_demo/RatVDay1NSD/RatV_Day1NSD_2021-10-0
)
recinfo = NeuroscopeIO(xml_file)
print(recinfo)
```

Save associated data in the folder where .xml resides

In [ ]:
```
filePrefix = xml_file.with_suffix('')
print(filePrefix)
```

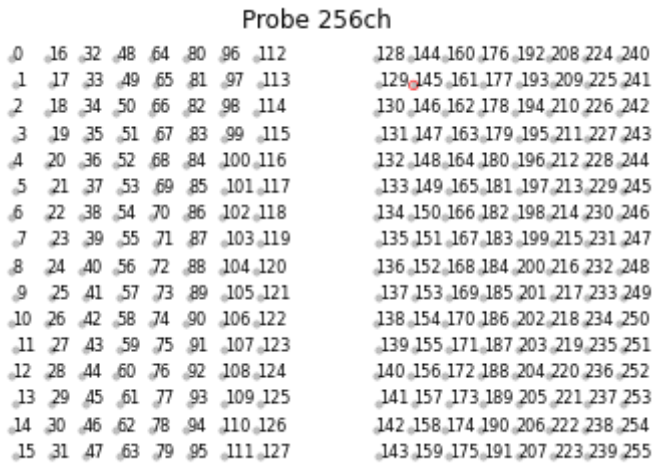
Set probe configuration

This recording sessions had two probes each having 128 channels. We can create probe configuration using NeuroPy's core objects: Shank, Probe and Probegroup.

In [ ]:
```
from neuropy.core import Shank, Probe, ProbeGroup
from neuropy.plotting import plot_probe

#Create Probe1
probe1_shanks = []
channel_groups = recinfo.channel_groups
for i in range(8):

    shank = Shank.auto_generate(
        columns=2,
        contacts_per_column=8,
        xpitch=15,
        ypitch=30,
        y_shift_per_column=[0, -15],
        channel_id=np.append(
            channel_groups[i][::2][::-1], channel_groups[i][1::2][::-1]
        ),
    )
    probe1_shanks.append(shank)
    shank.set_disconnected_channels(recinfo.skipped_channels)

probe1 = Probe(probe1_shanks)

#Create Probe2
probe2_shanks = []
for i in range(8,16):

    shank = Shank.auto_generate(
        columns=2,
        contacts_per_column=8,
        xpitch=15,
```

```
        ypitch=30,
        y_shift_per_column=[0, -15],
        channel_id=np.append(
            channel_groups[i][::2][::-1], channel_groups[i][1::2][::-1]
        ),
    )
    shank.set_disconnected_channels(recinfo.skipped_channels)
    probe2_shanks.append(shank)
probe2 = Probe(probe2_shanks)
probe2.move((probe1.x_max+500,0))

#Combine both probes into one ProbeGroup
prbgrp = ProbeGroup()
prbgrp.add_probe(probe1)
prbgrp.add_probe(probe2)

prbgrp.save(filePrefix.with_suffix(".probegroup.npy"))
plot_probe(prbgrp)
```

Out[ ]:
```
<AxesSubplot:title={'center':'Probe 256ch'}>
```



Specify epochs for your experimental paradigm

An experiment typically involves multiple epochs such pre sleep, running on track then another sleep epoch after track running.

In [ ]:
```
from neuropy.core import Epoch

epochs = pd.DataFrame(
    {
        "start": [0, 10000, 20000],
        "stop": [9000, 13600, 30000],
        "label": ["pre", "maze", "post"],
    }
)

paradigm = Epoch(epochs=epochs)
paradigm.save(filePrefix.with_suffix(".paradigm.npy"))
```

A base class that can load saved data

In [ ]:
```
from neuropy.io import NeuroscopeIO, BinarysignalIO
```

```python
from neuropy import core
from pathlib import Path

class ProcessData:
    def __init__(self, basepath):
        basepath = Path(basepath)
        xml_files = sorted(basepath.glob("*.xml"))
        assert len(xml_files) == 1, "Found more than one .xml file"

        fp = xml_files[0].with_suffix("")
        self.filePrefix = fp

        self.recinfo = NeuroscopeIO(xml_files[0])
        self.eegfile = BinarysignalIO(
            self.recinfo.eeg_filename,
            n_channels=self.recinfo.n_channels,
            sampling_rate=self.recinfo.eeg_sampling_rate,
        )

        if self.recinfo.dat_filename.is_file():
            self.datfile = BinarysignalIO(
                self.recinfo.dat_filename,
                n_channels=self.recinfo.n_channels,
                sampling_rate=self.recinfo.dat_sampling_rate,
            )

        self.probegroup = core.ProbeGroup.from_file(fp.with_suffix(".probegrou



    def __repr__(self) -> str:
        return f"{self.__class__.__name__}({self.recinfo.source_file.name})"


def ratN():
    basepath='/data/Clustering/sessions/RatN_Day1_test_neuropy'
    return ProcessData(basepath)
```

A.2 Theta-gamma coupling during novel experience

Colgin et al. [Colgin et al., 2009] were first to report that gamma oscillations observed in the hippocampus split into multiple bands, defined as slow (25-50 Hz) and fast (65-140 Hz) gamma. The authors also observed that activity in CA3 correlated strongly with slow gamma of CA1 pyramidal layer, whereas fast gamma oscillations were more coherent with the medial entrohinal cortex. In addition, they also suggested that slow and fast gamma occur at specific phases of theta oscillations, possibly serving different roles in routeing information to and from the hippocampus. Following this discovery, many studies have investigated the physiological properties of multiple gamma bands [Yamamoto et al., 2014, Amemiya and Redish, 2018, Belluscio et al., 2012, López-Madrona et al., 2020]. For example, Yamamoto et al. reported high synchrony between the entorhinal cortex and hippocampus in the fast gamma frequency range when animals made correct choices on a T-maze. More evidences have emerged in support of this idea and have claimed that fast gamma facilitates encoding while slow gamma is important for retrieval [Griffiths et al., 2019]. Some studies also suggest that slow gamma originates from the CA3 and plays an important role in pattern completion. However, some recent studies have argued against independent 'slow gamma' oscillations and have highlighted important caveats in earlier studies that have claimed multiple gamma bands [Sheremet et al., 2016, Sheremet et al., 2018a, Sheremet et al., 2018b]. Unlike pure sinusoids, theta oscillations during wake are not symmetrical and its amplitude and asymmetry increases with animal's velocity [Zhou et al., 2019]. As you need oscillations of higher frequencies to account for this asymmetry, spectral decomposition based on Fourier or wavelet, used by most of the studies, can show increased power in higher frequencies. To resolve these debates we investigated the existence of multiple gamma bands in hippocampal LFP.

Supplementary methods

Theta events and theta features estimation

Theta events were estimated by first bandpass filtering LFP during MAZE in theta frequency region (4-12 Hz). Subsequently, instantaneous theta amplitude was estimated using hilbert transform. Periods with theta amplitude exceeding 1 SD were classified as theta events. Theta events were then concatenated across sessions to obtain continuous LFP. From this LFP, theta defining features were calculated using a previously published method [Cole and Voytek, 2019, Belluscio et al., 2012]. Briefly, LFP oscillations from the hippocampus were bandpass filtered (1-25 Hz) and peaks and troughs were identified. Additionally, theta phases were estimated using hilbert transform of selected hippocampal LFP. From each cycle of theta, four features were extracted: amplitude, cycle-duration, rise-decay symmetry, and peak-trough symmetry. Here, rise and decay midpoints were defined as the time points at which the theta amplitude was halfway between the neighboring peak and trough. Now, rise-decay asymmetry was defined as fraction of theta in rising phase divided by total duration of theta cycle. Similarly, peak-trough asymmetry was defined as peak duration divided by duration of trough.

Gamma band

LFPs were bandpass filtered in the frequency range of interest and gamma band amplitude were estimated using Hilbert transform. From the resulting amplitude, periods with values above mean and peak exceeding at least 1 standard deviation were considered as putative high gamma events.

Wavelet analysis

For wavelet analysis, we closely followed previously used methods [Colgin et al., 2009, Belluscio et al., 2012]. LFP signals were convolved by a family of complex Morlet's wavelets,

defined for each frequency, as a function of time:

$$w(t, f) = A \exp(-t^2/2\sigma_t^2) \exp(2i\pi ft)$$

where central frequency, $\sigma_f = \frac{1}{2\pi\sigma_t}$ and the coefficient, $A = (\sigma_t\sqrt{\pi})^{-1/2})$. The wavelets were by defined by a constant ratio of $f/\sigma_f = 7$. Each frequency of the wavelet transform was individually normalized by the mean and SD of wavelet power across each session.

Preliminary Results

First, we assessed previously published methods to identify if recordings from our animals show independent 'slow gamma' oscillations. Using wavelet analysis, we observed distinct bands in the gamma frequency region similar to what has been reported in previous studies [Colgin et al., 2009, Tallon-Baudry et al., 1997, Belluscio et al., 2012]. However, some ambiguity does arise when we analyze LFP at multiple depths. As we move away from CA1 layer, gamma is observed as a continuous band ($\geq$ 30 Hz). One recent study by Lopez-Madrona et al. ([López-Madrona et al., 2020]) supported their claim on observing genuine slow-gamma oscillation by taking into account theta's asymmetry and other behavioral variables that govern theta power. They performed multiple regression analysis, where power in slow gamma band was found to be correlated with theta power, theta asymmetry and running speed. In that study, theta asymmetry was estimated using two quantities: the ratio between rise and decay phases in each cycle and the ratio between the duration of the peak and the trough [Cole and Voytek, 2018]. The authors reported that power in the slow-gamma band was mostly explained by theta power and other variables like theta asymmetry and running speed had negligible contribution. However, one important caveat of this analysis seems to be the exclusion of theta harmonic power as one of the variables in their multiple regression analysis. We carried out that analysis, but now including theta harmonic power, and found that power in theta-harmonic band explains significantly more variance than any

other variable. More importantly, we also note that all gamma bands are equally explained by theta and its harmonic.

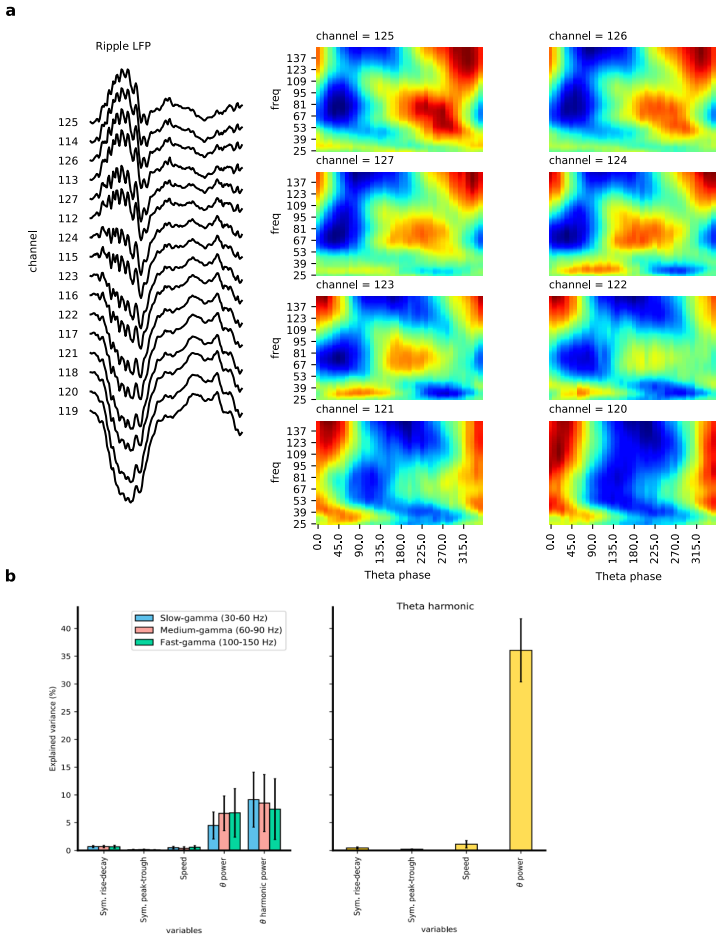


Figure A.1: Figure 5: a) Left panel shows an example ripple event across multiple electrodes. Right panels show the mean wavelet power between 25 and 150 Hz as a function of the waveform-based theta cycle phases (bin, 9 degrees) during MAZE b) Multiple linear regression with theta power, harmonic power, speed, and theta symmetry, measured as the ratio between duration of rise phases vs. decay phases (sym. rise-decay) and between duration of the peak and the trough (sym. peak-trough; see supplementary methods)

Slow gamma oscillation occurs at specific phases of theta. In fact previous studies have reported multiple gamma bands at descending phases of theta. To alleviate concerns regarding non-linearity of theta contributing to power in slow-gamma frequency range because of theta harmonics, we decomposed theta into constituent phases. We investigated if time

points at particular phases of theta concatenated across the session will make gamma more salient. To do so, we estimated theta phases from broadband theta LFP and then divided phases within each theta cycle into multiple bins. Next, theta was highpass filtered ($>$ 25 Hz) and LFP segments corresponding to each phase bin were concatenated across time. A schematic of the whole process is shown below (Fig. A.2*a*).

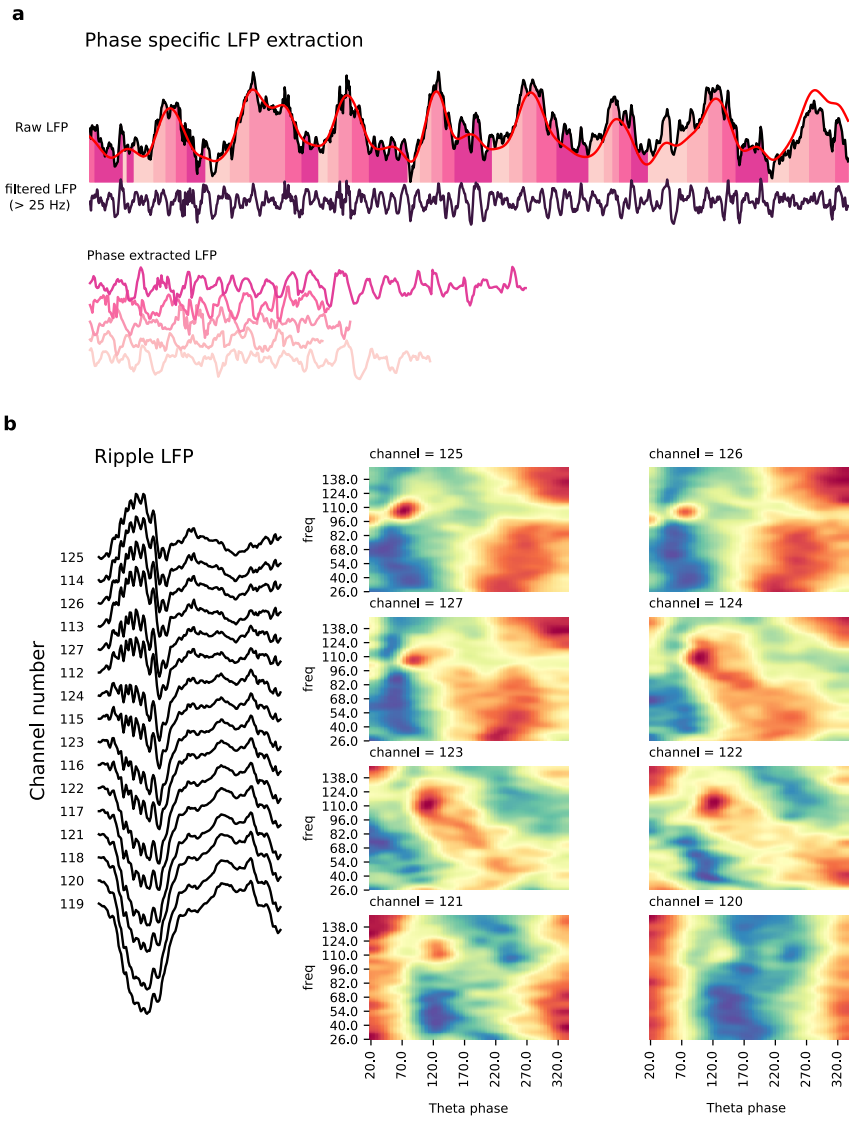


Figure A.2: Figure 5: a) Schematic of phase extraction process. Raw theta (black) and broadband theta (red) used to estimate theta phase. Phases were divided into bins (shaded region) which were used to extract corresponding timepoints from filtered LFP (purple) b) Raw LFP during ripple (left) with spectral decomposition for LFP at multiple depths.

Next, we calculated power spectral density for each phase specific LFP using Fourier analysis at multiple depths. We could only observe a continuous band in the gamma frequency region (25–90 Hz) and found no evidence for gamma splitting into multiple bands.

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