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The Role of Oriens-Lacunosum Moleculare Interneurons in Offline Processing of Spatial and Contextual Memory

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The Role of Oriens-Lacunosum Moleculare Interneurons in Offline Processing of Spatial and Contextual Memory

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Neuroscience

by

Michelle Frazer

2024

ABSTRACT OF THE DISSERTATION

The Role of Oriens-Lacunosum Moleculare Interneurons in Offline Processing of Spatial and Contextual Memory

by

Michelle Frazer

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2024

Professor Gina Rochelle Poe, Chair

The ability of an organism to survive often rests on the degree to which it is able to learn about its environment, separate useful information from noise, and file it away to be rapidly recalled at the appropriate time. For animals in the wild, this often amounts to recalling where food is buried, or what to do to avoid capture when a hawk flies overhead. For humans, our ability to retain and access memories over the years is arguably the crux of what it means to have a sense of self and form a cohesive narrative of our lives. Indeed, some of the most devastating psychiatric and neurological disorders strike directly at this ability. Alzheimer's disease and other dementias rob individuals of past experiences that form the fabric of their lives and personalities; psychiatric disorders such as schizophrenia dampen a person's mental acuity and flexibility, as well as the ability to distinguish between important and unimportant

information. Ultimately, understanding how memories are encoded and stably stored through time is an important window through which we can better understand the things that make us who we are, and the consequences that occur when this process is disrupted. As such, interrogating the mechanisms underlying memory formation and storage is one of the most pressing topics in neuroscience at the moment.

In chapter 1 of this dissertation, I will provide a discussion of concepts foundational to the study of learning and memory, aiming to describe the multiple scales across which neuroscientists study the formation and storage of information, from molecular mechanisms, cellular physiology, synaptic connections, and network activity. I will also discuss the unique aspects of hippocampal anatomy, cellular composition and physiology that have evolved to position this structure as the initial locus of memory traces.

Chapter 2 discusses the role of sleep in memory consolidation, aiming to describe the importance of each sleep stage and their unique physiology on facilitating strengthening and storing the labile memory traces formed during waking. In particular, I focus on what modern approaches allowing us to selectively modulate the activity of neural subsets can tell us about the circuit and cellular mechanisms underlying offline consolidation, which may have been obscured by methodological constraints in earlier sleep research.

In chapter 3, I describe experiments that investigate the role of a subpopulation of hippocampal interneurons, oriens-lacunosum moleculare (OLM) cells, in promoting sleep-dependent memory consolidation. By inhibiting this cell population selectively during offline periods between task acquisition and recall, we found that OLM cells in the hippocampus are necessary for offline spatial and contextual memory consolidation. Through *in vivo* recordings of brain activity with and without OLM inhibition, we linked this cell population to oscillatory activity during rapid eye movement (REM) sleep periods. These experiments are among the first to

posit a role for OLM cells in sleep-dependent consolidation and provide further evidence for the necessity of sleep to the formation of and maintenance of memories.

The dissertation of Michelle Frazer is approved.

Peyman Golshani

Hugh T. Blair

Christopher S. Colwell

Gina Rochelle Poe, Committee Chair

University of California Los Angeles

2024

To my many families.

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I had an idea before starting graduate school that it was going to be a long haul, but between moving the Poe lab across the country and getting through a global pandemic, I think it's safe to say I underestimated the challenges. Getting through them all has required the help of many, and this will certainly be an incomplete list of acknowledgements.

Thank you of course to my mentor Gina Poe, for your indefatigable optimism and your trust and belief in me that didn't waver, despite my own uncertainties. Thank you to my committee members, Peyman Golshani, Tad Blair, and Chris Colwell, who have been so generous with their time and resources over the years as I worked to complete this dissertation. A huge thank you to Jenny Lee and all the NSIDP administration for their guidance and assistance. My deep appreciation to the Kullander lab at the University of Uppsala, for giving us access to their transgenic mouse line, used in the experiments described within. I would also like to acknowledge the funding sources that made this work possible, particularly the Ruth-Kirschtein National Research Service Award (F31-MH125640-01) that allowed me to complete much of this work.

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Thank you to all members of the Poe lab, past and present, for making me laugh, offering support/commiseration and for never complaining that I eat way more than my fair share of the communal lab food. Thank you to my co-authors on the manuscript presented in

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Vita

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E. D., Sutton, M. A., Taylor, J. P., Mills, R. E., Charlet-Berguerand, N., & Paulson, H. L. (2013). CGG Repeat-Associated Translation Mediates Neurodegeneration in Fragile X Tremor Ataxia Syndrome. *Neuron*, *78*(3), 440–455.

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Chapter 1

Learning and memory at the circuit and cellular level

1.1 Systems of memory

Memory is not a singular concept; rather, it is an umbrella term that encompasses multiple systems with distinct functions. The main division of memory is between those memories that can be explicitly expressed, or “declarative memory,” and those that cannot, or “non-declarative memory.” Non-declarative, or implicit, memory refers to acquired skills or feelings that we are not able to access consciously. This system can be further broken down into procedural memory, which encompasses skills such as driving a car or riding a bike, and emotional memory, which involves changes in the valence of previously neutral stimuli following an experience, such as an aversion to a particular place following a negative experience (Dickinson and Eichenbaum, 2010). Declarative, or explicit, memory can be externalized and explained through language. In humans, declarative memory is made up of working memory, semantic memory, and episodic memory. Working memory refers to information that is held and manipulated in the short term; for example, performing mathematical computations on a series of numbers without using pencil and paper. Semantic memory is our internal encyclopedia, through which we can recall learned knowledge. Episodic memories are how we describe the events of our lives–when and where something took place, who was with us when it occurred, or how we fit an event into the narrative of our life.

Many of the early mechanistic theories of memory came from observing clinical cases in which patients presented with a memory disturbance and their treating physicians attempted to trace symptomology back to an underlying structural defect. Foundational work in the 1950s observing H.M., a patient who underwent bilateral medial temporal lobe (MTL) resection to treat

severe epilepsy, continues to define our understanding of memory systems as discrete processes, and solidified the hippocampus as an irreplaceable structure in episodic memory formation. Researchers working with H.M. found that he was frozen in the present, unable to form new declarative memories, though he retained memories from prior to his surgery, and had no personality or cognitive changes aside from the memory loss. Additionally, his semantic memories formed prior to the operation remained intact, his short-term memory was unimpaired, and he was able to acquire new motor skills (Dossani et al., 2015). H.M.'s case radically changed the way the field thought about how memory works, localizing the process of episodic memory largely to the MTL and specifically to the hippocampal formation. These studies launched the modern era of hippocampal research and ignited interest in the structure and function of this region, to understand the mechanisms underlying memory formation and retention more fully.

1.2 Hippocampal anatomy

The Rennaissance surgeon Giulio Aranzi was the first to use the name "hippocampus," noting the structure's similarity to the seahorse, while the French Enlightenment anatomist De Garengeot proposed the name *Cornu Ammonis*, in reference to the ram's horn associated with the Egyptian god Amun-Ra. The singular structure of the hippocampus made it a point of interest to early anatomists and helped identify its conserved presence in brains across mammalian species (Insausti, 1993). The hippocampal system includes the dentate gyrus, subiculum, presubiculum, parasubiculum, and the entorhinal cortex (EC). All mammalian species have a version of the hippocampal formation, with little variation in architecture across the phylogenetic tree, and largely conserved general function (Insausti, 1993; Squire, 1992). Comparative studies of hippocampal function in different species are useful in understanding how the role of the hippocampus changed with evolution and provide insight into structural

correlates of increased behavioral complexity; however, as the research I discuss only involves rodents, I will restrict my discussion of hippocampal anatomy to the rodent brain.

The unique structure of the hippocampal formation is critical to understanding how it synthesizes information and transforms disparate inputs into coherent spatial and contextual schema. The primary subregions of the hippocampus are the dentate gyrus (DG) and the CA fields (CA1,CA2, and CA3). Each region is defined by the cell types present as well as their afferent and efferent inputs and computational function. The classic trisynaptic loop of the hippocampus is one of the most well-characterized circuits in neuroscience and traces the flow of information from the entorhinal cortex (EC) to the DG granule cells, which synapse on to pyramidal cells in CA3 via the mossy fiber pathway. CA3 cells then project to CA1 pyramidal cells through a fiber bundle referred to as the Schaffer collateral, after which CA1 cells send information out of the hippocampus to the subiculum and EC. In addition to the trisynaptic loop, the perforant path delivers information directly from layer III of the EC to CA1.

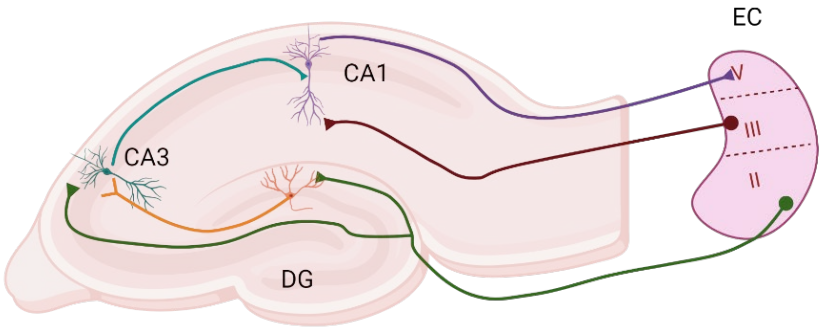


Figure 1.1: The hippocampal trisynaptic loop. Afferents from EC layer II synapse on to the granule cells of the DG via the perforant pathway (green). Granule cells project to CA3 pyramidal cells via the mossy fibers (orange). CA3 pyramidal cells synapse

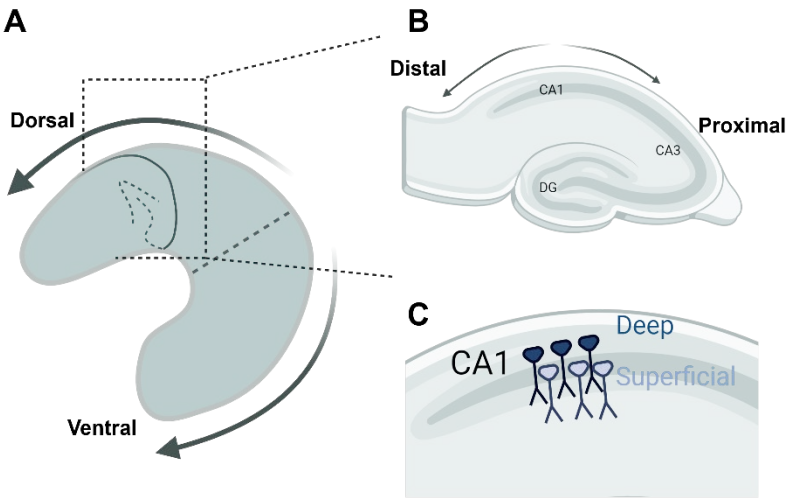


Figure 1.2: The three axes of the rodent hippocampus. (A) The longitudinal axis, connecting the dorsal and ventral poles. Also referred to as the septo-temporal axis. **(B)** The transverse, or proximodistal axis, with the most distal portion nearest to the EC, and proximal area nearest to CA3. **(C)** The radial axis, showing the layers of the hippocampus, and distinguishing between the deep and superficial pyramidal cell subgroups. Adapted from Geiller et al., 2017.

Hippocampal structure and function changes across its three spatial axes

The hippocampus can be divided along three structural axes–the longitudinal or dorsal-ventral axis, the transverse or proximo-distal axis, and the radial axis.

Hippocampal function along these axes can vary greatly, so much so that some have suggested that different areas of the hippocampus should be treated as separate regions.

A gradient of anatomical, physiological, and genetic changes along the longitudinal axis underlies a dorsal/ventral difference in function

The longitudinal axis is the “long” axis of the hippocampus, that follows the curve of the structure from its pole near the septum (dorsal) to its temporal pole (ventral) (Fig 1.2 A). Broadly, the dorsal hippocampus (dHPC) has been found to be largely responsible for spatial and cognitive functions, while the ventral hippocampus (vHPC) is related to emotional processing, mood, and anxiety responses (Faneslow and Dong, 2010). Lesions in the dHPC result in deficits in spatial encoding and memory, seen in rodents as a decreased performance

on tasks such as the Morris water maze, or the radial arm maze, while lesions in the vHPC are less likely to impair spatial tasks. The vHPC appears to be more important to emotional processing, as lesions in the vHPC reduce anxiety behaviors and impair hormonal stress responses (Bannerman et al., 2003; Moser and Moser, 1998). The differences in function along the longitudinal axis map on to the changes in connectivity from the dorsal to ventral pole. The dHPC is primarily connected to cingulate areas involved in spatial processing, such as the retrosplenial and anterior cingulate cortices, while the vHPC is densely connected with the amygdala, infralimbic and prelimbic cortices, all of which are involved in emotional regulation or fear (Strange et al., 2014). Differential gene expression patterns also distinguish between the dHPC and vHPC, including genes encoding for HCN subunits, which are important for voltage-dependent gating of *h*-channels (Bienkowski et al., 2018; Dougherty et al., 2013). The difference in HCN subunits likely contributes to the differences in excitability seen in pyramidal neurons in these two regions, with vHPC neurons intrinsically more excitable than those in the dHPC. Interestingly, these two regions also show a difference in long-term potentiation (LTP), or strengthening of synapses (discussed further below), in response to afferent inputs. dHPC neurons generate larger LTP following input into the dHPC than do vHPC neurons, though an acute stressor reverses this trend, resulting in larger LTP in vHPC cells and decreased LTP in dHPC (Maggio and Segal, 2006; Maggio and Segal, 2009). This change following stress again highlights the vHPC's role in anxiety and stress responses, further supported by the presence of anxiety-responsive cells in the vHPC, and the changes in vHPC LTP following early life stress in juvenile rats (Jimenez et al., 2018; Ivens et al., 2019). While functional distinctions do exist between the dorsal and ventral HPC, it is important to remember that these differences are truest in the most dorsal or ventral 25% of the HPC, while the change in connectivity, excitability, and gene expression gradually changes from one pole to the other (Strange et al., 2014). This graded shift from dorsal to ventral suggests that there is an intermediate region of the hippocampus that might be involved in both spatial and emotional functions to some degree,

and this is likely why some lesion studies have been inconclusive about dorsal vs ventral function–the resulting behavior following a lesion may change a great deal depending on the lesion's precise location along the longitudinal axis.

The spatial map of inputs into the hippocampus changes along the proximo-distal axis

The second of the hippocampus's three major axes is the transverse or proximo-distal axis, which imagines the hippocampus has been unfolded, and defines a location as being more distal if it is closer to the subiculum/EC, and more proximal if it is located toward CA3 (Fig 1.3). Some measure of functional dissociation exists along the transverse axis in each hippocampal subregion. The proximal CA3, closer to the DG, appears to perform pattern separation computations, similar to the DG, while the output of distal segments reflects pattern completion processes (discussed further below). The proximal and distal parts of CA3 also receive input from different DG regions and send recurrent collaterals to different locations, as well as increasing EC inputs as you move dorsally along the axis (Lee et al., 2020). A similar heterogeneity is seen across the transverse axis of the CA2, in which developmental genes such as *Sox5* are differentially expressed, the intrinsic physiology of principal cells changes, and principal cell modulation by oscillatory activity differs along this axis as well (Fernandez-Lamo et al., 2019). Arguably the largest differences along the transverse axis are found in the CA1 region, in which the temporoammonic pathway input from the

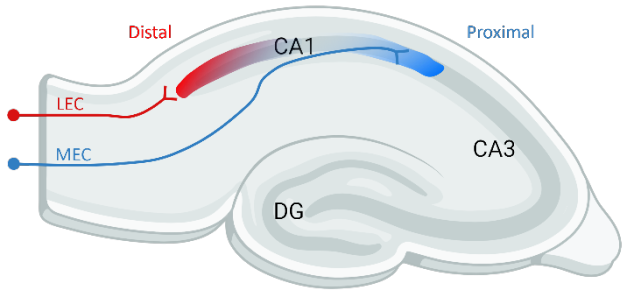


Figure 1.3: Functional diversity along the transverse axis
Distal CA1 cells are innervated primarily by LEC cells, conveying nonspatial information, while proximal CA1 PCs receive MEC inputs containing spatial information, resulting in place cell formation.

entorhinal cortex is spatially segregated. The proximal CA1 receives information from the medial entorhinal cortex (MEC), which passes on spatial information from its grid cells, border cells, and head direction cells, while the distal CA1 receives nonspatial contextual information from the lateral entorhinal cortex (LEC). This difference in afferent connectivity predicts that the proximal CA1 would be more important for processing spatial information; indeed, recent studies have shown that the proximal CA1 is more enriched with spatially selective cells with precise firing fields, while cells in the distal CA1 responsive to place had multiple firing fields and lower coherence (Henriksen et al., 2008). While the distal CA1 does not process spatial information as precisely as proximal cells, it appears to be crucial in distinguishing spatial events and contexts, through coding for object features such as texture, odor, or location (Igarashi et al., 2014). Distal CA1 cells may also be important for detecting novelty, as researchers found that exposure to a novel object selectively increased cFos expression in the distal CA1, an effect which was blocked selectively in the LEC terminals, but not MEC, by norepinephrine (NE) and dopamine (DA) (Ito and Schuman, 2012). Cell properties also vary across the CA1 transverse axis, as principal cells in the distal CA1 exhibit more burst firing, possibly indicating that this cell population encodes multiple streams of information (Jarsky et al., 2008). Neuromodulation is different between these groups as well. Acetylcholine (ACh) modulates proximal CA3-CA1 synapses, while NE and DA selectively modulate LEC terminals in the distal CA1 (Igarashi et al., 2014).

Heterogeneity of pyramidal cells along the radial axis

Four distinct layers lie along the radial axis of the hippocampus– from deepest to most superficial, the oriens, radiatum, pyramidale, and lacunosum moleculare. The afferent projections into the CA1 differ between these layers, as well as which subcellular compartments of the principal cells are present. Information directly from layer III of the entorhinal cortex (ECIII) arrives at the CA1 in the lacunosum molecular (LM), where pyramidal cell distal dendrites arborize, while intrahippocampal information from the CA3 region synapses on to the proximal dendrites of the radial layer (Fig 1.4). In addition to differences in afferent projections, studies now suggest that the pyramidal cell layer itself is made up of two functionally distinct subgroups, referred to as the deep (nearer to oriens) and superficial (nearer to the fissure) subgroups (Mizuseki et al., 2011). These groups have differing transcriptional profiles and are born at different ages in development (Dong et al., 2008). The deep cells have a higher firing rate and burst activity, and are more likely to become place cells, likely due to their pronounced Ih current and higher resting membrane potential (Mizuseki et al., 2011; Soltesz and Losonczy, 2018). In cue-poor environments superficial cells are more likely to fire in response to intrahippocampal inputs, while in cue-rich environments deep cells respond to extra-hippocampal information from the entorhinal cortex (Sharif et al., 2021). The functional connectivity differences between these subgroups support the idea that they process the LEC and MEC streams of information in

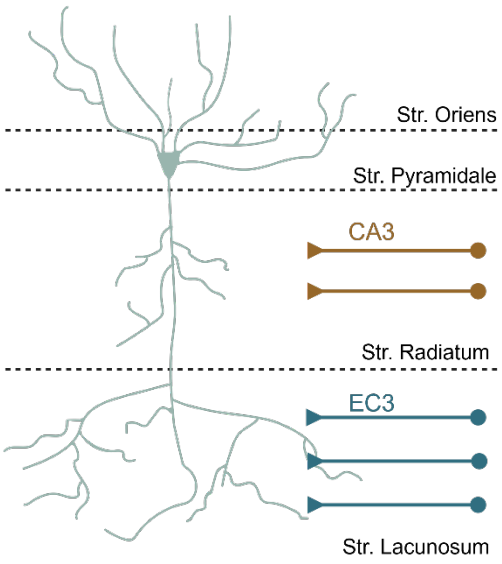


Figure 1.4 The main glutamatergic inputs at each layer of the CA1.

parallel, as LEC afferents preferentially target superficial cells while MEC afferents synapse on to deep cells (Masukar et al., 2017). Ultimately, this provides further evidence for multiple processing streams residing within the hippocampus, in order to complete complex computations.

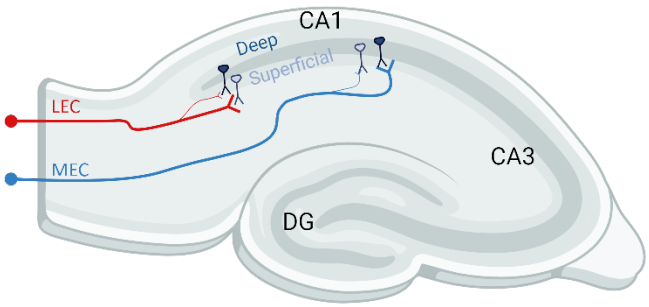


Figure 1.5: Pyramidal cells in CA1 are a heterogenous population.
Deep and superficial PCs respond differently to spatial cues; deep cells are tied to landmarks, while superficial cells represent the context as a whole (Geiller et al., 2017). LEC (non-spatial) information preferentially innervates superficial PCs, while MEC (spatial) afferents favor deep PCs (Masurkar et al., 2017).

1.3 Hippocampal Computations

DG in pattern separation

The DG is a trilaminar area containing a densely packed principal cell layer of granule cells. The major input to the DG is the perforant pathway, from layer II of the EC, which can be divided into lateral EC (LEC) and medial EC (MEC) components, providing spatial and non-spatial

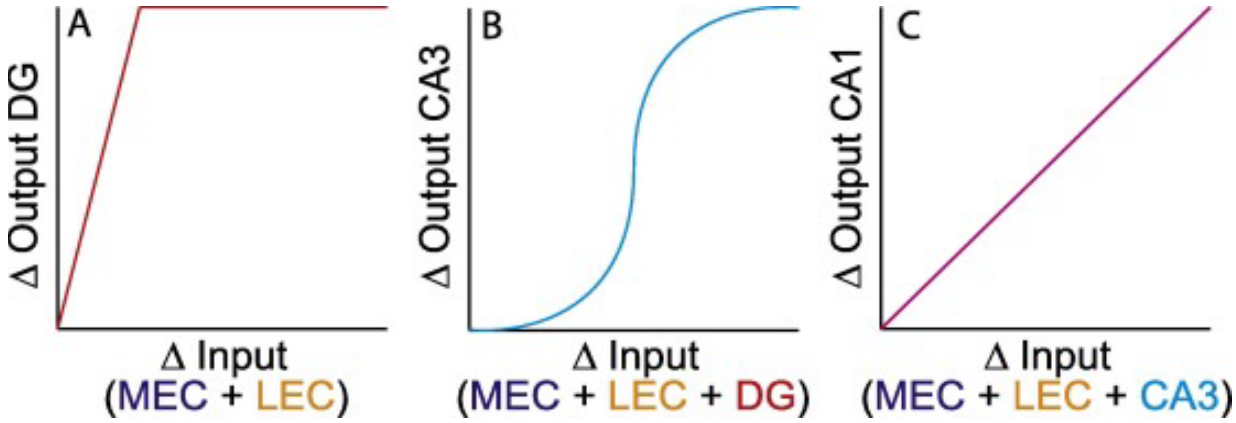


Figure 1.6: Hypothetical input-output curves for each subregion of the hippocampus. The x-axis represents the difference between the inputs to the region, and the y-axis is change in the resulting output. **(A**) Small differences between two inputs to the dentate gyrus result in large differences between the output, representing its pattern separation function. **(B)** The bimodal output of CA3 represents its putative function to complete patterns. When the difference between two inputs is small, the CA3 output shows minimal difference and the pattern is completed; however, after a certain threshold, differences result in pattern separation**. (C)** The CA1 demonstrates a linear relationship between the change in input and output. Adapted from Knierim and Neunuebel 2016.

information, respectively. Theoretical models of hippocampal computations have posited that the DG may have a pattern separation function, in which it receives highly correlated experiences from the EC and transforms them into non-overlapping ensembles in the DG, through a process known as expansion recoding (Marr 1969). The resulting output allows for greatly reduced interference between the representations, so that they can be retrieved separately (Knierim and Neunuebel 2016). Behavioral studies have provided evidence supporting this role for the DG. In one study, rats were trained on a delayed match-to-sample task, in which they were presented with a row of 15 food wells and received a reward when displacing an object covering the baited well. In a choice condition, two identical objects covered

two wells, and the rat must make the correct choice to remove the object from the original location to receive the food reward. Spatial separations between the correct and incorrect object, finding that rats with lesioned DG had lost the ability to discriminate between short spatial separations, while still maintaining a correct choice between longer spatial distances (Gilbert et al., 1998). In a more recent study, creating a transgenic mouse line with NMDA receptor 1 (NR1) knocked down selectively in the DG demonstrated the necessity of this region for discriminating between contexts. Despite having intact hippocampal memory in tasks such as the Morris water maze, these animals had severe deficits in discriminating between contexts after undergoing fear conditioning. Additionally, CA3 place cell activity in NR1 knock-out mice showed deficits in context-dependent rate remapping, suggesting that the altered DG dynamics resulted in aberrant activity along the DG-CA3 mossy fiber pathway (McHugh et al., 2007). Taken together, these studies indicate an important role for the DG in computing differences between inputs.

CA3: Pattern separation and completion

The CA3 region receives input via the mossy fiber projections from the DG, as well as perforant path input from the EC, and its defining network of recurrent collateral connections between its principal cells. This unique circuitry has given rise to several theories regarding the function of CA3; primarily, that it allows for rapid encoding of novel information and associations during learning, as well as providing a mechanism for pattern completion via attractor networks (Kresner 2007; Amaral and Witter, 1995). Numerous behavioral studies strongly support the role of CA3 in rapidly developing novel associations. Blocking NMDA receptors in CA3 through chemical or genetic means in rats and mice results in a decreased performance on spatial tasks when performed in novel environments (Lee and Kesner, 2002; Nakazawa et al., 2003). Pattern completion refers to the ability of a system to take an incomplete input and retrieve a stored

pattern. Theoretical models of this aspect of CA3 activity predict that similar inputs to the CA3 will result in similar outputs, even as the difference between inputs increases beyond the difference between their outputs. At a certain point of dissimilarity, the output will shift in a non-linear fashion to a state in which the difference between outputs is greater than between inputs. The state change displayed in this model suggests the presence of attractors, as inputs are pulled into one of two output states that are mutually exclusive (Knierem and Neunuebel 2016). Supporting evidence from animal studies shows these attractor dynamics at play in electrophysiology, behavior, and gene expression. Rotating the environment in which an animal runs a maze, for example, results in more coordination between CA3 place cells than in CA1, indicating that the CA3 is completing the pattern provided by the familiar cues, and outputting a cognitive map more similar to the original than shown in the CA1 (Lee et al., 2004). When a familiar maze is moved into an entirely new room, however, researchers found a greater dissimilarity between place cells in the CA3 versus those in the CA1, indicating that the difference in inputs was large enough to push the CA3 output towards a different attractor, resulting in a greater decorrelation between the old and new CA3 cognitive map than those of the CA1, exemplifying the role of CA3 in the DG-CA3 pattern separation function (Leutgeb et al., 2004). In a study utilizing NR1 knockout mice selectively expressed in the CA3, the performance of the animals on a spatial task in which familiar cues were removed was markedly inhibited, indicating that the CA3 specifically plays a role in pattern completion necessary for normal spatial learning (Nakazawa et al., 2004).

CA1 as a novelty detector

Computational models of the CA1 have posited that input differences are transformed into output differences in a linear fashion, unlike in the DG or CA3. A wide range of behavioral and electrophysiological studies support the role of CA1 in acquiring spatial memory. Genetically

modified mice lacking NR1 selectively in CA1 display impaired spatial memory, while retaining non-spatial learning (Tsien et al., 1996). Lesion studies in CA1 additionally suggest that damage to this region and its connections results in impaired retention of spatial tasks, though acquisition is unaffected (Lee and Kesner,2003). Positioned between the two main glutamatergic streams of the hippocampus, the CA3 Schaffer collaterals and the EC3 temporoammonic pathway, the CA1 is hypothesized to function as a "comparator" between intrahippocampal information and external inputs from the EC (Vinogradova, 2001). CA1 pyramidal may perform a match-mismatch operation to determine whether an input is novel or familiar, after comparing incoming information to intrahippocampal representations. Accurate spatial encoding relies on both input streams, as disruption of CA3 inputs results in impaired spatial recall, while selective EC3-CA1 disruption or downregulation causes a loss of long-term spatial memory and decreased ability to discriminate between spatially displaced objects (Burns, 2002; Remondes and Schuman, 2004; Vago and Kesner, 2008). As both CA3 and EC3 inputs synapse on to the same pyramidal cells, the final output of CA1 principal cells can shift depending on the relative contributions of each input, in the context of temporal segregation of inputs by hippocampal oscillatory activity.

1.4 Synaptic mechanisms of memory

Synapse strengthening underlies learning

Early pioneering work on the gill withdrawal reflex of *Aplysia* helped establish some of the basic principles of synaptic responses to environmental changes. The simplicity of this system allowed researchers to isolate the stages of memory formation and the basic building blocks involved, ultimately discovering mechanisms that are applicable to learning across the animal kingdom. Reflexive behavior exists in most organisms, and even simple animals can change behaviors in response to their environments and maintain these changes for both short and long

periods of time; in other words, they have the capacity to learn and remember. In *Aplysia*, the synaptic connection between sensory neurons of the siphon and motor neurons of the gill can store implicit memories that dictate the gill's withdrawal reflex. A series of studies showed that the path from tail stimulation to gill withdrawal is mediated by the release of serotonin by modulatory neurons, which increases cyclic monophosphate (cAMP) in the sensory cell. The resultant signaling cascade causes the sensory neuron to release glutamate into the synaptic cleft on to the motor neuron, strengthening their synaptic connection. Manipulations such as repeated applications of serotonin to the sensory neuron or pairing the stimulation to a tail shock both further enhance synaptic strength. These environmental changes increase cAMP concentration, leading to a stronger sensory-motor synapse, and result in a lasting change in synaptic strength, called long-term facilitation. Protein synthesis inhibitors abolish long-term facilitation, an insight that led to the discovery that increased concentration of cAMP phosphorylates nucleic transcription factors, producing the gene expression necessary for long-term memory (Davis and Squire, 1984). Both pre- and post-synaptic changes are necessary for lasting plasticity. Increased release of glutamate by the presynaptic neuron results in the activation of post-synaptic metabotropic glutamate receptors (mGluRs) and the release of stored Ca^{2+}, which causes the insertion of new glutamate receptors in the post-synaptic cell, long-term structural reorganization of the cell that results in lasting changes in synaptic strength.

Plasticity in the mammalian synapse supports complex behavior

Hebbian plasticity is a model for learning and memory in which changes in synaptic strength are determined by the timing of pre- and postsynaptic firing (Hebb, 1949). In this model, presynaptic activity immediately preceding postsynaptic activity strengthens the synaptic connection, in a process called long-term potentiation (LTP). While multiple forms of LTP exist, the most well-

studied and representative form of LTP occurs at the CA1 synapse. CA1 LTP is dependent on N-methyl-D-aspartate (NMDA) receptors in the postsynaptic cell. NMDA receptors act as "coincidence detectors," as their activation requires both membrane depolarization and glutamate binding. This ensures that NMDA receptors are only active at synapses in which the postsynaptic cell is depolarized concurrently with presynaptic release of glutamate.

In the classic example of 100Hz stimulation to CA1 fibers, the tetanizing stimuli give rise to postsynaptic depolarization, fulfilling the initial requirement of NMDA activation. Simultaneous release of glutamate fully activates NMDARs, resulting in a Ca^{2+} influx into the cell. The increased levels of Ca^{2+} activate a signaling cascade, initially binding to calmodulin-dependent kinase II (CaMKII), which causes structural changes in the synapse resulting in spine enlargement. Other Ca^{2+}-activated secondary messengers increase AMPA receptors postsynaptically, which results in a heightened response to glutamate following further presynaptic activity. The late phase of LTP involves protein synthesis, similarly to the *Aplysia* long-term facilitation. Protein kinases are activated, resulting in the CREB-1 transcription factor directing increased gene transcription and protein synthesis to maintain LTP (Kandel et al., 2014).

1.5 Stages of memory formation

As early as the beginning of the 20th century, psychologists were theorizing that forming and maintaining a memory is a time-dependent process that could be derailed by interference. Müller and Pilzecker (1900) proposed the preservation-consolidation hypothesis after finding that the ability of subjects to retain a new memory was disrupted upon learning new information (McGaugh 2000). This suggested to researchers that newly formed memories are initially in a fragile state and become strengthened through time. Research now suggests that memories are initially encoded into a labile memory state, which is then strengthened through the process of consolidation, and moved into long-term storage from which it can later be retrieved.

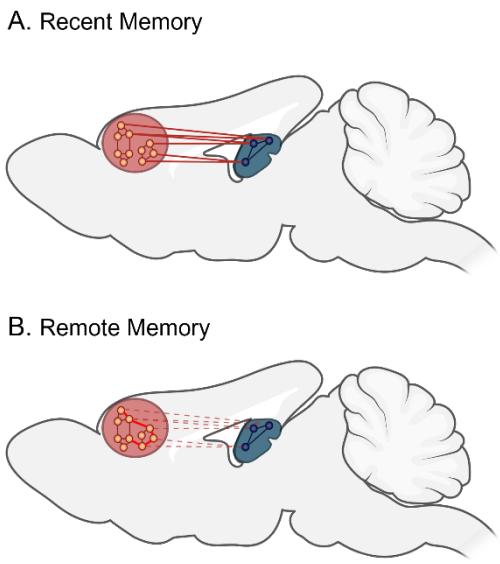


Figure 1.7: Theoretical models of systems consolidation.
(A) Information is initially stored in both hippocampal and neocortical representations during learning and recent memories. **(B)** As consolidation occurs information from the hippocampus is integrated into cortical modules and gradually becomes hippocampus-independent.

Encoding is an NMDAR-dependent process

Encoding is the process by which information is transformed into neural representations. The accuracy of this processes is integral to an animal's ability to make sense of its surroundings and have reliable information on which to base its decisions. The discovery that principal cells in the dorsal CA1 fire in response to an animal's location and encode spatial representations was key in establishing the principles underlying encoding (O'Keefe and Dostrovsky, 1971). Subsequent studies have shown that each environment an animal encounters is encoded by many of these place cells in the dorsal CA1, with different cells encoding different areas within

the area. Additionally, studies show that when alterations are made in the ability of an animal to properly form these cognitive spatial maps, their ability to learn and their performance on navigation tasks suffers. Early studies linking encoding to cellular processes in the hippocampus utilized the Morris water maze, a hippocampus-dependent task in which a rat was placed in opaque water and had to find an escape platform. Through repeated trials, rats were able to find the hidden platform reliably by using visual cues in the room to navigate. Hippocampal lesions severely restricted the ability of rats to perform this task, showing its dependence on the hippocampus, and further studies demonstrated that cellular mechanisms of the hippocampus, particularly the CA1 region, underlie successful spatial encoding (Morris et al., 1982). The finding that D(-)-2-animo-5-phosphonovaleric acid (AP5), a NMDA glutamate receptor (NMDAR) antagonist, infused into the dHPC during task acquisition resulted in impaired performance on the watermaze suggested a role for NMDARs in encoding spatial memory (Morris et al., 1986). NMDARs are also necessary for long-term potentiation (LTP), the process by which repeated stimulation of hippocampal input fibers results in the strengthening of downstream synapses (Bliss and Lomo, 1973; Collingridge et al., 1983). The presence of AP5 in rats undergoing water maze training also resulted in a loss of LTP in the DG of these animals, indicating that NMDAR-dependent synaptic plasticity is crucial to spatial encoding in the hippocampus; interestingly, NMDAR blockade after acquisition of the task did not impair performance on the maze (Morris et al., 1989). Creating strains of mice in which NMDA-dependent LTP mechanisms are altered has similarly provided evidence of their importance to memory acquisition. Genetically modified mice that lacked calcium-calmodulin-dependent protein kinase type II (CaMKII), an enzyme important for the generation of LTP, were found to have impaired spatial memory, while their non-spatial memory was relatively intact (Silva et al., 1992a; Silva et al., 1992b). As the CaMKII knockout was global, introducing the possibility that a lack of the enzyme in neocortical regions could be mediating the decrease in spatial performance, a more targeted transgenic line was created that had a CA1-specific deletion of NMDAR1, a NMDAR subunit necessary for NMDAR

activity (Tsien et al., 1996). The NR1-KO mice failed to exhibit LTP in the CA1, and were similarly deficient in learning the water maze task (Tsien et al., 1996). Further studies in transgenic lines with mutant CaMKII or NR1 knockout show a deficit in hippocampal place cells, in which spatial maps are less precise, less stable, and spatial ensembles are less correlated (Rotenburg et al., 1996; McHugh et al., 1996). These studies demonstrate that impairing the ability of single neurons to participate in synaptic plasticity can result in population-level dysfunction and behavioral abnormalities. Ultimately, these studies suggest an important role for NMDAR-mediated synaptic plasticity in the encoding of spatial memories. Over the years, studies have found that NMDAR-mediate memory encoding plays a role in a range of behaviors, including fear conditioning, novelty versus familiarity tasks, and others (citation).

Systems consolidation stabilizes memories by creating disparate representations across several cortical areas

The process of stabilizing labile memory traces can be viewed from a systems perspective as well as cellular mechanisms (Figure 1.7). Cellular mechanisms underlying consolidation refer to the changes in gene expression, protein synthesis, or receptor expression that stabilize synaptic plasticity into short term memory. Systems consolidation, on the other hand, is a longer process through which memories become hippocampus-independent, and are reorganized and redistributed over multiple neocortical regions (Squire et al., 2015). Memories represented across the disparate cortical areas are thought to be more stable, as they are connected to multiple pre-existing networks. This increased stability with time may cause the observed phenomenon of retrograde amnesia, in which disease or hippocampal damage is more likely to cause a loss of newer memories than older ones (Manns et al., 2003). In animal studies, researchers have found that hippocampus-dependent tasks such as fear conditioning and the Morris water maze are affected by post-training hippocampal lesions as well as reversible CA1-NMDAR lesions and AMPA/kainate receptor blockade, without affecting previously learned

remote memories (Kim and Faneslow, 1992; Riedel et al., 1999;Anagnostaras et al. 1999;Shimuzi et al., 2000). Under other circumstances, however, post-training lesioning or inactivation affected both recent and remote memories, complicating the narrative that manipulations occurring after task acquisition can be directly linked to consolidation processes alone (Lehmann et al., 2007;Winocur et al., 2005). While these studies suggest that the hippocampus is necessary for a period of consolidation after training, they provide little insight into what mechanisms occur during consolidation to stabilize memory traces. Reactivation of the cells involved with a memory may be one mechanism, as well as differing population activity occurring in offline brain states. Hippocampal sharp wave ripples (SWRs) are of particular interest, as they involve the replay of hippocampal sequences, thought to result in increased synaptic strength of the ensembles, and are implicated in hippocampal-cortical interaction (Oliva et al., 2020; Pedrosa et al., 2022; Zhang et al., 2021). Increasing certain oscillatory patterns during consolidation has also been shown to increase the strength of consolidated memories (Kanta et al., 2019). Because of the unique brain states that occur during sleep, particularly in the hippocampus and neocortex, much of the recent work to understand memory consolidation has focused on offline processing that occurs during sleep, which will be discussed extensively in Chapter 2.

Retrieval involves the reactivation of previously stored engrams

The process of retrieval allows an organism to access stored information, typically when it is relevant to its current environment. An early study examined the expression of *Arc*, an indicator of neuronal activation, in neurons following a protocol where an animal was sequentially exposed to two environments–either an identical environment (AA') or a different environment (AB). By utilizing a time delay between the two event exposures they were able to determine whether a neuron had been reactivated in both experiments, by looking for the presence of *Arc* RNA from the first exposure (located in the nucleus) and the second exposure (located

cytosolically) (Guzowski et al., 1999). In animals that were exposed to the AA' context, there was a greater presence of double-labeled cells in the CA1, indicating that the re-exposure to context A activated the same set of neurons. Researchers using fear conditioning have also found that the cell ensembles encoding the fear memory strongly overlap with the ensembles reactivated during retrieval (Reijmers et al.,2007;Lacagnina et al., 2019;Denny et al., 2014). Importantly, the strength of the overlap correlates with the behavioral expression of the memory, and only inhibiting the unique cell ensembles for the fear memory trace results in an inhibition of the fear response in the appropriate context.

More research is needed to delineate the underlying mechanisms of memory processing

Ultimately, there is an abundance of data outlining the stages of memory processing, noting the importance of the hippocampus, NMDARs, and cellular mechanisms strengthening synaptic connectivity to each stage. There are still many unanswered questions, however, regarding the circuitry involved in memory formation and consolidation, how memories are tagged for storage, which synaptic plasticity mechanisms are behind selective strengthening and weakening of connections between ensembles, and the role of behavioral state-specific activity in coordinating these processes across spatial regions and temporal scales.

1.6 Rhythmic population activity in the hippocampus

When large groups of neurons fire synchronously in a periodic manner, their currents sum together producing large, rhythmic fluctuations in local field potential (LFP) recordings. These oscillations are thought to connect disparate areas of the brain, allowing for the exchange of information between regions performing complex cognitive tasks. As discussed above, the process of encoding and storing new information requires connections between neurons and communication between structures. Rhythmic activity of neuronal populations is thought to be one mechanism to coordinate the activity of distributed cell ensembles during memory processing. In this section, I will focus primarily on the rhythmic activity of the hippocampus and how it may facilitate memory computation. The rhythmic activity in the hippocampus serves to organize neural ensembles, provide periods of population-level synaptic potentiation/depotentiation, and facilitate consolidation. The dominant rhythm of the hippocampus changes depending on the behavior and activity state of the animal, suggesting that different oscillatory patterns serve unique functions.

Theta rhythmic activity orchestrates hippocampal population activity during active behavior

When a rodent is moving, the dominant rhythm in the hippocampus is the large-amplitude 4-12 Hz theta oscillation. The theta rhythm is thought to be generated by inputs from the medial septum (MS) inhibitory interneurons, which fire rhythmically at theta frequencies (Toth et al., 1997), though isolated hippocampal preparations retain the ability to synchronize at the theta rhythm, suggesting that intrinsic cellular properties of hippocampal cells can also support theta generation (Goutagny et al., 2009). Early studies found that the loss of hippocampal theta led to

severe impairment in spatial learning, implicating this oscillatory activity in the mnemonic functions of the hippocampus (Winson 1978).

The theta rhythm temporally organizes cell firing

The theta rhythm may support encoding in several ways. Several studies in hippocampal slices have noted that LTP is optimally elicited by patterns of stimulation separated by 200 ms, or 5 Hz, and that stimulus trains outside of this interval produced less potentiation (Larson et al., 1986). In slice preparations, LTP was heightened following stimulation during cholinergic agonist-induced theta frequency oscillations, and stimulation coincident with the peaks of theta rhythm led to the most pronounced and longest-lasting effect on plasticity (Huerta and Lisman, 1993, 1995). Intriguingly, stimulation occurring during the trough of the theta oscillation led to long term depression (LTD) of the synapse, and depotentiation of a previously potentiated synapse, indicating that theta plays a role in bidirectional synaptic plasticity (Huerta and Lisman, 1993, 1995, 1996). Providing periods of increased potentiation may serve to group data together temporally. For example, during navigation, sequences of activity representing an animal's position are compressed into a single theta cycle, providing the right timeframe for spike timing-dependent plasticity (STDP), increasing synaptic strength between temporally-related cell assemblies (Buzsaki, 2006).

Theta phase segregates encoding and retrieval processes

The phases of theta represent times of minimal and maximal excitability of the cell population; however, the local theta phase depends upon the location along the radial axis. There is a 180° phase shift between pyramidal layer theta in the stratum radiatum and distal dendrites of the lacunosum molecular, resulting in the peak excitability of the soma being out of phase with that of the dendrites. The two main glutamatergic inputs into the CA1, from the CA3 and EC, are also segregated in time and space. CA3 inputs are maximal at the trough of theta measured at the stratum radiatum (SR), while EC inputs are strongest at the peak. Each input is localized to a different layer of the hippocampus, with CA3 inputs synapsing at proximal dendrites of the SR layer, while EC inputs arrive at the distal dendrites of the stratum lacunosum molecular (SLM). This sharp delineation between phases has led to the theory that each phase of theta serves a unique purpose; specifically, that encoding occurs at the peak of SR theta, while retrieval occurs at the trough (Hasselmo et al., 2002). In this theory, during encoding, EC inputs would dominate during the phase of maximal LTP, resulting in strengthened assemblies representing incoming sensory information. During the retrieval phase, however, CA3 activity dominates, and weak EC input serves to bring retrieval cues into the network; however, because LTP is absent, the retrieved information is not encoded, and prior associations are able to be extinguished.

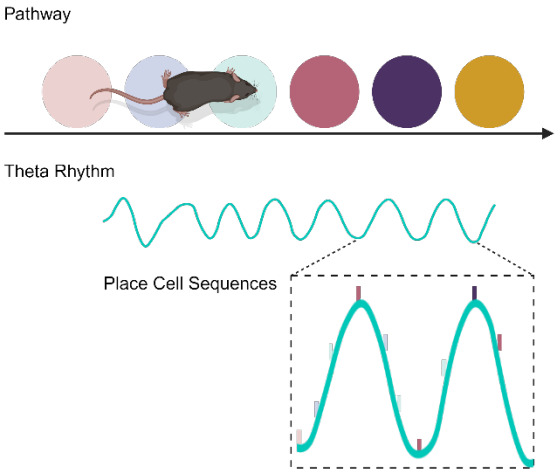


Figure 1.8: Theta rhythms group individual representations temporally.
In this figure, each place cell and corresponding place field is color-coded. The sequence is represented in time by the spiking in relation to theta phase. As an animal traverses through a place field, spikes occur at earlier theta phases in each cycle, resulting in early and late theta phases that represent earlier and later locations in the path. Adapted from Colgin 2016.

One prediction of this theory is that the preferred phase of firing of CA1 pyramidal cells should shift towards the pyramidal layer theta peak in novel contexts, in order to bias the system towards encoding rather than retrieval. When this was tested experimentally, pyramidal cells did

shift their firing phase earlier in the theta cycle, towards the peak, though to a smaller degree than initially posited by computational models (Douchamps et al., 2013; Manns et al., 2007). This shift was abolished by the administration of scopolamine, indicating that acetylcholine (ACh) also plays a role in segregating encoding and retrieval states. The consequences of disrupting the phase of firing shift can be inferred from scopolamine's previously established role in affecting encoding but not retrieval. Behavioral experiments manipulating firing phase, however, are beginning to offer evidence to support the encoding/retrieval phase theory's importance in memory consolidation. Using closed-loop optogenetics to activate parvalbumin-positive interneurons, Siegel and Wilson were able to disrupt pyramidal cell activity at the phase or trough selectively. Using a choice task requiring well-defined periods of encoding and retrieval, they found that activating PV+ interneurons in the troughs of retrieval phase or the peaks of encoding phase lead to increased task performance, suggesting this occurs because increased inhibition led to the filtering out of irrelevant stimuli (Siegel and Wilson, 2014). More work is required to delineate the exact processes underlying the relationship between encoding and retrieval, but the previous study suggests that manipulations of ensemble activity will differentially affect behavioral performance according to theta phase and task.

Theta-Gamma Coupling

In addition to the theta rhythm, hippocampal activity is also coordinated by a faster (approximately 30-100 Hz) gamma oscillation. Gamma rhythms are nested within theta cycles, which may allow the hippocampal-entorhinal network to organize sequences of events temporally (Colgin, 2015). Further studies into theta-gamma coupling suggest that its presence is necessary for optimal memory performance and has revealed that at least three distinct gamma bands exist, associated with different memory processes. Many studies find that during behavior, unique gamma frequency bands reliably couple with different theta phases, as slow

gamma (~25-80 Hz) coupling peaks during descending theta and mid gamma (~65-120 Hz) peaks at the theta peak, with theta measured at the pyramidal cell layer (Colgin et al., 2009; Schomburg et al., 2014). These gamma bands reflect the two main excitatory inputs into the CA1. Slow gamma occurs when intrahippocampal CA3 inputs arrive, synapsing on to the proximal dendrites of CA1 pyramidal cells. Mid gamma arises from the layer 3 entorhinal cortical (EC3) afferents that terminate in the SLM layer, innervating CA1 distal dendrites. With the spatial segregation provided by the isolated layers of the hippocampus (gamma coherence between layers is low, while within-layer coherence is high), as well as the temporal segregation provided by the theta cycle, theta-gamma coupling appears to be another mechanism by which memories can be formed, stored, and retrieved while minimizing interference between disparate representations.

Theta-gamma coupling in the hippocampus appears consequential for determining hippocampal output and memory task performance. High spatial resolution electrophysiology recordings of the hippocampus suggest that the output of CA1 pyramidal cells, place cell action potentials, is under the dual control of EC3 and CA3 inputs, mediated by theta-gamma coupling (Fernández-Ruiz et al., 2017). When CA3 inputs into CA1 are strongest, slow gamma is maximal, and PC firing occurs on the descending theta phase, nearer to the maximal coupling between slow gamma and theta. Similarly, when EC3 inputs dominate, PC spiking occurs near the peak of theta, where EC3 inputs occur, and fast gamma is maximal. Thus, the coupling of CA1 theta and the gamma band of either afferent projections influences the theta phase of PC spiking (Fernández-Ruiz et al., 2017;Mizuseki et al., 2009).

Sharp Wave-Ripples

Hippocampal sharp wave-ripples (SWRs) occur when a large fraction of CA1 pyramidal cells are simultaneously depolarized (the “sharp wave” in LFP traces) by activity in the CA3. The CA3

activity that triggers the sharp wave also activates interneuron-coordinated pyramidal ensembles in CA1 that constitute the 150-250 Hz ripple activity (Joo and Frank, 2018). SWRs appear most frequently during non-REM sleep, but can also occur during periods of waking when an animal is at rest. The frequency of SWTs is increased following reward learning or being in a novel context. These all suggest a role for SWRs in memory consolidation, which is born out through studies that find disrupting SWRs leads to hippocampus-dependent memory consolidation impairments, as well as studies showing that inducing SWRs during sleep can enhance memory consolidation and subsequent task performance.

1.6 Hippocampal interneurons

Inhibitory control of hippocampal oscillatory activity

The oscillatory activity key to hippocampal function is regulated in large part by GABAergic interneurons (INs), which serve as pacemakers to coordinate spike timing across circuits. Though INs make up only a small fraction of cells within the hippocampus, they are highly specialized, with at least 21 unique classes of INs that are categorized by the location of their cell body, the target of their axonal arbor, electrophysiological properties, and molecular markers (Freund and Buzsaki, 1996; Klausberger and Somogyi, 2008). As a single IN typically receives input from multiple pyramidal cells and outputs onto many cells, they are well-positioned to synchronize activity across hippocampal circuits (Cobb et al., 1995). At the simplest level, they participate in feedforward or feedback inhibitory circuits. Feedforward inhibition occurs when afferent projections from another brain region synapse on to INs, resulting in the silencing of that IN's target population. Feedback inhibition, on the other hand, primarily involves local circuit activity, in which pyramidal cell firing excites nearby interneurons, which then inhibit the same pyramidal cell population. Feedback inhibition is thought to be

important for the generation and maintenance of gamma and theta oscillatory activity in the hippocampus (citation needed). Due to the complex and heterogeneous nature of hippocampal INs, the role played by each IN subtype in coordinating rhythmic activity has yet to be fully understood. In this work, I seek to more fully understand the role of oriens-lacunosum moleculare (OLM) interneurons in the oscillatory activity present during offline processing, thought to be crucial for learning and memory consolidation.

Spiking and resonance properties of OLM cells suggest they play a role in theta-dependent processing

Several avenues of study provide evidence that OLM cell activity is modulated by theta oscillations, with some computational models suggesting that they could play a role in generating and maintaining theta in the hippocampus (Chatzikalymniou and Skinner, 2018; Gloveli et al., 2005). Additionally, their unique membrane properties may tune them to have increased theta resonance. Decay time constants of EPSPs in OLM cells have been found to be slower than those in other SOM+ non-OLM cells in the hippocampus which, alongside longer after hyperpolarization periods, results in a tonic firing rate within the theta frequency range (Goldin et al 2007). Postsynaptic kainate receptors (KA-Rs) are prevalent specifically in OLM interneurons in the alveus, and mediate theta frequency entrainment (Cossart 2002; Goldin et al 2007). OLM cells increase their activity significantly during theta activity (Varga et al 2012), firing phasically at the trough of the extracellular theta rhythm (Klausberger 2003). As EC3 inputs arrive at the distal dendrites of pyramidal cells at this phase of theta, OLM cell activity likely serves to phase modulate input from the perforant path, and could potentially provide rhythmic hyperpolarization to facilitate backpropagation and burst discharge activity in place cells. Other computational models have posited a role for OLM cells in creating gamma- and theta-coherent assemblies (Tort et al 2007).

OLM interneurons are necessary for hippocampus-dependent learning and memory

Recent studies indicate that OLM cells may have a unique function as a “switch” between the two major glutamatergic information streams into the CA1, gating whether the TA or SC inputs ultimately have greater influence on the CA1 output. When OLM cells arborize in the SLM, they synapse on to the distal dendrites of PCs, directly inhibiting the TA path inputs from EC layer III. In addition to attenuating EC3 input, *in vitro* studies suggest that they inhibit bistratified cells that synapse on to proximal cells receiving input via Schafer collaterals (SCs), effectively disinhibiting these inputs and biasing the pyramidal cell firing towards CA3 afferents. Given the critical role of the timing of EC3 and CA3 input into the CA1, and its potential role as a comparator discussed above, the morphology and function of OLM interneurons position this group of cells as potentially playing a critical role in the formation and retrieval of memories. Studies investigating the role of OLM cells in learning and memory suggest that they play an integral role in hippocampal-dependent memory tasks. Ablating OLM cells specifically results in a loss of ability to discriminate between novel and familiar locations in the NOPR task, while the performance on the hippocampus-independent NOR task remains intact. Similarly, OLM-ablated animals have a reduced performance on the Y-maze spontaneous alternation task, further indicating that loss of OLM cell functionality results in an inability to distinguish between novel and familiar spatial contexts (Haam 2018). Optogenetic inhibition of SOM+ interneurons in the dorsal hippocampus during fear learning results in decreased retention of contextual information relating to the fear memory; as an estimated 40% of SOM+ GABAergic hippocampal cells are OLM interneurons, this provides evidence for their role in hippocampal-dependent fear memory as well. Further work has refined this, providing evidence that the location along the dorsal-ventral axis of the hippocampus determines the role of OLM cells in memory formation, and in which way modulation of these cells will affect behavior.

1.8 Studying memory in the rodent brain

As discussed above, episodic memory in humans combines 'what-where-when' information about an event into one coherent representation (Dere et al., 2005; DeVito and Eichenbaum, 2010). Whether animals, particularly rodents, can form episodic-like memories is difficult to assess, and requires behavioral tasks contingent upon an animal's ability to correctly recall this information simultaneously. In the work presented here, I focused on two hippocampus-dependent tasks, novel object place recognition (NOPR) and fear conditioning (FC) as a proxy for episodic-like contextual memory.

Novel Object Place Recognition

NOPR leverages the tendency of mice to preferentially explore novel parts of their environment, giving researchers a way to measure a mouse's perception of novelty and familiarity. At its simplest, this task asks mice to encode a memory of objects and their location, and then recall this information after a period of time in order to discern when one of the objects is displaced. Hippocampal damage, including specific inactivation of the CA1 or CA3 regions, results in NOPR task impairment (Assini et al., 2009; Langston and Wood, 2009; Stupien et al., 2003). The NOPR task is sensitive to hippocampal dysfunction and is impaired in mouse models of neurodegenerative disorders, epilepsy, and aging (Zhang et al., 2023; Kim et al., 2020; Wimmer et al., 2012). The acquisition, consolidation, and retrieval of this task is also negatively affected by sleep deprivation, indicating that offline consolidation during sleep is critical to short- and long-term storage of object location memories (Heckman et al., 2020). NOPR is thus an important behavioral paradigm for testing the role of hippocampal function and sleep-dependent consolidation in forming and maintaining spatial and contextual memories.

Fear Conditioning

An animal's ability to recognize threats and establish patterns to determine which situations might prove dangerous is critical to its survival. Fear conditioning has proven a useful paradigm for understanding the neurobiological mechanisms underlying how animals create associations between external stimuli and threats. This technique relies on classic Pavlovian conditioning, in which an animal is taught to form an association between an unconditioned stimulus (US) and a conditioned stimulus (CS) in a given context. The most common version of this used in behavioral neuroscience uses a foot shock as the US, which produces a reflexive fear response in rodents. The shock is typically paired with a tone as the CS, and the fear conditioning chamber the rodent is placed in provides the context. In this scenario, the contextual information–which can include visuospatial cues, odors, textures, or other sensory input–is encoded into a unified representation (context encoding), which is then associated with the US (context conditioning; Maren et al., 2013). The underlying circuitry involved in encoding fear memories involves disparate parts of the brain interacting to process relevant stimuli and establish a fear response. Central to this process is the amygdala. Lesions of the amygdala preclude acquiring or expressing fear responses, and synaptic plasticity of the amygdala is necessary for fear conditioning (Sanders et al., 2003). Research into the role of the hippocampus in fear conditioning suggest that it plays a role in establishing a contextual representation that becomes associated with the US. Early studies into the circuitry involved show that lesions in the hippocampus disrupt fear conditioning, especially the contextual freezing response. Intriguingly, whether the lesion was induced pre- or post-training affected the degree to which fear memories were impaired. When the hippocampus was lesioned after training, the freezing response to a tone CS remained, while the contextual fear was disrupted (Kim and Fanselow, 1992). The effect of post-training lesions on contextual memory disappeared, however, when they did not immediately follow training, indicating that after a

period of time, fear memories become independent of the hippocampus (Kim and Fanselow, 1992; Sachetti et al., 1999). This indicates that the hippocampus participates in the storage of the contextual representation as well as forming and associating it with the US. This was further established by studies in which rats were pre-exposed to the shock context before undergoing fear conditioning and hippocampal lesions. The pre-exposure allowed the animal to form a contextual representation that it was able to later connect to the shock, despite the damage to the hippocampus post training; further supporting the role of the hippocampus, the restoration of context fear was eliminated if the hippocampus was pharmacologically inactivated during the pre-exposure to the context (Young et al., 1994). NMDA receptor antagonists infused into both the dorsal and ventral hippocampus lead to selective impairment of contextual memory, and post-training infusion of scopolamine results in similar contextual memory deficits, indicating that learning and plasticity related mechanisms need to be functioning in the hippocampus in order to accurately store context representations (Sanders et al., 2003). Taken together, this large body of work supports the theory that contextual fear memory requires synaptic plasticity in the hippocampus to encode fear contexts, consolidate the memory of conditioning, and maintain the context/fear representation for retrieval up until the memory has been moved into non-hippocampal-dependent long-term storage. As I will discuss further below, the consolidation of contextual fear memory is also dependent on offline processing during sleep.

Chapter 2

Shining a light into the mechanisms of sleep for memory consolidation

2.1 Introduction

Sleep is a period of heightened vulnerability for an organism--an extended stretch of time during which an animal is oblivious to its surroundings, cut off from environmental cues that would alert it to a potential food source, an approaching mate, or an impending danger. And yet, despite all of the potential survival-related drawbacks that such a state entails, every animal sleeps. From drosophila to laboratory rats to humans, evolution has conserved this unguarded state across time. Clearly, the function of sleep is important enough to outweigh the danger of being in such an unguarded state. Researchers have proposed a range of potential roles for each sleep state, including recovery from oxidative stress in the brain (Ramanathan et al., 2002; Gopalakrishnan et al., 2004), energy conservation (Lima et al., 2005), a period of heightened protein synthesis (Seibt et al., 2012; Ramm and Smith, 1990), and the stimulation of a sleeping brain to facilitate transitions into waking (Vertes and Eastman, 2000). A somewhat more controversial proposition, however, is that sleep is an important stage of time for the consolidation of memories created during waking.

Figure 2.1

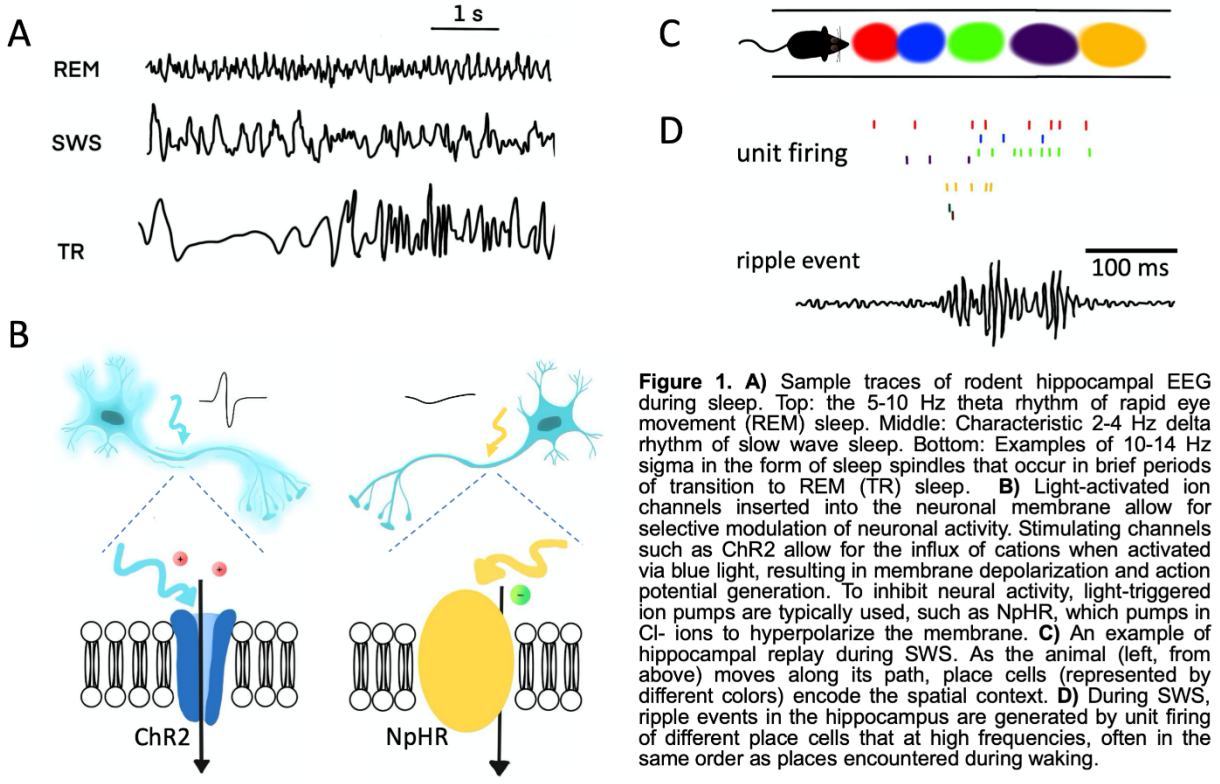


Figure 1. A) Sample traces of rodent hippocampal EEG during sleep. Top: the 5-10 Hz theta rhythm of rapid eye movement (REM) sleep. Middle: Characteristic 2-4 Hz delta rhythm of slow wave sleep. Bottom: Examples of 10-14 Hz sigma in the form of sleep spindles that occur in brief periods of transition to REM (TR) sleep. **B)** Light-activated ion channels inserted into the neuronal membrane allow for selective modulation of neuronal activity. Stimulating channels such as ChR2 allow for the influx of cations when activated via blue light, resulting in membrane depolarization and action potential generation. To inhibit neural activity, light-triggered ion pumps are typically used, such as NpHR, which pumps in Cl- ions to hyperpolarize the membrane. **C)** An example of hippocampal replay during SWS. As the animal (left, from above) moves along its path, place cells (represented by different colors) encode the spatial context. **D)** During SWS, ripple events in the hippocampus are generated by unit firing of different place cells that at high frequencies, often in the same order as places encountered during waking.

Sleep is divided into multiple stages (Fig. 1A), each of which is identified by stereotyped behavior, electrical activity, and neurochemical composition. The broadest distinction splits sleep into two stages, rapid eye movement (REM) and non REM (NREM) sleep. REM is characterized by muscle atonia, desynchronized waking-like EEG activity, and a 5-10 Hz hippocampal theta rhythm. NREM displays 2-4 Hz slow wave delta activity, accompanied by fast sharp-wave ripple (SWR) events in the hippocampus. Determining the role of each sleep stage and its signatures in the consolidation process is an area of intense investigation within the field of sleep research.

Experimentalists have been investigating the role of sleep in learning and memory storage for years, finding that depriving animals of normal sleep nearly always results in adverse outcomes in their ability to store and retain new information. For example, early studies of REM deprivation in rodents resulted in decreased performance on hippocampal-dependent working memory tasks, especially contextual/spatial mazes that require established cognitive maps of the animals' environment (Youngblood et al., 1997,1999; Smith and Rose, 1996; Smith et al., 1998). Physiological studies indicate that REM deprivation results in a loss of excitability in the rodent hippocampus (McDermott et al., 2003,2006), and reduced long-term potentiation (LTP)(Ribeiro et al., 2002). Accumulated evidence demonstrates that NREM is beneficial for declarative memory in humans and may provide a time for communication between disparate brain areas, particularly the hippocampus and prefrontal cortex through the generation of sleep spindles, which are correlated to memory recall (Schabus et al., 2004; Demanuele et al., 2017). Conversely, REM seems to be an important period for learning procedural tasks (Mednick et al., 2003; Karni et al., 1994).

While the body of evidence supporting an important role for sleep in memory consolidation grows, several critiques have plagued the field since its inception and proven themselves difficult to fully resolve. Limitations in the specificity of past methods as well as incongruous and conflicting study results have led some prominent sleep researchers to altogether reject any role for sleep in memory processing, instead viewing sleep solely through the lens of homeostatic regulation and systemic physiological function (Vertes and Eastman, 2000; Siegel, 2001; Vertes and Siegel, 2005). New techniques such as optogenetics enable researchers to target light-activated ion channels to control the activity of select brain areas, neural populations, or specific memory traces (Fig. 1B). Using light-activated channels such as channelrhodopsin (ChR2) and halorhodopsin (NpHR) allows for precise spatial and temporal neural activation or inhibition in order to tease apart the function of specific structures and cell populations (Pastrana, 2011). Modulating neurons at such a granular level during sleep can tell us how activity outside of waking encoding (offline) shapes the connections and circuits of a memory ensemble. In this review, we aim to look at the ways in which optogenetics has allowed us to more specifically delineate the role of sleep in the consolidation of waking experiences (Table 1). We find that optogenetic techniques enable the modulation of various facets of sleep, while avoiding many of the confounding variables obscuring the results of earlier studies.

2.2 Stress is not the culprit: sleep feature disruption using optogenetics causes learning and memory deficiencies without the stress of behavioral sleep deprivation methods

One of the biggest critiques of early sleep studies was the difficulty in dissociating sleep loss from the stress induced by methods of sleep deprivation as playing the causal role in subsequent learning deficits. Methods to disrupt REM can result in hypothalamic-pituitary-adrenal (HPA) axis activation and increased cortisol levels, which have been shown to negatively impact hippocampal dependent memory consolidation (Guzman-Marin et al., 2005). These methods led researchers to argue that the behavioral deficits seen were a result of impaired hippocampal function due to stress rather than sleep loss. Indeed, stress does impair hippocampal neurogenesis just as chronic sleep disruption and sleep deprivation does, and perhaps the two mechanisms are the same (Guzman-Marin et al., 2005; Mirescu et al., 2006). With the use of optogenetics, the studies discussed herein target specific sleep stages to modulate sleep features in a manner that does not introduce stress as a confounding variable.

One set of experiments used an optogenetic protocol to fragment sleep without inducing stress in the animal (Rolls et al., 2011). By selectively expressing ChR2, an excitatory optogenetic ion channel (Fig. 1B), in hypocretin/orexin (Hcrt) neurons in the lateral hypothalamus (LH), investigators were able to decrease latency to arousal in both REM and NREM, resulting in fragmented sleep periods. Stimulation of the LH at 60 s intervals with blue light resulted in Hrct neurons firing action potentials, and was enough to fragment sleep, as measured by microarousal periods. Overall sleep amount, quality and composition of sleep was unchanged, and no evidence of sleep debt occurred following the stimulation protocol, indicating that only the continuity of sleep was disrupted. Recent research suggests that this arousal effect might be mediated through extensive projections to the locus coeruleus (LC), an important structure for sleep and arousal regulation (Bourgin et al., 2000; Carter et al., 2013; Espana et al., 2005). To ensure that the animal was not under stress due to stimulation, investigators tested cortisol levels in the plasma of both ChR2 and control mice following the 4 h stimulation protocol, finding no difference between stimulated and unstimulated animals. Similarly, animals that underwent

the optogenetic stimulation did not show increased anxiety behavior in an open field maze. Taken together, these findings show that the optogenetically induced sleep fragmentation protocol did not increase physiological markers of stress or behavioral indications of increased anxiety.

The Hcrt-stimulation induced sleep disruption paradigm allowed researchers to directly test the effect of discontinuous sleep on learning and memory. Using the novel object recognition (NOR) task as a measure of hippocampal- and sleep-dependent memory (Palchykova et al., 2006; Chen et al., 2014; Cohen et al., 2013), they ran animals through the optogenetic stimulation protocol during the crucial 4 h window during the light period immediately following training on the task (Smith and Rose, 1996). In animals with Hcrt neurons expressing ChR2, the blue light stimulation resulted in a significant impairment in NOR performance as compared to control animals. Importantly, this effect was only seen when stimulation occurred in the light period following task acquisition, not in the dark period hours later (outside the critical consolidation window), highlighting the role of Hcrt neurons in sleep dependent consolidation rather than a result of aberrant Hcrt activity alone. Additionally, this effect was abolished when stimulation intervals were increased to 120 s apart, indicating that there is a minimum length (>60 s) of sleep “quanta” necessary for adequate memory consolidation in the rodent. Another study found that 60 s long inductions of SWS repeatedly for 30 min within 30 min of learning stabilized the memory such that discrimination was maintained for as long as 6 h after object place exposure and novel arm Y maze task (Lu et al., 2018). They produced SWS by optogenetically stimulating the GABAergic neurons in the parafacial zone, thereby increasing delta and reducing EMG activity as is consistent with the induction of SWS. The 60 s long inductions of SWS also stabilized contextual fear memory and improved context discrimination in these animals.

It would be interesting to know what that minimum undisturbed sleep quanta for memory stabilization and consolidation is in the human. Even as few as 5 airway obstruction related

arousals per hour (equating to once every 12 min) is associated with memory deficits in humans (Kloepfer et al., 2009).

Table 1. Comparison of studies using optogenetics to investigate the role of sleep in memory consolidation

Study authors, date	Species	Optogenetic manipulation, brain region	Sleep state	Physiological results	Behavioral results
Kumar et al., 2020 [43]	Mouse	Inhibition & excitation of adult born neurons (ABN) in DG	REM	Inhibiting ABN ↑ dendritic spines w/o Δ in spine density, head diameter, & neck length	Inhibition & excitation both impaired CFC memory consolidation
de Sousa et al., 2019 [54]	Mouse	Post-learning activation of RSC	Iso; sleep	↑ 100 Hz in ACC and HPC in sleep or iso	↑ freezing within fam &new context despite hippocampal inactivation
Davis & Vanderheyden 2020 [59]	Rat	Excitation of MCH neurons	24 hr	Initial ↓ REM & NREM in dark phase; then daily ↑ REM & NREM	↓ cued fear freezing
Izawa et al., 2019 [44]	Mouse	Inhibition & excitation of MCH neurons & terminals in HPC	REM	Stim of MCH inputs ↓ CA1 pyramidal activity & ↑ inhibitory currents	Inhibition ↑ memory consolidation; excitation ↓ memory consolidation
Kim et al., 2019 [52]	Rat	Inhibition of primary motor cortex neurons	SWS	Inhibition during delta ↑ SO-spindle nesting ↑ reactivation in SO-nested spindles	↑ motor performance after delta replay inhibition; ↓ after inhibition during SO
Swift et al., 2018 [31]	Rat	Activation of LC neurons	sleep	↓ delta power; ↓ theta power; ↓ sigma power	↓ spatial encoding; ↓ reversal learning
Lu et al., 2018 [60]	Mouse	Activation of GABA neurons of parafacial zone to induce SWS	SWS	Induced slow waves; ↓ EMG tone; ↑ delta power. When stim w/in 30 min of learning:	↑ 6 h novel arm Y maze discrimination; ↑6 h CFC; ↑ 6 h NOPR discrimination
Ognjanovski et al., 2018 [33]	Mouse	Inhibition of PV neurons	SWS	↓ delta power; ↓ theta power	↓ freezing following CFC
Gulati et al., 2017 [53]	Rat	Inhibition of primary motor cortex neurons	SWS	Inhibition during SO upstate = ↓ rescaling of indirect neuronal activity	↓ BMI task performance
Latchoumane et al., 2017 [61]	Mouse	Induced spindles in PV inhibitory neurons in TRN	SWS	↑ ripples co-ocurring with spindles induced in SO upstate	↑ CFC; ↑ NOPR performance
Boyce et al., 2016 [45]	Mouse	Silence medial septal neurons	REM	↓ theta power in HPC	↓ NOPR performance, ↓ CFC recall
Kovacs et al., 2016 [40]	Mouse	SWR-timed silencing of CA1 pyramidal neurons for 3 h	sleep/rest	↓ pyramidal cell activity;↓ SWR; ↑rebound on inhibition release; No Δ in place cell dynamics	Passive exploration. No learning tests.
Miyamoto et al., 2016 [62]	Mouse	Inhibition of M2 fibers in S1	NREM	↓ M2-S1 causality; reactivated S1 neurons	↓ texture recognition
van de Ven et al., 2016 [39]	Mouse	Inhibition of SWR in principal hippocampal neurons	sleep/rest	↓ assembly pattern reactivation during novel environment re-exposure	No Behavior Tested
McNamara et al., 2014 [63]	Mouse	Activation of dopaminergic neurons in VTA or CA1	Waking	↑ ensemble reactivation strength during SWR in sleep	↑ maze performance
Rolls et al., 2011 [23]	Mouse	Activation of hypocretin neurons	All stages	↑ sleep fragmentation; TR-specific ↓ delta power and ↑ theta power	↓ NOR performance in light phase (sleep phase)

Abbreviation Key: ABN = Adult born neurons; **DG** = dentate gyrus; **CFC** = contextual fear conditioning; **HPC** = hippocampus; **CA1** = Cornus Ammonis 1 region; **RSC** = retrosplenial cortex; **MS** = medial septal nucleus; **ACC** = anterior cingulate cortex; **Iso** = isoflurane; **LC** = locus coeruleus; **MCH** = melanin-concentrating horomone; **NOR** = novel object recognition; **NOPR** = object place recognition; **PV** = parvalbumin; **REM** = rapid eye movement sleep; **SWS** = slow wave sleep; **TR** = transition to REM; **TRN** = thalamic reticular nucleus; **M2** = secondary motor cortex; **S1** = primary somatosensory cortex; **SWR** = sharp-wave ripple; **VTA** = ventral tegmental area

The ability to separate the effects of sleep deprivation from the methods used to sleep deprive and the negative effects they can cause allows research to move towards understanding the role that each component of sleep plays in the memory consolidation process. Work in our lab has used optogenetics to change baseline activity in neuromodulatory systems during sleep without disrupting overall time spent in sleep or sleep architecture itself (Swift et al., 2018). Unlike the Hcrt study, we did not induce arousals from sleep, yet found similar memory consolidation deficits related to changes in the electrophysiological features of each sleep state. We optogenetically modulated the locus coeruleus (LC) activity during sleep, and provided

correlational evidence for the role various sleep signatures play in sleep-dependent consolidation. Typically, the LC quiets during NREM periods and is silenced during REM sleep (Aston-Jones and Bloom, 1981). Maintaining LC activity at waking levels throughout sleep after learning resulted in a decrease in the 1-4 Hz delta band and in the 10-14 Hz sigma band corresponding to sleep spindles. Longer spindles, which increased in control learning animals, were nearly eliminated when LC activity was maintained during sleep. In REM sleep, theta power was significantly decreased during LC stimulation. These changes were correlated with performance in incorporating changes made in a spatial memory task. Greater reductions in delta and theta band power were directly related to an increase in switching away from reliance on the hippocampal map. Similarly, spindle-ripple coupling predicted confusion between the original and modified food positions on the maze. The ability to adjust the frequency of LC activation with optogenetics during sleep allowed us to keep normal sleep bout lengths, yet still observe changes in memory consolidation.

The fact that changing the underlying physiology of sleep without disrupting other aspects of sleep affects memory consolidation suggests that the brain activity associated with each sleep state, and the particular neurochemical composition associated with each phase of sleep, is critical in supporting memory consolidation. Additional studies have found that optogenetic modulation of neuronal subtypes during sleep can similarly disrupt memory consolidation. Researchers investigating the inhibition of parvalbumin interneurons (PV+) during NREM sleep found that this intervention abolished the typical post-learning sleep increase in delta and theta power in the hippocampus (Ognjanovski et al., 2018). In another group of animals that were sleep deprived, a similar decrease in delta and theta power was observed. These delta and theta power decreases were concurrent with disrupted contextual fear memory in both groups. However, activating hippocampal parvalbumin interneurons rhythmically at theta frequency during sleep deprivation stabilized CA1 population activity measured during subsequent sleep

and rescued appropriate post-sleep hippocampus-dependent fear behavior. These findings suggest the necessity of sleep at least partially lies in the population activity engendered during the state, and provides evidence that this activity directs the consolidation of memories.

2.3 Optogenetics allow us to target and modulate specific sleep signatures, to test their causal relationship to learning and memory

NREM sleep

The phenomenon of hippocampal replay present in NREM sleep has long intrigued sleep researchers as a possible mechanism by which labile memory traces in the hippocampus are stabilized and sent to the neocortex for long-term storage. Replay consists of cells active during waking task acquisition (Fig. 1C) being reactivated during sleep (Fig. 1D) with conserved temporal order. Ripples (Fig. 1D) take place in the CA1 region of the hippocampus indicating fast, synchronous excitatory inputs from the principal cells of the CA3. Replay of waking sequences occurs at the peak of ripples (Wilson and McNaughton, 1994; Kudrimoti et al., 1999). Recruitment into ripple activity is at least in part associated with the strength of connections between cells formed during wakefulness--cells that are significantly more likely to fire together during waking are more likely to fire together during ripple associated replay events (Wilson and McNaughton, 1994; Pavlides and Winson, 1989). Thus cell participation in ripple events is heavily biased by waking patterns of activity. These findings served as the basis for the theory that SWR and replay activity therein are mechanisms for strengthening and storing memories.

Indeed, when SWR activity is electrically disrupted during sleep following acquisition of a new hippocampus-dependent task, a significant impairment is observed in an animal's ability to perform those tasks, likely due to aberrant consolidation (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010). Whether this performance impairment was due to the loss of SWRs

specifically, or simply the disruption of hippocampal population activity due to the electrical stimulation, was not testable until optogenetics became a common technique.

Studies utilizing optogenetics found that selectively inhibiting pyramidal cells of the CA1 once SWRs were detected resulted in a significant decrease in an animal's ability to reinstate assembly activity representing a novel environment (van de Ven et al., 2016). Intriguingly, inhibiting SWRs did not result in a change in assembly activity representing already consolidated familiar environments, suggesting the necessity of hippocampal SWR activity for consolidating new information. In addition, novel assemblies that stabilized more rapidly than others during waking acquisition were also unaffected by the disruption of offline reactivation, perhaps because their original online activity was enough to result in synaptic stabilization, obviating the necessity of consolidation. These findings support the hypothesis that offline activity is important for resolving unfinished business, strengthening weak connections made during waking.

A second study using a similar paradigm in which principal CA1 cells express an inhibitory optogenetic ion channel investigated the effect of SWR suppression during the 3 hours following passive exploration of a novel open field. They explored the ability of hippocampal place cells to subsequently encode the same open field environment after inhibiting pyramidal activity for 500 ms triggered after the detection of the ripple. No explicit learning task was given for the animals in the environment. Surprisingly, no significant differences were found between the place cell stability, coherence, or likelihood of remapping in the novel environment between animals with and without CA1 pyramidal activity during spontaneous SWR in this offline period (Kovacs et al., 2016). Given the task impairment seen in Girardeau et al., as well as the change in assembly activity in novel environments of the van de Ven paper, one might conclude that disrupting the place cell code during the consolidation of novel memories underlies the spatial memory deficits seen following SWR inhibition. On the surface, this conclusion is not born out by Kovacs et al.

However, they did observe a post-inhibition rebound in pyramidal cell activation that appeared to be coincident with strong local field potential (LFP) depolarization and, in their example, occurred in the ripple frequency. The apparent rebound depolarization-coupled ripple activity could itself have stabilized the memory code. Also, the authors point out that passive exploration may be relatively resistant to memory disruption by SWR inhibition as compared with active learning. For instance, a task in which learning is involved (and tested), such as an appetitive maze, should require the encoding and association of more information than is required when wandering in an open field. Another possibility is that ripples are involved primarily in the transmission of information to long-term cortical storage, and their disruption does not have an effect on spatial map stabilization within the hippocampus. Future experiments should be able to distinguish between these possibilities.

REM sleep

The question of whether REM sleep has a role in learning and memory has been particularly controversial (Vertes and Eastman, 2000; Siegel, 2001). In rodents, early sleep deprivation studies indicated that REM sleep is necessary for proper memory formation; however, as discussed above, the methods available at the time came with numerous caveats, leaving some sleep researchers skeptical of whether REM deprivation was truly causing memory impairments. Similar to our ability to optogenetically disrupt sleep as a whole, we now can design experiments selectively targeting REM sleep independently to isolate its role in memory processing without causing stress to the animal or affecting other sleep states. What we have learned about REM sleep thus far suggests that it serves several key functions in consolidation.

REM sleep is for forgetting

REM sleep appears to be a time during which synapses are pruned, creating circuits with more sparse and more specific firing. Recent studies in mice show that the pruning of dendritic spines in the motor cortex (M1) during REM sleep is coincident with the improvement on a rotarod task (Li et al., 2017; Zhou et al., 2020). Similarly, the firing of adult-born neurons (ABNs) in the dentate gyrus becomes more sparse in response to contextual fear conditioning (CFC) following a period of REM sleep (Kumar et al., 2020). These data provide evidence that one of the functions of REM sleep is to pare down excessive activity following learning and suggests a role for REM sleep in “forgetting” or weakening memories.

Studies of melanin-concentrating hormone (MCH) neurons in the hypothalamus provide further evidence for the function of REM in forgetting (Izawa et al., 2019). Optogenetically inhibiting REM-active MCH neurons during REM periods following the learning phase of a novel object recognition (NOR) task improves the animals’ ability to discriminate between new and familiar objects. The converse was also true--when MCH cells were optogenetically activated during REM periods, the animals’ ability to discriminate between novel and familiar objects was diminished, as was their performance in an second task: contextual memory following fear conditioning. As this study also found that REM periods normally involve MCH cell activity, REM sleep appears to play a role in forgetting. It may be that the normal MCH activity during REM serves the function of schematic integration and extraction of gist, losing details as memories are moved to long-term storage or schematized.

REM sleep is for remembering

To complicate matters further, however, evidence also exists that REM sleep promotes consolidation as well. The inhibition of theta activity by silencing medial septal GABAergic projections to the hippocampus during REM sleep significantly disrupted consolidation of CFC and novel object place recognition (NOPR) tasks (Boyce et al., 2016). This result raises the possibility that REM sleep is a time for both strengthening and weakening synapses, due to the unique electrophysiological and neurochemical properties of the hippocampus in REM sleep. Hippocampal theta activity during REM sleep segregates the population activity into temporal cycles (Fernandez-Ruiz et al., 2017; Dragoi and Buzsaki, 2006; Foster and Wilson, 2007; Gupta et al., 2012), which may be key to allowing both synaptic strengthening and weakening during this period of time. Previous studies from our lab show that spatial representations fire differently in relation to theta phase based on their novelty or familiarity (Poe et al., 2000). New place cells fire at peak population activity, resulting in synaptic strengthening, while place cells encoding familiar spaces fire closer to the low point of activity, likely weakening the associated synapses through the principles of heterosynaptic depotentiation (Poe et al., 2000). In addition to the theta rhythm, the neurochemical composition of the hippocampus during REM sleep, particularly the complete lack of noradrenaline and high levels of acetylcholine, could make it an optimal time for restructuring synaptic connections and integrating new information into old memory schema (Poe, 2017; Kim et al., 2019).

Population activity and timing is key in sleep-dependent consolidation

Through the temporal precision afforded by optogenetic techniques, sleep researchers have been able to tease apart the function of various sleep signatures and oscillatory activity. What we are finding suggests that the effect that a particular sleep stage or signature has on a neural ensemble is highly dependent on the population activity surrounding the ensemble. We have a

great deal of evidence that reactivation of an ensemble during sleep strengthens the circuitry involved (Wilson and McNaughton, 1994; Kudrimoti et al., 1999); however, it was initially unclear if this strengthening was simply a result of activity-dependent plasticity that could occur during any brain state. Furthermore, activity within sleep stages must be tightly temporally regulated in order to properly consolidate information. Studies suggest that the timing of action potentials of cells in an ensemble in relation to oscillatory population activity is critical to whether the connections of a memory trace will be strengthened or weakened. In a series of studies utilizing a brain-machine interface task in rats, researchers determined that whether an ensemble reactivated in closer proximity to a slow oscillation (SO) or to a delta wave had an effect on whether the memory was consolidated or weakened (Gulati et al., 2017). Ultimately, they found that perturbing spiking activity during the up states of SOs led to an impairment of memory consolidation, whereas disrupting spiking during the up state of a delta wave actually boosted sleep-dependent memory consolidation. Further exploration suggests that one underlying mechanism of the changes in consolidation are dependent on nesting of spindles within SOs or delta waves. SO-spindle coupling appears to preserve memory reactivation, as reactivation strength and duration increased with SO-spindle coupling, and changes to SO-spindle coupling correlated with task performance. Interestingly, when researchers optogenetically perturbed SO spiking activity, a rapid reduction in reactivation strength followed. The converse was true when delta activity was disrupted, resulting in significantly stronger and longer lasting reactivation, supporting the idea that inhibiting delta waves increased the strength of a memory. Ultimately, inhibiting SO activity abolished any significant rescaling of the neural network involved in the task used, resulting in impaired performance. In another study, this group inhibited the specific neural ensemble controlling the task selectively during the up states of NREM sleep, which also impaired task performance as well as the ability of the ensemble to develop sparse coding of the task (Sousa et al., 2019). Finally, another group showed the same relationship between SO-spindle coupling and spindle-nested ripples that indicate cell activation. When they increased

sleep spindles by activating parvalbumin interneurons in the reticular nucleus of the thalamus, they increased spindle-ripple coupling in the upstate of SO's and increased memory on both contextual fear memory and novel-object place recognition (Latchoumane et al., 2017). These data all support the hypothesis that sleep is a time during which synaptic connections are both strengthened and weakened in order to efficiently and accurately encode an animal's environment.

2.4 Neural ensembles require offline processing to accurately consolidate new information

Optogenetic techniques allow for further exploration into the circuit-level changes underlying disrupted memory consolidation following abnormal sleep dependent activity. Alteration of sleep state activity described in the experiments above broadly results in memory impairment suggesting a relationship between processing during sleep and integrity of memory ensembles. Although they provide invaluable insights, direct manipulation of neural ensemble activity during sleep is required.

Rhythmic activity like the theta oscillation is important for memory consolidation

So far, studies suggest that ensembles undergo reshaping during offline processing that can lead to both strengthening and weakening of synaptic connections. The participation of these ensembles in the local rhythmic activity present in sleep appears to be crucial for proper memory formation, in a manner that is highly specific. In another experiment by Kumar et. al., optogenetically modulating the activity of adult-born neurons (ABNs) elucidated the role of these cells in consolidating contextual fear memories during REM sleep (Kumar et al., 2020). Their study shows that the tuning of ABNs representing the CFC memory during REM sleep is critical to the animal's ability to recall this memory the following day. Inactivating the ABN ensemble during REM sleep resulted in poor consolidation of the CFC memory, suggesting the necessity

of ABN activity in the DG during REM sleep for memory retention. Silencing ABN activity during NREM sleep following learning did not produce the same effect, indicating a role for REM activity of ABNs in the processing of fear memories.

Non-ensemble cells are not involved

Intriguingly, when ABNs that were not part of the memory encoding process were randomly stimulated during REM sleep, consolidation was also impaired. This suggests that interfering with the integrity of the ensemble active during REM processing is critical to proper consolidation. Similarly, it provides evidence of the causal nature of sleep reactivation to memory consolidation--it suggests a role for the unique population activity of sleep stages in shaping ensemble circuits, which directly contributes to the retention of a memory.

Cortical ensembles also participate in offline processing for memory consolidation

The necessity of ensemble reactivation during sleep appears to be critical to brain regions outside of the hippocampus as well. When looking at CFC in the retrosplenial cortex (RSC), researchers found a similarly important role for reactivation in learning and memory (Sousa et al., 2019). Isolating stimulation to cells active during a CFC task, enabled tightly controlled temporal and spatial modulation of the ensemble presumed to represent the CFC memory trace. To examine the necessity of activation during offline processing to memory integrity, researchers optogenetically stimulated the fear memory ensemble during both anesthesia and natural sleep; ultimately, both conditions produced similar results. This study in particular focused on examining the potential role of sleep-dependent processing in transferring memories to long term storage. Predictably, animals with activity in their hippocampi blocked 24 h after learning, did not form the proper association between the context and the shock, indicating an improperly consolidated fear memory. This was not the case in the animals that underwent optogenetic stimulation of the RSC memory trace during either anesthesia or sleep right after

learning--these mice displayed normal freezing behavior despite the 24 h later hippocampal block, suggesting that high-frequency activation of the trace ensemble was able to create a hippocampus-independent memory of the fear trace, effectively accelerating the process of systems consolidation. Intriguingly, the optogenetic stimulation protocol was only effective when the animals were in a non-waking state. Stimulation of the fear trace while animals were awake and behaving did not protect against memory impairment following hippocampal block, indicating that sleep state activity, or anesthesia plus artificially stimulated activity, is necessary for systems consolidation. Which aspects of sleep are crucial for this process, however, are uncertain, as these experiments did not differentiate between REM or NREM sleep. Additionally, as the researchers note, their stimulation protocol did not maintain the temporal patterns of activity. Given the difference in effectiveness of the optogenetic stimulation between wakefulness and sleep, future research into whether stimulation during only REM or NREM is still effective in inducing systems consolidation would provide more evidence into the role of each sleep stage in long-term memory storage.

Similar work in the visual cortex assessed the effect of offline stimulation of the neural ensemble on memory consolidation (Clawson et al., 2020). Rhythmically activating this trace while an animal was anesthetized biased surrounding neurons towards responding to the same cue during subsequent waking, suggesting a role for offline activity in modifying responsiveness of visual cortical neurons to allow visual discrimination. Inhibition of the cue ensemble during REM and NREM sleep resulted in a generalized behavioral response to all gradients. It will be interesting to observe in future studies what, if anything, distinguishes the roles of REM and NREM sleep in consolidation.

2.5 Conclusions and Future Directions

In summary, applying optogenetic techniques to sleep research has provided an exciting new avenue by which to understand the mechanisms involved in sleep-dependent memory consolidation. We are now able to say with confidence that the negative effects of sleep deprivation on memory consolidation are not solely due to the anxiety- and stress-inducing methods of sleep deprivation. Instead, we have begun to determine the specific functions of sleep signatures such as ripples coupled with spindles nested in slow oscillations, and theta. The studies we covered here included demonstrations of sleep's involvement in hippocampus-dependent learning such as spatial learning and contextual fear learning, perceptual learning like visual pattern and texture discrimination learning, motor learning tasks, and even neuroprosthetic learning, the consolidation of all of which are disrupted by specific sleep feature manipulations: delta, theta, spindles, spindle-ripple coupling, slow oscillation spindle coupling during REM and non-REM sleep. In fact, using optogenetics to selectively disrupt these features of sleep without altering overall sleep architecture or structure consistently finds learning and memory disruptions, unlike the gross sleep deprivation or disruption studies which have produced more variable results.

Despite these advances, the mysterious nature of sleep still remains, as do challenges to the sleep-memory hypothesis. For example, we are still unsure of the degree to which experimental sleep studies done in rodents (where the vast majority of our knowledge of sleep's electrophysiology comes from) are applicable to humans. Because of the difficulty of obtaining human intracranial electrophysiological data, for example, we cannot be fully confident that sleep signatures important to rodent learning and memory, such as hippocampal replay or REM sleep theta, are present in human sleep, let alone critical for our sleep-dependent processing. In order to link sleep disturbances to disease and psychiatric illness, more studies will need to be

done to demonstrate that sleep-dependent consolidation processes in laboratory animal models are analogous to human sleep. Without the ability to target specific neural ensembles in the human brain, as is possible when using optogenetics in animal models, researchers instead use targeted memory reactivation (TMR) as a method to modulate electrophysiological activity during sleep. TMR involves the re-exposure of sensory cues (odor or sound) during sleep that were present during wakeful learning. Unlike the use of light in rodent optogenetic studies, TMR uses cue presentations as a method to selectively reactivate neural ensembles associated with the stimulus. Since the discovery that TMR modulates neural activity in the sleeping brain at the time of cue presentation and improves memory of associated items at subsequent testing by Rasch et al., researchers have sought to elucidate to what extent findings in rodent studies apply to humans. For a full review of recent publications related to this topic, see Cellini and Mednick, 2018 and Mankin and Fried, 2020. The use of intracranial recordings to explore sleep in select patient populations as well as new non-invasive technology such as magnetoencephalography in healthy participants will help shed light on some of these questions.

The power of optogenetics as a technique comes from allowing researchers to design experiments that target neurons for modulation in a temporally and spatially specific manner. For sleep research, this means being better equipped to test the underlying mechanisms of sleep without adding confounding variables such as stress or sleep architecture changes into experiments. From just the last few years of research into sleep using optogenetics, we have gathered convincing evidence that without sleep, memories are not processed correctly, largely due to the disturbance of activity patterns underlying sleep signatures such as ripples and spindles. We are beginning to see evidence of sleep-dependent processing occurring in various regions of the brain such as the visual cortex and retrosplenial cortex and, through optogenetic interruption, learning that when the electrophysiology of sleep is disrupted memory

consolidation is impaired as well. The specificity allowed by optogenetic techniques means we are able to narrow down our inquiries to single neuronal ensembles representing a memory trace and observe the impact of sleep-dependent consolidation on that memory alone.

Chapter 3

Chemogenetic manipulation of OLMα2 interneurons disrupts offline processing of spatial and contextual information

Abstract

Accurate storage and maintenance of information is critical to the survival of organisms across the animal kingdom. A large body of research supports a correlation between sleep and memory; however, the underlying mechanisms through which sleep supports memory consolidation are not yet fully delineated. Here, we use mice to interrogate the role of hippocampal oriens-lacunosum moleculare (OLM) interneurons in facilitating offline processing and consolidation of spatial and contextual information. While multiple studies posit a crucial role for OLM neurons in encoding information in the hippocampus during awake behaving, their role in offline processing has not yet been determined. We used chemogenetic methods to selectively inhibit these cells in the dorsal or ventral hippocampus during offline periods following task acquisition. We found that OLM inhibition in the dorsal hippocampus negatively affected performance on spatial and contextual fear tasks, while ventral hippocampus inhibition only impacted spatial task consolidation. Using *in vivo* electrophysiology recordings across the sleep/wake cycle, we determined that inhibition of OLM cells altered the oscillatory activity during rapid eye movement (REM) sleep without significantly affecting other behavioral states, providing further evidence for the role of OLM cells in maintaining the hippocampal theta rhythm. Together, these studies provide more evidence for the importance of REM sleep theta in stabilizing hippocampal memories and suggest a role for OLM interneurons in maintaining the oscillatory activity necessary for offline consolidation.

3.1 Introduction

Memory formation and storage occurs over time, and each behavioral state plays a unique role in the process. While an animal is awake and behaving, active cells are encoding information from the environment to a labile trace in the hippocampus. Strengthening this trace for long-term storage requires additional offline processing, including sleep-dependent mechanisms. Disruption in sleep-dependent consolidation results in impaired recall of behavioral tasks requiring the hippocampus, as well as diminished long-term plasticity (LTP) in the CA1 region (Havekes et al., 2016; Graves et al., 2003; Prince et al., 2014). While there is a great deal of evidence supporting the role of sleep in memory consolidation, the underlying mechanisms involved are less understood.

Sleep is comprised of multiple stages, broadly defined in rodents as rapid eye movement (REM) sleep and non-REM (NREM) sleep. Each state is characterized by unique oscillatory activity and neurochemical composition and is hypothesized to have a specific function in the memory consolidation process. Oscillatory activity in the hippocampus temporally regulates information arriving from disparate brain areas, allowing for perception, learning, and memory formation. This rhythmic activity is precisely controlled by GABAergic interneurons, cells that participate in feedforward and feedback inhibition to regulate the dynamics of neuronal populations. The hippocampus contains a wide diversity of interneuron subtypes, with each class of cell displaying unique firing patterns, target location, and resonant oscillatory properties (Cobb et al., 1995; Klausberger et al., 2003; Lapray et al., 2012). This heterogenous cell population allows for the development of complex oscillatory activity within hippocampal circuits. Disordered interneuron activity can result in impaired memory encoding and is thought to play a role in the etiology of numerous psychiatric and neurologic conditions, such as Rett's disease,

temporal lobe epilepsy, autism and schizophrenia (Steullet et al., 2017;Ito-Ishida et al., 2015; Hoffman et al., 2016; Canitano and Pallagrossi 2017).

Oriens-Lacunosum Moleculare (OLM) cells are a dendrite-targeting subtype of somatostatin-positive interneurons. These cells have soma in the stratum oriens, while their axons arborize in the stratum lacunosum-moleculare, synapsing on to the distal dendrites of CA1 pyramidal cells and inhibiting inputs from the temporoammonic pathway (TA). Their activity also results in the disinhibition of proximal pyramidal cell dendrites, resulting in a bias towards intra-hippocampal Schaffer collateral (SC) inputs from the CA3. Taken together, this suggests that OLM cells can act as a "switch" between the two main glutamatergic inputs into the CA1 (Leao et al., 2012). Ablation of these cells in the CA1 results in decreased spatial memory abilities (Haam et al., 2018), while manipulating the activity of these cells during behavior can bidirectionally impact learning in both spatial and fear-based tasks, in a manner dependent on the location of the cells stimulated on the dorso-ventral axis (Siwai et al., 2018; Mikulovic et al., 2018). The role OLM cells may play in offline memory consolidation, however, has yet to be investigated. Given that these cells contain nicotinic acetylcholine receptors (Leao et al., 2012), fire phase-locked to hippocampal theta rhythm (Klausberger and Somogyi, 2008; Varga et al., 2012), and may play a role in theta generation itself (Gu et al., 2020; Mikulovic et al., 2018; Ferguson et al., 2015), it stands to reason that OLM cells could be a key component of REM-sleep theta and the aspects of memory consolidation in which it plays a role (Boyce et al., 2016).

To better understand the role of OLM cells in offline consolidation, we used a transgenic mouse line with Cre in neurons expressing the *Chrnα2* acetylcholine receptors, resulting in highly specific targeting of OLM cells (Leao et al., 2012). Our experimental paradigm involved two single-trial hippocampus-dependent behavioral tasks, during which we manipulated the offline OLMα2 cell activity selectively with chemogenetics (Fig. 3.1). We also recorded cortical EEG and hippocampal LFP during offline periods to analyze effects on sleep architecture and

oscillatory activity (Fig. 3.2). While inhibition of dorsal hippocampal (dHPC) OLMα2 cells did not impact sleep architecture, we did find a significant decrease in REM sleep theta power. As hippocampal function changes along its longitudinal axis, we also inhibited OLMα2 cells in the ventral CA1 (vCA1) following task acquisition, finding that inhibition resulted in impaired performance on an object location task but not contextual fear memory. Similar to dorsal CA1 (dCA1) inhibition, inhibiting vCA1 OLMα2 cells does not affect sleep architecture but does disrupt the spectral properties of REM sleep. These data provide evidence that OLMα2 cells play a role in offline consolidation, possibly through maintaining REM theta. They also suggest that their role in offline processing changes along the hippocampal longitudinal axis, as seen in their change of function during waking (Siwani et al., 2018).

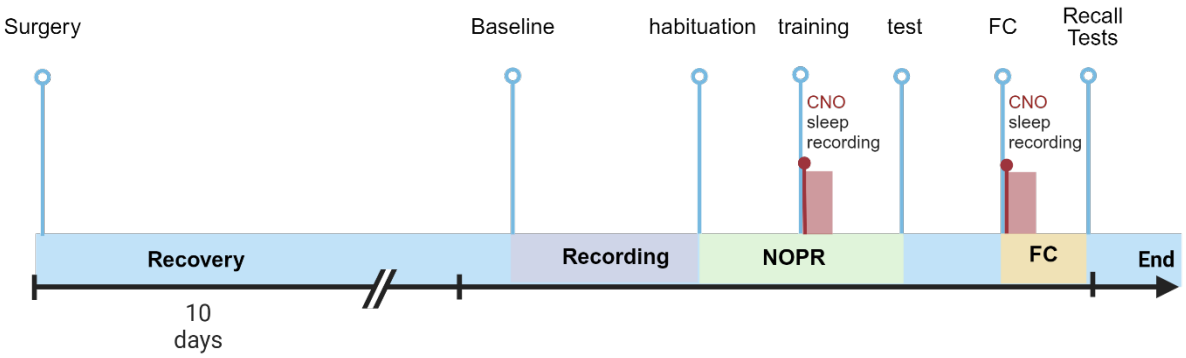
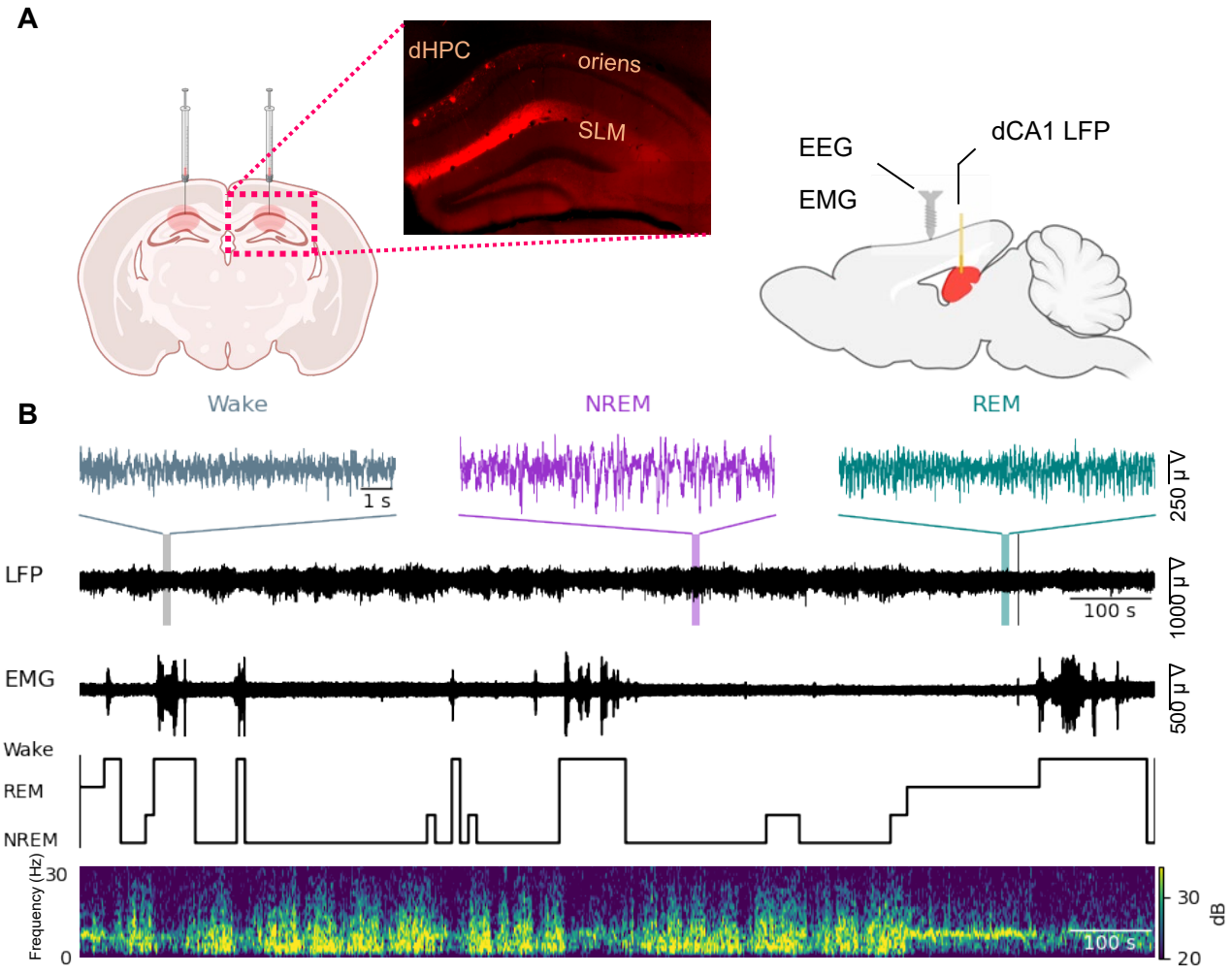


Figure 3.1: Experimental Timeline.
For the experiments described, male *Chrna2*-Cre mice at P60 underwent bilateral virus injection in the dorsal or ventral hippocampus, as well as EEG/EMG and LFP electrode implantation. Following 10 days of recovery, mice were recorded from for 2 days of baseline sleep. NOPR was performed first, comprised of habituation, training, and testing on consecutive days. Immediately following training, animals were given an injection of CNO and recorded from for 4-hours. Fear conditioning was similarly performed. Following fear recall tests, animals were transcardially perfused, and hippocampal sections were stained and imaged.

3.2 Results

Figure 3.2: Experimental setup and data collection.



(A) Chrna2-Cre mice receive bilateral injections of AAV1-DIO-hm4D(Gi) or AAV1-DIO-mCherry control vectors in the dCA1 (-1.7, +/- 1.0, -1.5). OLM cell bodies are labeled in the oriens, and axon arborization is seen in the str. lacunosum-moleculare. Polysomnography recordings are made from EEG screws in the parietal cortex (-2.1, +/- 2.4) and neck EMG wires. Spectral analysis is performed on dCA1 LFP recordings. **(B)** Example polysomnography analysis across the sleep-wake cycle, with representative segments of wake (gray), NREM sleep (purple), and REM sleep (teal) epochs highlighted.

Object location memory is impaired when dorsal OLMα2 cells are pharmacogenetically inhibited during offline consolidation

The ability of rodents to recognize when an object has moved from a familiar location requires the integration of spatial and contextual information, as well as the ability to distinguish between novelty and familiarity. As such, these tasks can be used as a proxy for episodic-like memory.

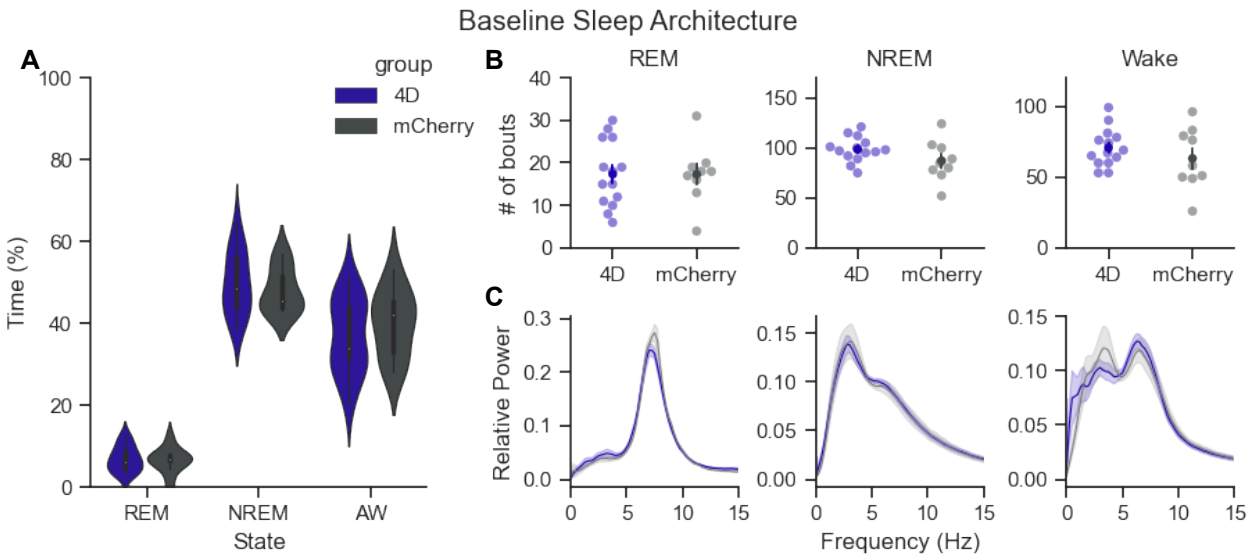


Figure 3.3: Baseline sleep architecture and dCA1 LFP spectral properties are unchanged between groups prior to CNO administration.
(A) The percentage of time spent in each state during baseline recording without CNO is not statistically different between the groups (two-sample t-test with Bonferroni correction, p=ns). **(B)** The number of epochs of each activity state do not differ between groups under baseline conditions (t-test, p=ns). **(C)** Mean spectral properties of each state are similar between groups under baseline conditions.

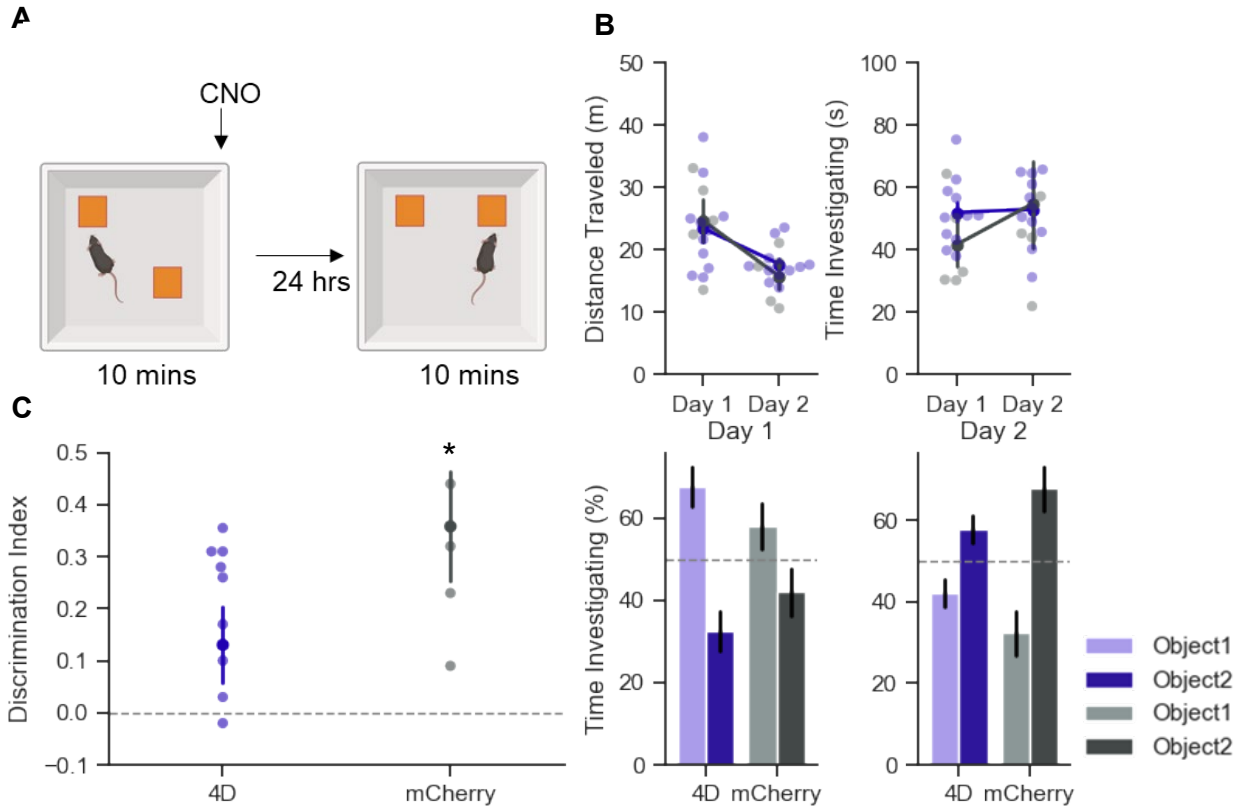


Figure 3.4: Inhibition of OLMα2 interneurons following NOPR acquisition results in impaired novel object location recall.

(A) Mice are placed in a familiar chamber with two novel objects and allowed to explore for 10 minutes. Immediately after completion of the task, they receive an intraperitoneal injection of CNO (5 mg/kg), returned to their home cage, and recorded from for 4 hours. The next day, mice are brought back to the chamber in which one of the objects is displaced and allowed to explore again for 10 minutes. **(B)** The distance traveled and total time investigating both objects remains unchanged between groups on both days**. (C)** Left: The preference for the moved object, expressed through the discrimination index ((Moved $Object_{Time}$ – Stationary $Object_{Time}$)/ (Moved $Object_{Time}$ +Stationary $Object_{Time}$)). A DI of zero indicates no object preference. Control animals significantly prefer the moved object, while OLMα2-4D do not ($p^* = 0.03$, one-sample t-test). Right: Percent time investigating each object.

This task is dependent on a functional hippocampus (Oliveira et al., 2010) and is disrupted by sleep deprivation (Havekes et al.,2014). Several studies have provided evidence that impairing OLMα2 cells during acquisition of spatial tasks impairs performance, suggesting that these cells are necessary during the encoding of hippocampus-dependent tasks (Haam et al., 2018; Siwani et al., 2018). The role of OLMα2 neurons in offline processing, however, has not been investigated. To investigate their role in spatial memory consolidation, we used a novel object placement recognition (NOPR) task in which animals explore two identical objects during a 10-

minute training period. During testing, mice are reintroduced to the NOPR chamber with one of the objects displaced. This task utilizes mice's preference for engaging with novelty in their environment; when presented with a moved object and a stationary object, mice will spend more time investigating the novel location. If they have not formed a stable memory of the context and location of the objects, however, mice will spend equal time investigating both objects as novel. To test the effect of OLMα2 inhibition during consolidation on maintaining contextual memories, *Chrn*α2-*cre* male mice were injected with either AAV1-DIO-hm4Dgi or AAV1-DIO-mCherry in the dorsal CA1 region (AP: -1.7, ML: +/- 1.0, DV: -1.6) and implanted with dCA1 LFP electrodes, cortical screws, and EMG wires for sleep-state recording. Following 7 days of recovery and 3 days of handling, mice underwent 2 days of baseline recording, to ensure that they exhibited regular sleep cycles. Importantly, baseline recording did not find any differences in sleep architecture or spectral properties between the two groups without other manipulations (Fig. 3.3). To begin the NOPR task, mice were habituated to the chamber without any objects during a 10-minute session. On Day 1, mice were placed in the habituated chamber with two identical objects (Object 1 and Object 2) and allowed to explore for 10 minutes. Tracking was performed during the training session, and the amount of time animals spent investigating each object (defined by having their nose within a 2 cm radius of the object) was calculated. Immediately following acquisition of the task, animals received an intraperitoneal injection of clozapine-N-oxide (CNO) (5 mg/kg) to inhibit the OLMα2 cells and were returned to their home cage for 4 hours of sleep state-recording. After 24 hours, mice were returned to the chamber in which one of the objects (Object 2) had been moved to another quadrant and were allowed to explore for another 10-minute testing session (Fig 3.4A). There were no differences between groups in either the distance traveled or the total time spent investigating the objects ($T_{Object1} + T_{Object2}$), indicating that the CNO did not affect locomotion or exploratory behaviors. The discrimination index (DI) was calculated for the test session to quantify the preference towards the moved object. While both groups spent above 50% of the time investigating the moved object

($100*T_{Object2}/T_{Total}$) , only the OLMα2-mCherry controls showed a significant preference for the moved object demonstrated by the DI (Fig 3.3C, one-sample t-test of DI mean vs theoretical mean of 0, mCherry: p*= 0.027 vs 4D p-value: 0.1017), indicating that OLMα2-mCherry animals were able to form and retain a memory of the objects and their locations between the training and testing session. OLMα2-4D animals, however, did not demonstrate a significant preference for the moved object. This suggests that inhibiting the OLMα2 cells during consolidation impaired object location memory, leading OLMα2-4D mice to explore both objects as though they were new. As both groups received 5 mg/kg CNO injections following the 10-minute training session, the difference in DI was not due to the presence of CNO during consolidation.

Inhibition of OLMα2 cells does not affect sleep architecture but results in decreased theta power during REM sleep

To investigate the mechanisms underlying the observed impairment in object memory, we next analyzed the 4 hours of sleep recorded following the task, during the presence of CNO. The 4-hour post task window was chosen as this period is critical to offline consolidation (Havekes et al., 2014), and encompasses the time during which CNO activates the inhibitory hm4D(Gi) receptors. The hm4Dgi-mediated inhibition did not affect sleep architecture, as the percentage of time spent in each sleep state and the number of bouts of each state during the 4-hour recording period remained unchanged between the two groups. The only significant effect on the spectral properties of dCA1 LFP during the 4-hour recording session was a decrease in the power of the 5-10Hz theta band during REM sleep (Fig 3.4C, mCherry vs 4D binned band power, two-sample t-test = 0.04). This suggests that the chemogenetic inhibition of OLMα2 cells

affects the strength of theta oscillations during REM sleep, which may contribute to offline consolidation (Boyce et al., 2016).

Oscillatory activity patterns of different frequencies are able to interact with one another through a process called cross frequency coupling (CFC). CFC is thought to integrate local circuit processing, reflected by high-frequency oscillatory activity, with low-frequency rhythms that span brain regions, resulting in functional systems that can transfer information between large- and small-scale networks (Canolty and Knight, 2010; Colgin, 2015; Lisman and Jensen, 2013). One coupling pattern with functional implications is the phase-amplitude coupling (PAC) between theta phase and gamma amplitude. This occurs when the phase of the slower theta rhythm modulates changes in gamma amplitude, the strength of which correlates with learning, and is disrupted in disease states (Tort et al., 2009; Vivekananda et al., 2020; Goutagny et al., 2013). Interestingly, theta-gamma coupling strength is significantly increased in the hippocampus during REM theta compared to waking, which may indicate that it is critical to the memory consolidation function of REM sleep (Scheffzuk et al., 2011). To explore whether our manipulations impact PAC during REM theta states, we calculated comodulograms as previously described (Tort et al., 2008). This involves breaking both the slow and fast oscillatory activity into narrow-filtered band pairs**,** and using a measure called the modulation index (MI) to assess the strength of the coupling. Further discussion of the mathematical principles behind MI calculation can be found in the Methods. As shown in Fig 3.6A, we observed theta-gamma coupling patterns in the CA1 and parietal cortex consistent with previous studies (Bandarabadi et al., 2019). Hippocampal CA1 LFP recordings targeted to the stratum radiatum during REM sleep display strong theta-gamma coupling in the 60-100 Hz frequency, while parietal cortical EEG recordings show strong coupling between ~110 to 150 Hz in addition to the slower frequency band (Fig 3.6A). To determine whether inhibition of OLMα2 cells affected theta-gamma coupling, we compared comodulograms from REM sleep during the post-task recording

period. To facilitate comparison across animals, comodulograms were normalized to baseline for each animal before quantification. Then, the average MI was computed by averaging all MI values at the intersection of the theta and gamma frequency bands. For the sleep recording post NOPR, we saw a trend toward decreased PAC in the dCA1 LFP recordings, but this result was not significant (t-test, $p= 0.0598$). The coupling between both the low and high gamma bands and theta phase in the PCx EEG remained unchanged by manipulation of OLM cell activity (one-way ANOVA, $p= 0.87$).

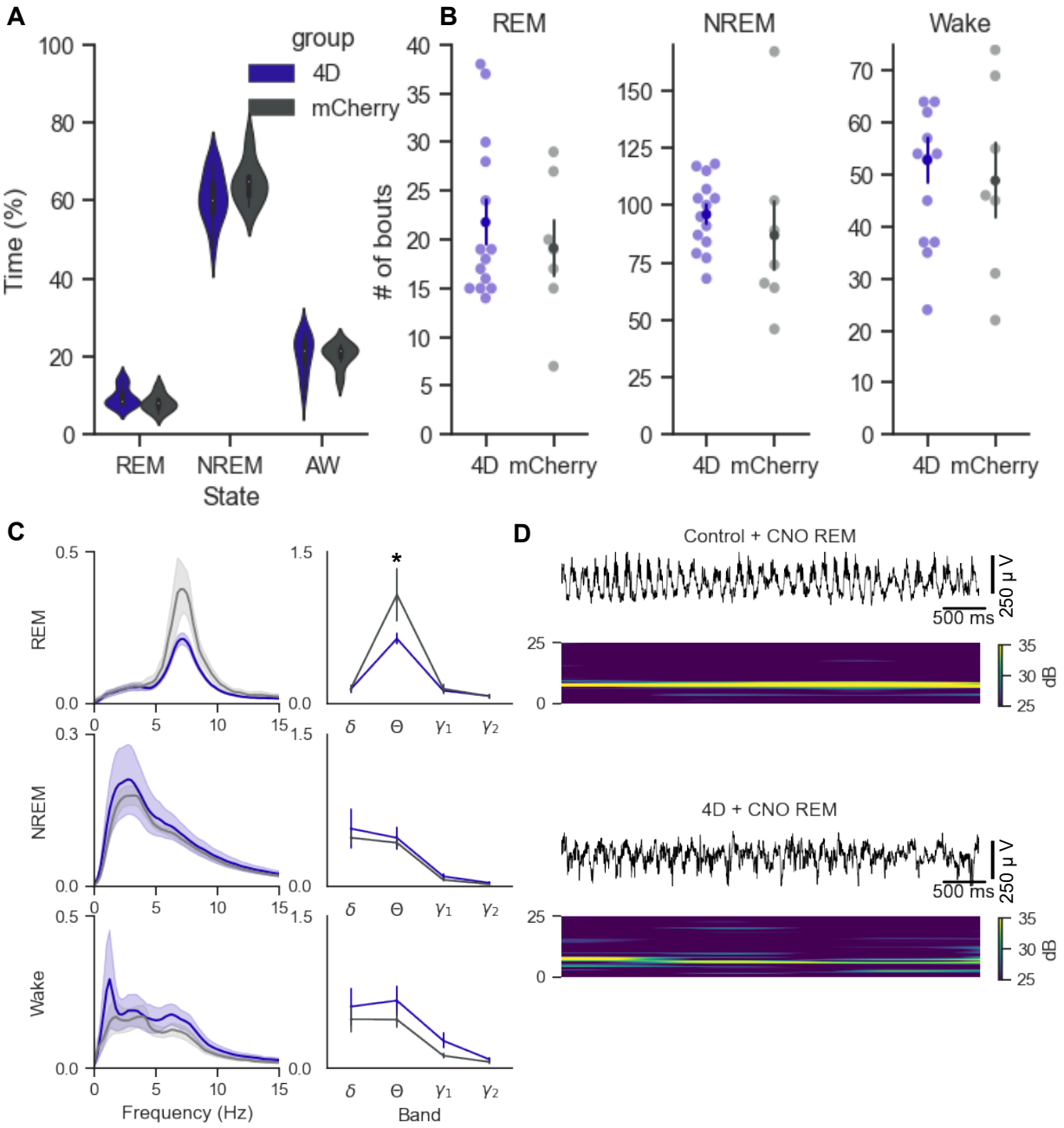


Figure 3.5: Inhibition of OLMα2 interneurons does not affect sleep architecture but does impact REM power spectral properties.

(A) Both 4D and mCherry mice spend the same percentage of time in each activity state following NOPR and CNO administration. **(B)** The number of bouts of each activity state is similarly unchanged between groups. These results indicate that sleep architecture is unchanged by chemogenetic inhibition of OLMα2 cells. **(C)** Mean power spectral density plots of each sleep state. Binned band power for delta (0.5-2 Hz), theta (5-10 Hz), slow gamma (30-80 Hz), and fast gamma (80-120 Hz) for each state and group. The only significant difference occurred during REM sleep, in which 4D animals had significantly reduced theta power ($p^* = 0.041$). **(D)** Example traces of REM sleep in mCherry (upper) and 4D (lower) animals post NOPR acquisition with CNO. Decreased theta power is evident in the spectrogram of 4D animals.

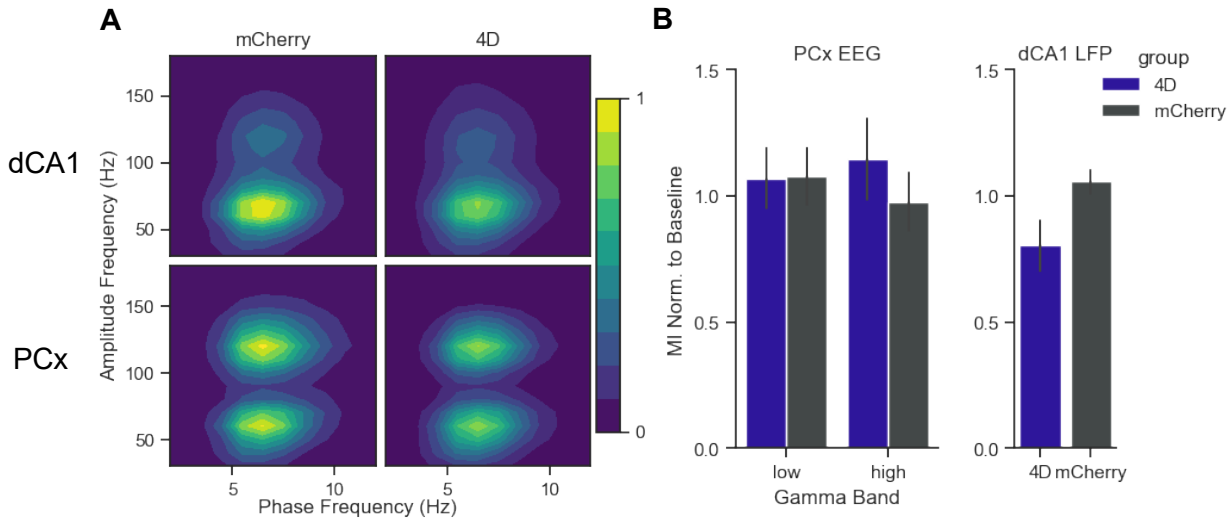


Figure 3.6: Inhibition of OLMα2 neurons following NOPR lowered but did not significantly reduce theta-gamma coupling in the dCA1 region during REM sleep.
(A) Comodulograms of mCherry (n=7) and 4D (n=10) during post-NOPR REM sleep. Each animal's comodulogram was normalized to baseline before combining. **(B)** Theta-gamma in the PCx EEG was unchanged between mCherry and 4D animals in low and high gamma (one-way ANOVA, p=0.87). Theta-gamma coupling in the dCA1 was decreased from baseline, but not significantly (p = 0.0598).

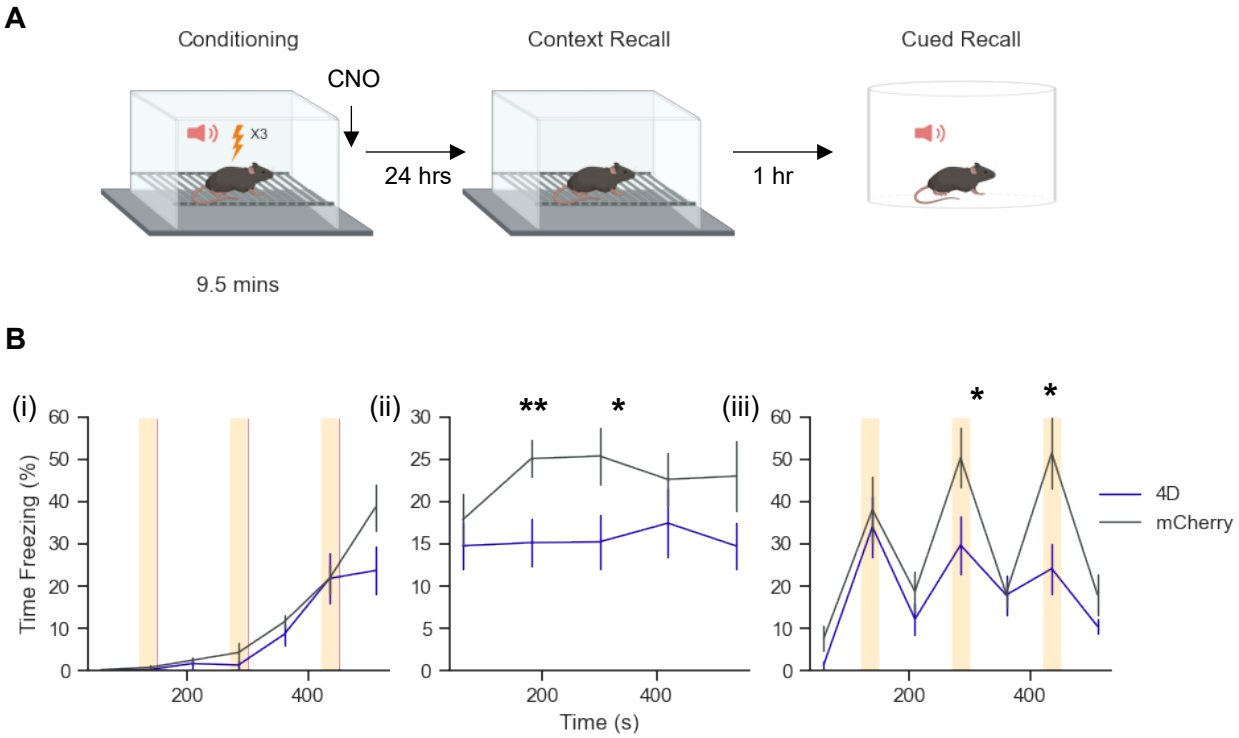


Figure 3.7: Inhibiting dorsal OLMα2 neurons during offline processing impairs hippocampus-dependent fear memory recall.

(A) Fear conditioning paradigm. During Day 1, animals receive 3 tone-shock pairs, composed of a 30 second tone and a co-terminating 2 second 0.6 mA foot shock. Immediately following conditioning, animals are given a 5 mg/kg CNO injection and returned to their home cage for polysomnography recording. 24 hours later, mice are returned to the conditioning context and freezing behavior is observed for 10 minutes. They are returned to their home cage for 1 hour, then introduced to a novel context and re-exposed to the conditioned tone and observed for freezing behavior. **(B)** When binned by 120s, the 180-300s time bins were significantly different between the two groups (two-way ANOVA, adjusted Holm-Bonferroni's multiple comparisons test, adjusted p**-value = 0.011; p*= 0.038). Following contextual recall, animals were returned to their home cage for at least 1 hr, before being moved to Context B, a novel environment with unique visual cues and odor presentations. Conditioned tones were presented in the same pattern as during day 1, and animals were monitored for freezing. On the first tone presentation, both groups froze at the same rate, though upon the second and third tone, 4D animals froze less than mCherry controls (two-way ANOVA, adjusted Holm-Bonferroni multiple comparisons, *p <0.05).

Inhibition of dorsal hippocampal OLMα2 cells during offline consolidation impairs contextual fear memory recall

We next investigated whether OLMα2 cell activity is necessary for contextual memory processing. Formation of contextual fear memories is hippocampus- and sleep-dependent and is also sensitive to a 4-hour post-training window (Graves et al., 2003). Investigations into the

underlying circuitry of encoding and consolidation of fear memory suggest that the activity of somatostatin-positive (SOM+) interneurons in the dorsal CA1 is necessary during acquisition and encoding of fear and hippocampal theta rhythm during REM sleep is required for consolidation (Boyce et al., 2016; Lovett-Barron et al., 2014). To investigate this further, OLMα2-4D and OLMα2-mCherry controls were fear conditioned in a distinct context, with three co-terminating tone-shock events over the course of 9.5 minutes (Boyce et al., 2016). There was no difference in the freezing response between groups during the first day of the task (Fig 3.7 B(i)). Immediately following the end of the acquisition session, both groups received CNO (5 mg/kg i.p.) and were recorded during the 4-hour consolidation window.

The following day, mice were tested for recall of both the memory of the fear context and the auditory cue. Mice were first placed in the conditioning context, with identical visual and odor cues, and observed for 10 minutes without tone or shock. After binning the freezing data, we found that OLM-hm4Dgi animals froze less significantly less than mCherry controls during the 3 minute to 6 minute time period of the context recall session (Fig 3.5B(i), two-sample t-test,** = 0.01, * = 0.04) . Following the context recall, mice were returned to their home cage for one hour, after which they were placed in a novel context and played the same pattern of tones heard during conditioning without the accompanying shock. Both groups displayed freezing in response to the tones; however, OLM-hm4Dgi animals decreased their freezing behavior with each successive tone, and significantly differed from controls by the third tone presentation (Fig 3.5B(iii)). This may indicate facilitated fear extinction, and further investigation into these results is needed to delineate what role these cells may have in sensitization to auditory fear cues.

We found no difference in the sleep-wake architecture between groups resulting from the presence of CNO. In analyzing the spectral profile of each sleep state in both groups, we found that there was a significant decrease in the relative power of the theta band during REM sleep (Fig 3.6C, p*=0.04). The spectral profile of NREM was not significantly different between animals with and without chemogenetic inhibition of OLM cells.

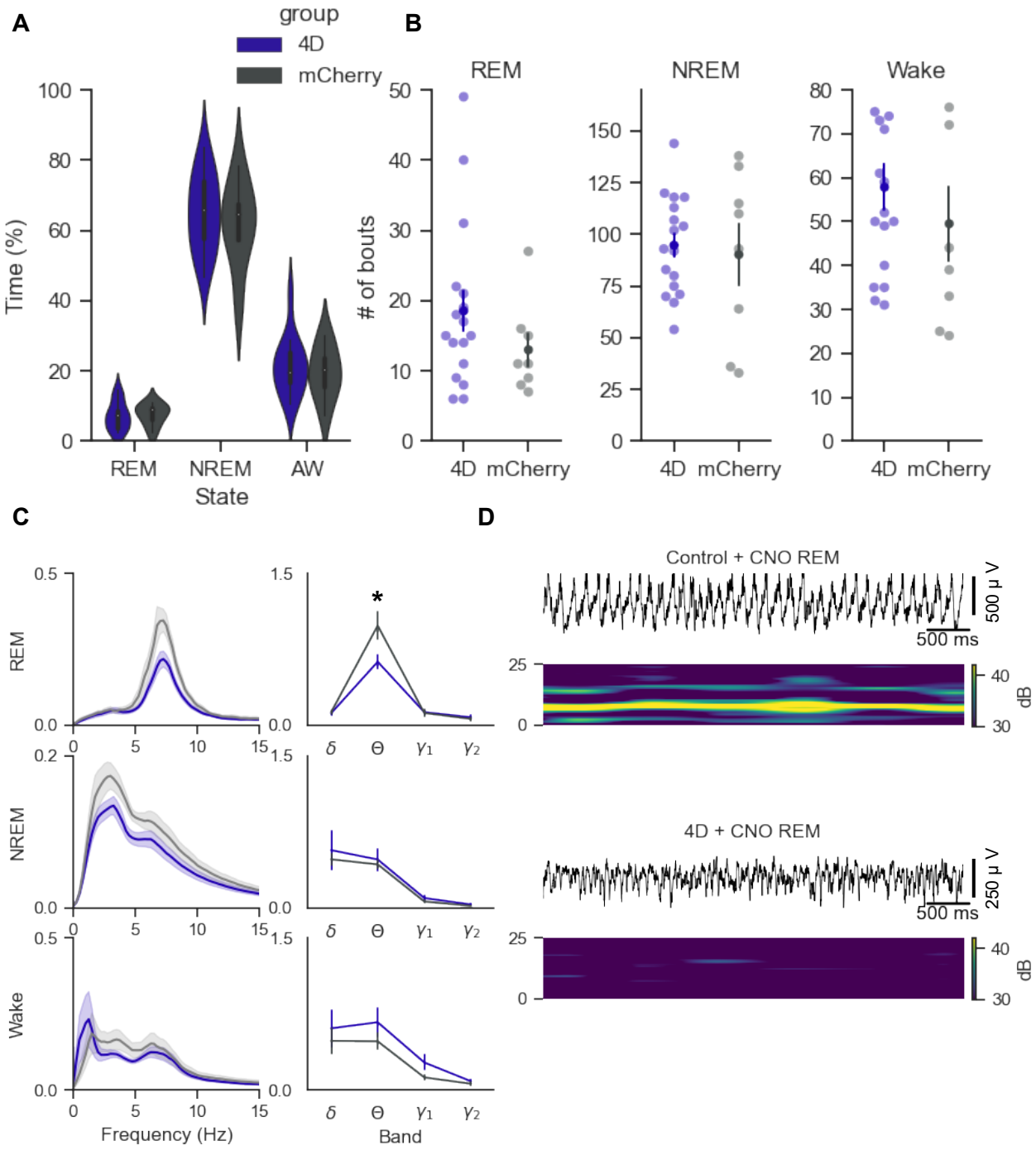


Figure 3.8: Inhibition of OLMα2 neurons following fear conditioning does not affect sleep architecture but decreases REM theta power.

(A) The percentage of time spent in each sleep state is not different between 4D and control animals receiving CNO**. (B)** CNO did not cause any significant changes in the number of epochs for each stage during the 4-hour recording period**. (C**) Mean power spectral density plots for each sleep state. Theta power was significantly reduced in OLMα2-4D animals during REM sleep compared to controls (p* = 0.02, two sample t-test), while other bands across the sleep states remained unchanged. **(D)** Example traces and spectrograms for control (top) and OLMα2-4D (bottom) theta rhythm during REM sleep.

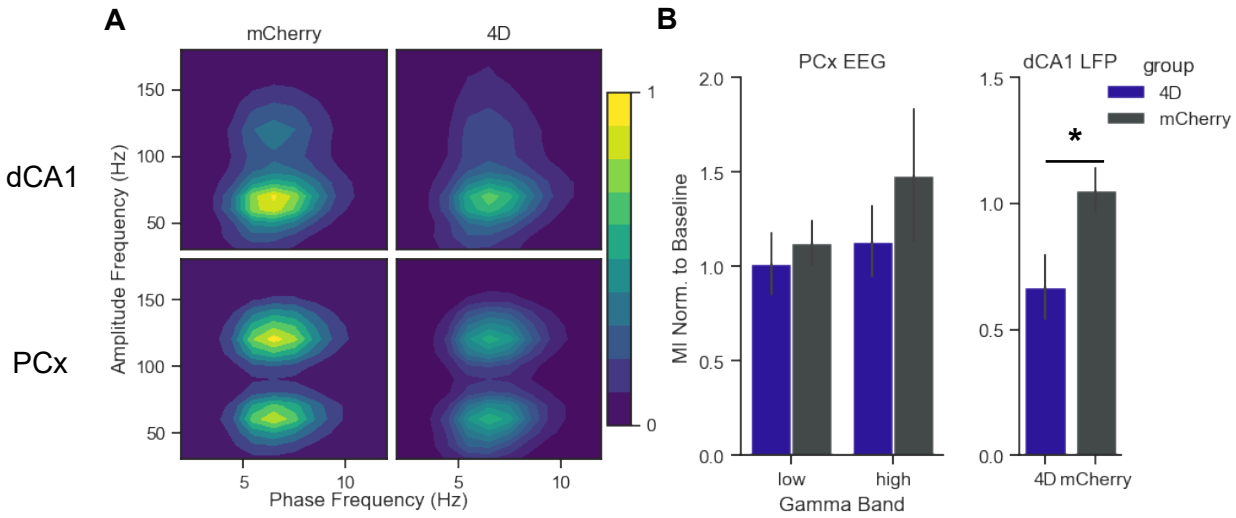


Figure 3.9: Inhibition of dOLMα2 neurons following fear conditioning alters REM theta-gamma coupling in the dCA1 region.

(A) Normalized comodulograms of LFP (top) and EEG (bottom) phase-amplitude coupling during REM sleep in OLMα2-4D animals and mCherry controls, following fear conditioning and CNO administration. **(B)** Coupling strength between theta phase and high/low gamma band is unchanged between groups in the parietal cortex EEG screw recordings (one-way ANOVA, p=0.868), but was significantly reduced in the dCA1 LFP (t-test, p*=0.0482).

Ventral hippocampal OLMα2 cells

Hippocampal function changes along the dorsoventral axis, with the dorsal pole relating predominantly to spatial information processing, while the ventral portion is more involved with anxiety and fear responses (Faneslow and Dong, 2010). Their connectivity differs as well, as the dHPC is connected to the anterior cingulate and retrosplenial cortices, while the vHPC is functionally connected to the amygdala and hypothalamus (Strange et al., 2014). The density of OLMα2 interneurons also increases across the longitudinal axis, with over 75% of identified Chrna2-OLMα2 interneurons residing in the ventral hippocampus (Siwani et al., 2018). Optogenetic manipulation of OLM α2 cells in the intermediate and ventral hippocampus both modulate spatial memory and fear behavior (Mikulovic et al., 2018; Siwani et al., 2018), suggesting that this population plays a role in encoding hippocampus-dependent tasks during waking; however, the role of ventral OLMα2 cells in sleep-dependent consolidation has not been investigated. In order to investigate this, we injected Chrnα2-Cre animals with AAV1-DIO-hm4Dgi in the ventral hippocampus, to selectively express inhibitory DREADDs in ventral OLMα2 cells. Prior to administering CNO, baseline recordings indicate that sleep architecture and power spectral signatures between the 4D and mCherry control groups were not significantly different (Fig 3.10).

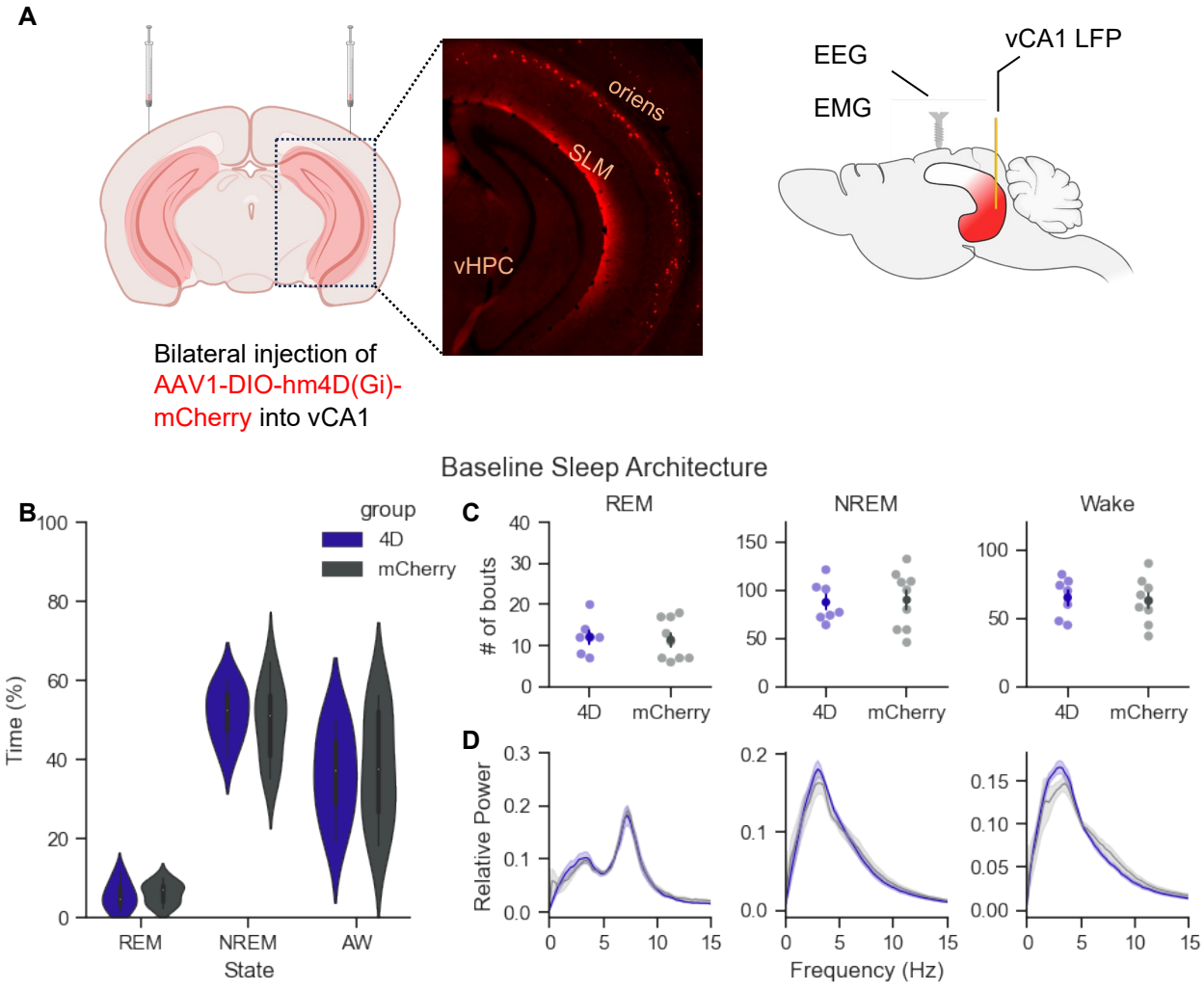


Figure 3.10: OLMα2 inhibition and recording in the ventral hippocampus.
(A) Experimental paradigm in which the hm4Dgi inhibitory DREADD or mCherry control virus were bilaterally injected into the ventral CA1 (AP:-3.2,ML +/- 3.8, DV: -3.6/3.0/2.5) and parietal screws, vCA1 LFP, and neck EMG wires implanted for polysomnography recording. **(B)** The percentage of time spent in each sleep state was unchanged between groups in baseline recordings. **(C**) The number of epochs of each sleep state were similarly unchanged between groups during baseline recording, as well as **(D)** the mean power spectral profile of each sleep state.

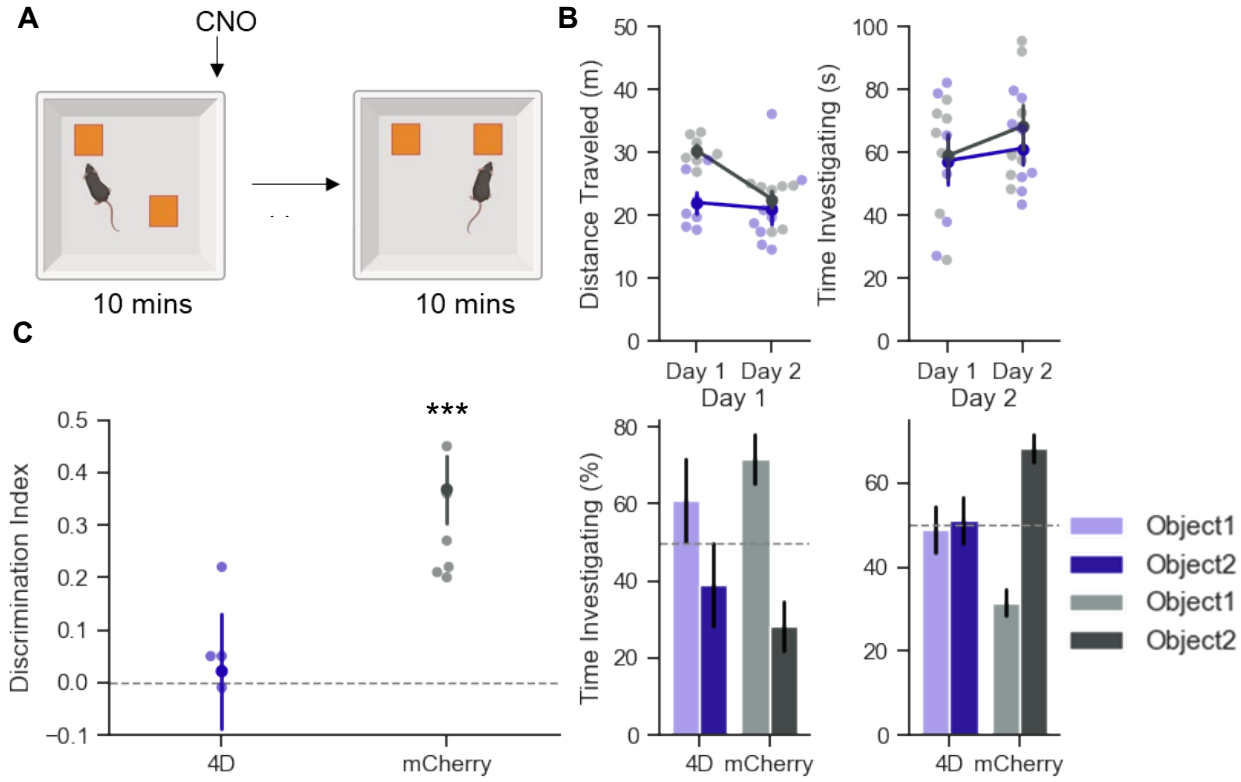


Figure 3.11: Offline silencing of vHPC OLMα2 cells impairs object location memory.
(A) NOPR tests were performed as described above with a 4-hour retention interval for sleep recording. **(B)** The distance traveled, and time spent investigating both objects (T_{Obj1}+T_{Obj2}) was not significantly different between groups on either day. **(C)** Left: The preference for the moved object (Obj2) was not significant for the 4D animals (one-sample t test versus 0, p=0.85), while mCherry animals demonstrated a significant preference from zero (one-sample t test vs 0, p***=0.0006). Right: There was no significant preference for Obj2 on Day 1 of the NOPR task. On Day 2, 4D animals investigated both objects equally, while mCherry animals spent significantly more time investigating the moved Obj2.

Inhibiting ventral OLMα2 cells after spatial task acquisition significantly impairs memory recall

To elucidate the role of ventral OLMα2 neurons in spatial memory consolidation, we again utilized the NOPR task with OLMα2 animals expressing either an inhibitory DREADD (4D) or an mCherry control in the ventral hippocampus. The function of the hippocampus changes along the dorsal-ventral axis, with the starkest difference between the dorsal and ventral pole. In the broadest terms, the dorsal hippocampus is characterized as relating primarily to spatial and cognitive tasks, while the ventral hippocampus is associated with fear responses and

processing emotional memory. However, more evidence is emerging suggesting that there is a gradient along the longitudinal axis, resulting in a gradual change in function between the two poles of the hippocampus, and the possibility that the ventral hippocampus could also play a role in spatial processing. We bilaterally targeted Cre-dependent AAV1-DIO-hm4Dgi or mCherry control to the ventral CA1 region (-3.2, +/- 3.8, -3.6/3.0/2.5) and implanted EEG screws in the parietal cortex, LFP probes in the ventral CA1 (-3.2, +/- 3.8, -3.6) and EMG wires in the neck muscles for sleep state identification. The NOPR task was conducted as described above, though the inter-task interval was changed from 24 hours to 4 hours to create a more robust memory. During the training period in which the animals were introduced to the objects, neither group of animals displayed a preference towards Object 2, though the mCherry group spent more time investigating Object 1. Immediately following the training session, mice were given a 5 mg/kg CNO i.p. injection and recorded from for the entire 4-hour period. The sleep architecture between the OLMα2-4D and OLMα2-mCherry groups was not significantly different, either in percentage of time spent in each sleep state or in number of epochs of each state during the recording period (Fig 3.12). Following the 4-hour recording period, Object 2 was displaced in the NOPR chamber, and animals were returned

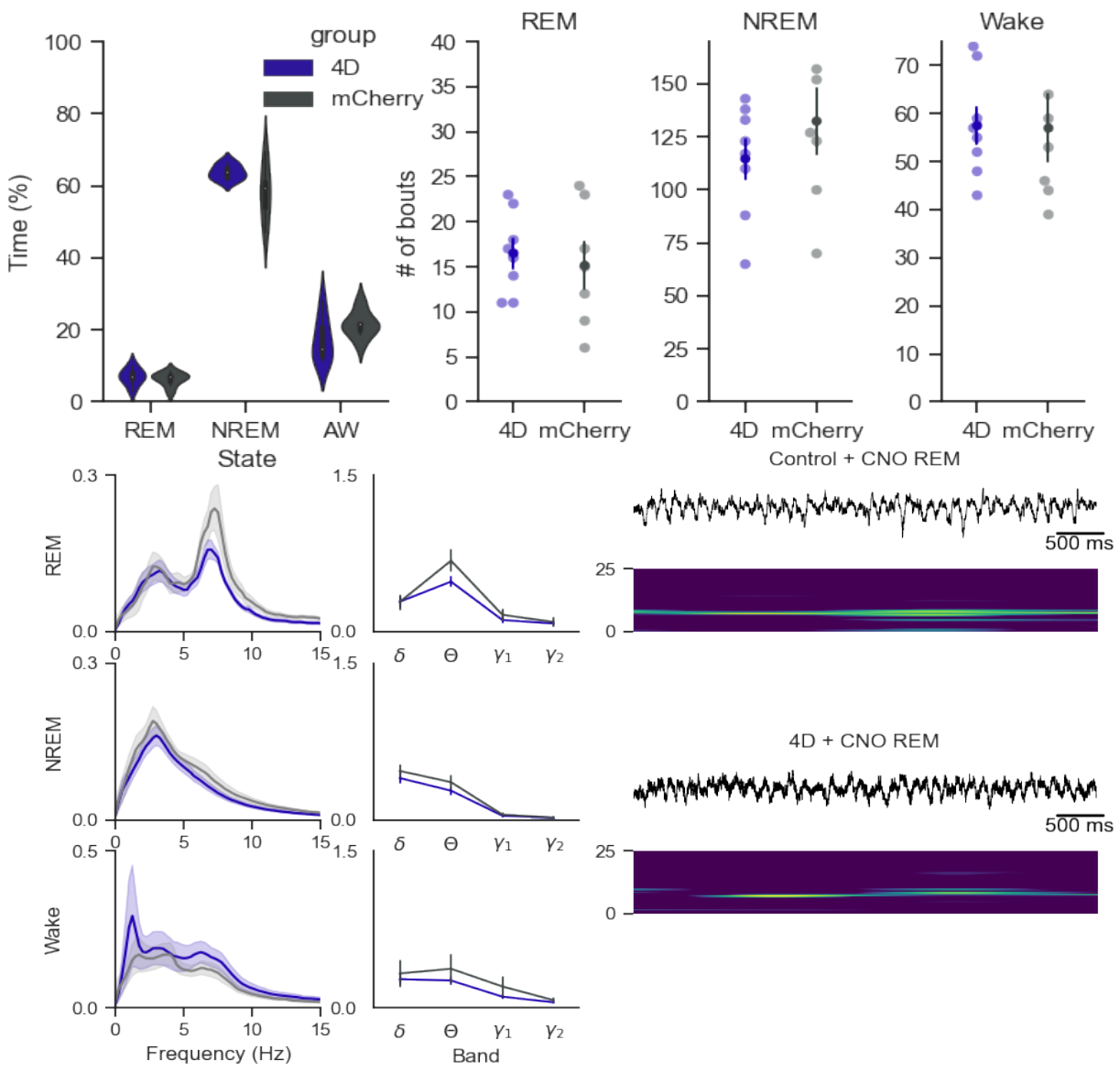


Figure 3.12: Inhibition of vHPC OLMα2 interneurons following NOPR does not affect sleep architecture or spectral properties of sleep states in the vCA1.

(A) Both 4D and mCherry mice spend the same percentage of time in each activity state following NOPR and CNO administration. **(B)** The number of bouts of each activity state is similarly unchanged between groups. These results indicate that sleep architecture is unchanged by chemogenetic inhibition of OLMα2 cells. **(C)** Mean power spectral density plots of each sleep state. Binned band power for delta (0.5-2 Hz), theta (5-10 Hz), slow gamma (30-80 Hz), and fast gamma (80-120 Hz) for each state and group. None of the band power ranges were significantly different, though REM sleep theta was reduced ($p = 0.097$). **(D)** Example traces of REM sleep in mCherry (upper) and 4D (lower) animals post NOPR acquisition with CNO.

and allowed to investigate another 10 minutes. Inhibiting vCA1 OLMα2 cells significantly decreased the animals' ability to discriminate between the familiar and novel object locations. While OLMα2-4D animals maintained a discrimination index that did not deviate significantly from zero (DI= 0.02 +/- 0.11, one sample t test versus 0, p=0.85), OLMα2-mCherry controls clearly demonstrated a preference for the novel object location (DI = 0.37 +/- 0.06, one sample t test versus 0, p***=0.0006). This suggests that inhibiting OLMα2 vCA1 cells following NOPR acquisition impaired the ability of mice to retain their memory of the object locations. The elimination of REM theta has been shown to impair spatial and fear memory consolidation in the dorsal HPC, and similar mechanisms may be at play in this instance. Additionally, the dorsal and ventral poles are thought to coordinate at key times during learning and memory processes. One way in which this occurs is through theta-gamma phase-amplitude coupling (PAC) between the dorsal theta rhythm and ventral gamma. To investigate this in our experiments, we found the modulation index (MI) as described previously, between vCA1 LFP gamma (80-160 Hz) amplitude and parietal cortical EEG theta (5-10 Hz) phase during baseline and following NOPR training and CNO administration. We constructed a comodulogram plot from all REM epochs and normalized the post-NOPR comodulogram to baseline to allow for comparison between animals. In both OLMα2-4D animals and mCherry controls there was no group differences in dHPC-vHPC theta-gamma coupling during REM sleep (Fig 3.13A,C). To investigate local vCA1 theta-gamma coupling, we performed the same process on REM epochs from vCA1 LFP recordings, finding a low level of coupling that was not significantly different between the groups (Fig 3.13B,C). These results suggest that theta-gamma coupling between the dorsal/ventral poles, as well as locally in vCA1, is not significantly affected by vOLMα2 during REM sleep.

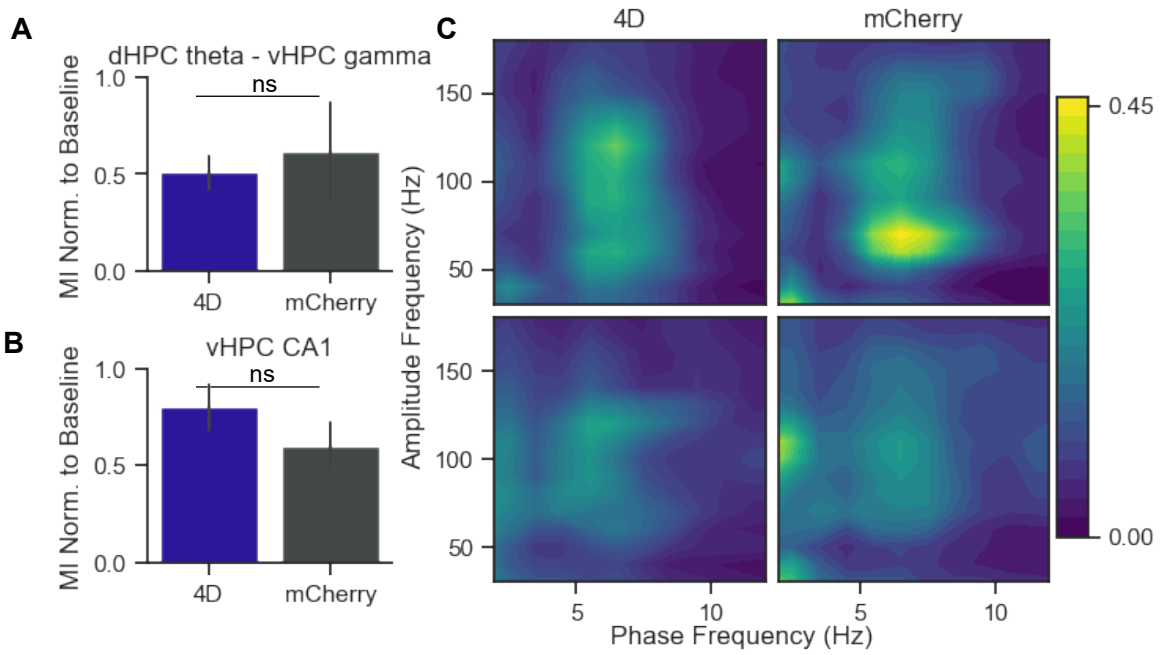


Figure 3.13: Inhibition of ventral OLMα2 neurons following NOPR did not significantly affect local CA1 theta-gamma coupling or dorsal HPC theta – ventral HPC gamma coupling.

We assessed coupling between the dorsal and ventral HPC by finding the degree of modulation of ventral hippocampal gamma amplitude by dorsal theta oscillation. **(A)** We did not find a significant difference in normalized modulation index between groups following NOPR (two-sample t test, p=0.66). **(B)** There was similarly no difference between local theta-gamma coupling in the vCA1 between groups (two-sample t test, p=0.26). **(C)** Normalized average comodulograms of 4D and mCherry animals, for both dHPC-vHPC coupling (top) and vCA1 coupling (bottom).

Fear memory consolidation is unchanged following post-conditioning OLMα2 silencing

The ventral hippocampus is critical to contextual fear memory consolidation. In behaving animals, optogenetic manipulation of ventral OLMα2 cell activity was able to enhance or suppress fear response to predator odor. Inhibition of these cells was anxiogenic during a center-crossing task, while activation of the cells had an anxiolytic effect and resulted in decreased fear behavior expression. Both responses appeared to be mediated through the effect of OLM activity on type 2 theta—inhibition correlated with decreased theta and increased anxiety responses, while activation enhanced theta and decreased anxiety behaviors (Mikulovic et al., 2018). Other studies found that inhibition of intermediate OLM cells did not affect conditioned place preference memory, while activation resulted in decreased latency to enter a

shock context (Siwani et al., 2018). These data suggest that theta in the ventral hippocampus plays an important role in generating anxiety responses and processing fear-related information. To investigate the role of vOLMα2 cells in fear memory consolidation, we again silenced this population chemogenetically following fear conditioning. During the initial fear conditioning process, the tone-0.6 mA shock pairs were sufficient to induce freezing behavior in both groups (Fig. 3.14 B(i)). Immediately following conditioning, mice received a 5 mg/kg CNO injection and EEG/LFP was recorded for 4 hours. LFP recordings from the vCA1 during vOLMα2 suppression showed that theta band power was significantly decreased during REM sleep (two sample t test, $p^*=0.03$). Sleep architecture was unaffected, and the power spectra of other behavioral states was similarly not impacted. The following day, context and cued recall was assessed. Animals were returned to the conditioning chambers for 10 minutes, and freezing behavior was observed. The amount of freezing for each group was combined into 120s bins for comparison, and we found no significant difference between 4D or mCherry animals at any time point (Fig 3.14 B(ii),two-way ANOVA, time x treatment $p=0.577$). For the cue recall test, mice were placed in a novel context, and presented with the 30s tones used during conditioning. Prior to the introduction of the tones, mice exhibited no freezing behavior, and tone presentation elicited freezing, indicating recall of the associated fear memory. Freezing in response to the tones was not different between 4D and mCherry animals during the cued recall (Fig 3.14 B(iii), two-way ANOVA, time x treatment $p = 0.88$). Ultimately, these results do not provide evidence that vOLMα2 cell activity is necessary during offline sleep for fear memory consolidation.

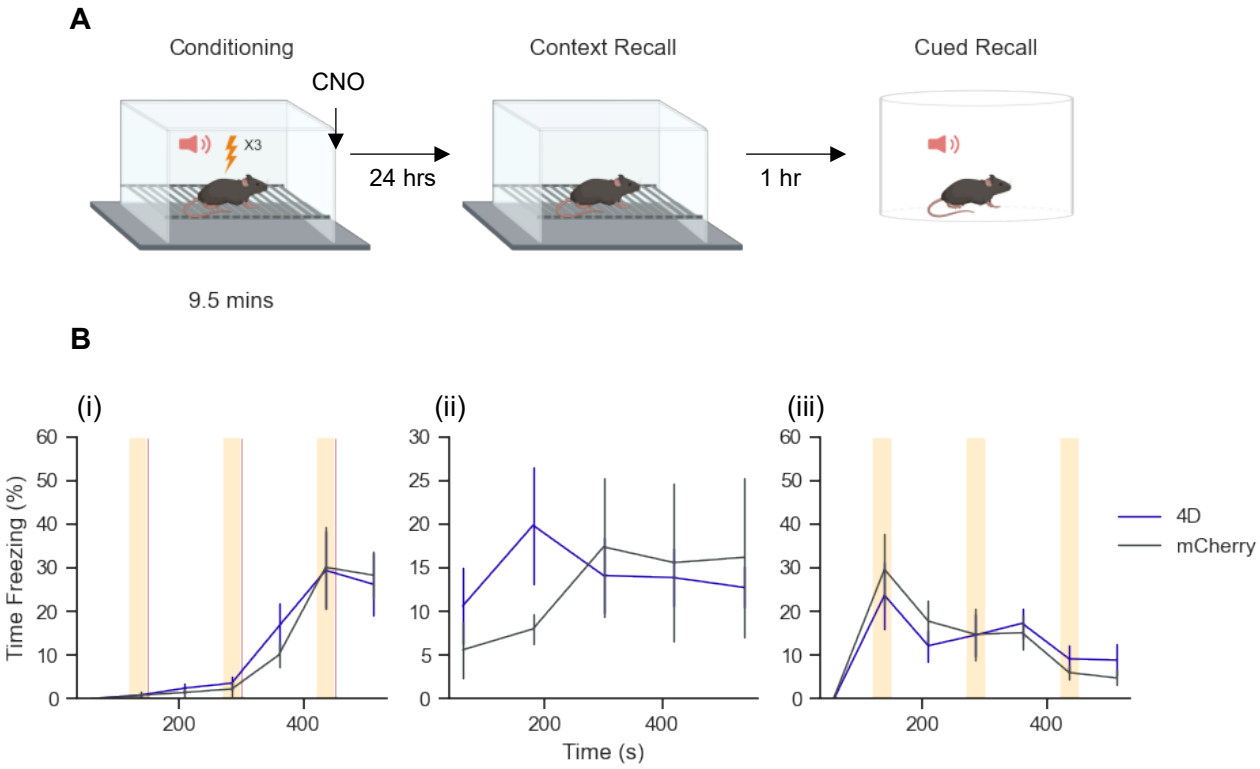


Figure 3.14: Inhibition of vHPC OLMα2 cells following fear conditioning does not impair context or cued recall.

(A) The same conditioning protocol is used with the vHPC group as described above. **(B)** (i) Both groups displayed increased fear behavior over the course of the conditioning session indicating learning with no effect of group (two-way ANOVA, p=0.98). (ii) When reintroduced to the freezing context, there were no significant differences in freezing behavior between groups (two-way ANOVA, p=0.58). (iii) Similarly, cued freezing response was unchanged between groups (two-way ANOVA, p=0.88).

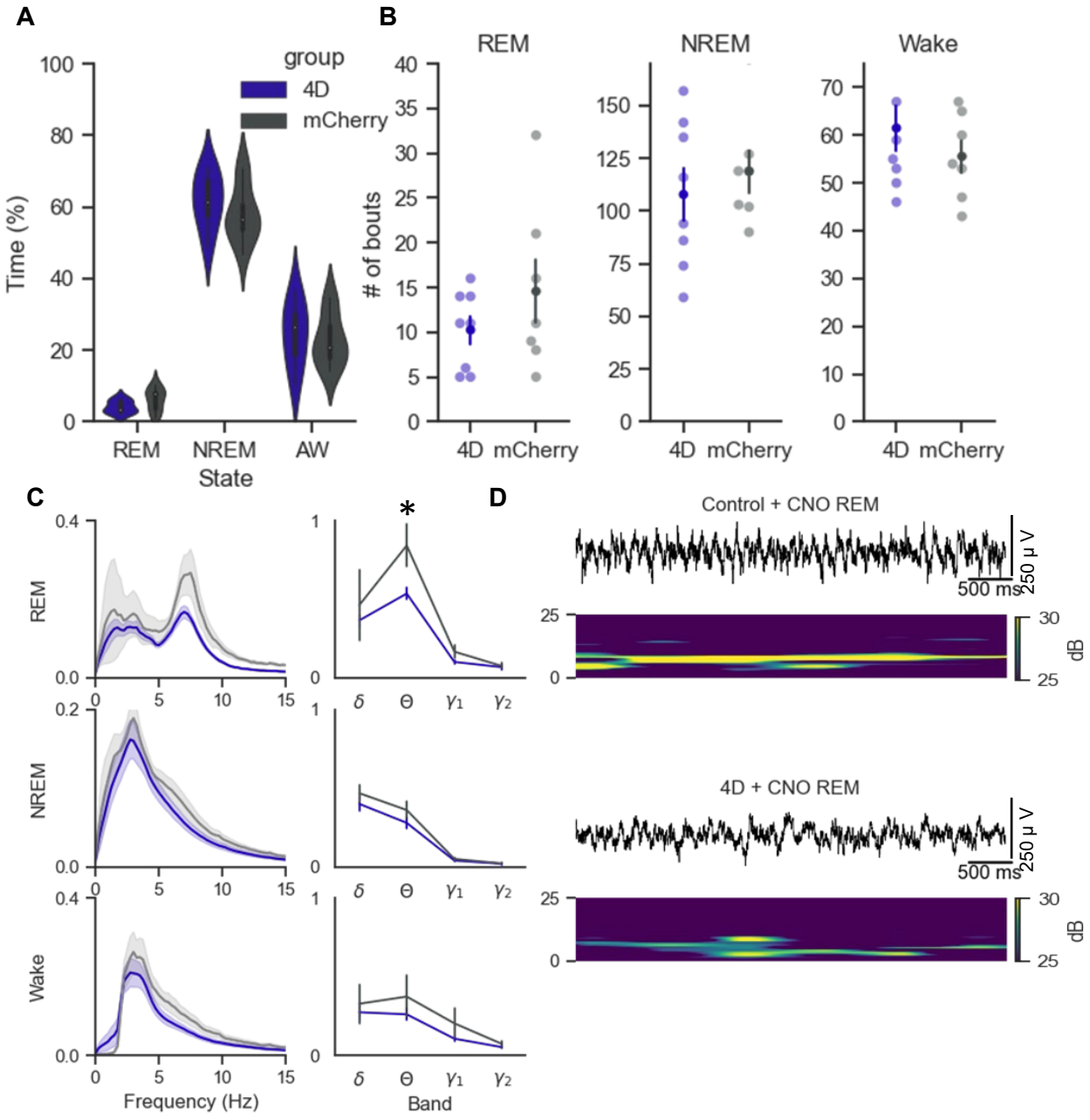


Figure 3.15: Inhibition of vHPC OLMα2 cells following fear conditioning reduces theta rhythm power in vCA1 without affecting sleep architecture.

(A) vHPC-OLMα2 inhibition did not change the amount of time spent in each sleep state (two-way ANOVA, p=0.52) nor **(B)** the number of bouts of each state occurring during the 4-hour recording window (two-sample t-tests between groups, all ns). **(C)** Only the theta power during REM in the vCA1 was significantly changed during the period of OLM inhibition (two-sample t-test, p*=0.03). **(D)** An example vCA1 LFP trace and spectrogram of a control animal (top) and OLMα2-4D animal (bottom) during REM sleep in the post-FC recording with CNO present, demonstrating the loss of typical REM theta power.

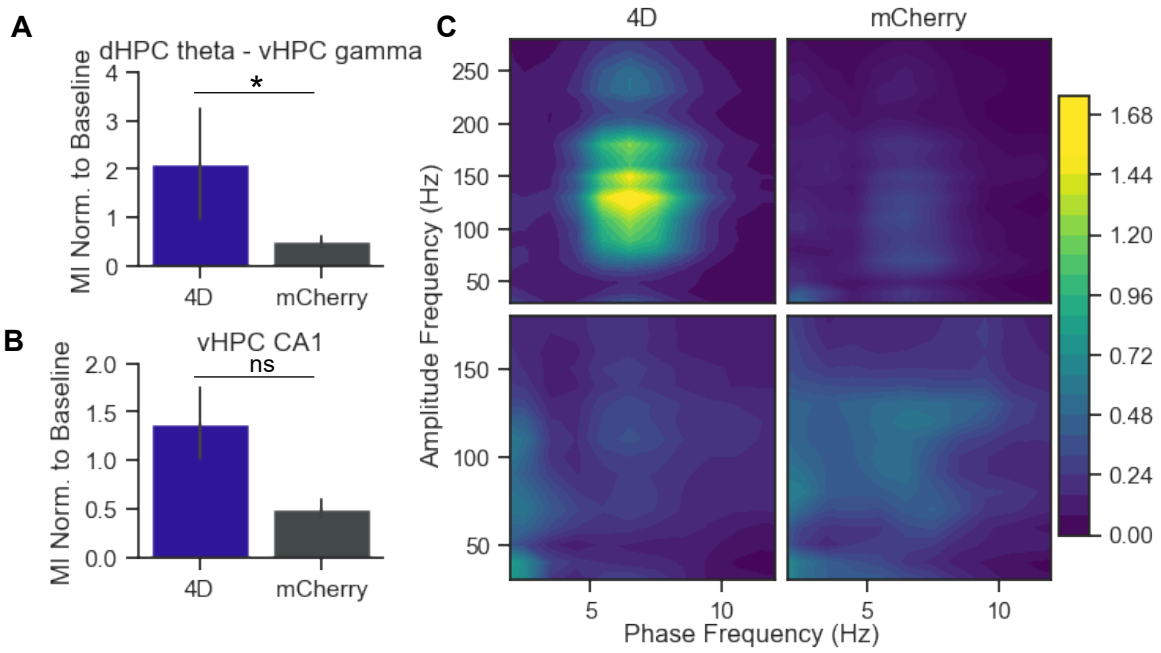


Figure 3.16: Inhibition of ventral OLMα2 neurons following FC increased vCA1 theta-gamma coupling. **(A)** There was a significant difference between dHPC-vHPC gamma coupling in the vCA1 between groups (two-sample t test, p*=0.03). **(B)** We did not find a significant difference in normalized modulation index between groups following FC in vCA1 local coupling (two-sample t test, p=0.24). **(C)** Normalized average comodulograms of 4D and mCherry animals, for both dHPC-vHPC coupling (top) and vCA1 coupling (bottom).

3.3 Discussion

Taken together, our results provide evidence that OLMα2 cells in the CA1 region of both the ventral and dorsal hippocampus play a role in offline consolidation of spatial and contextual information. Chemogenetically silencing OLMα2 cells in the dorsal and ventral CA1 impaired animals' ability to distinguish between novel and familiar object locations. Inhibition of dCA1 OLMα2 neurons also impaired contextual fear memory recall without abolishing freezing in response to auditory cues, indicating selective interference with hippocampus-dependent tasks. Surprisingly, inhibition of OLMα2 in the vCA1 did not affect contextual fear memory recall, despite the importance of vHPC processing to contextual fear memory. However, this result mirrors studies done in behaving animals, in which optogenetic inhibition of intermediate OLMα2 interneurons during waking did not affect fear-related memory recall (Siwani et al., 2018).

Our results also provide more evidence for the functional distinction between the dorsal and ventral poles of the hippocampus. Previous studies have found that the location of OLMα2 modulation along the longitudinal axis is critical for determining the resulting change in behavior. Changes in connectivity, underlying cellular physiology, and receptor composition result in the gradient of functional differences seen along the dorsoventral hippocampal axis. OLMα2 cells in the dorsal HPC, for example, have a stronger expression of Ih current, leading to a more depolarized resting potential of OLMα2 cells in the dorsal compared to ventral hippocampus. Ventral OLMα2 cells may also be more strongly modulated by 5HT3AR agonists (Chittajallu et al., 2013). Nicotine also differentially affects dorsal vs ventral OLMα2 cells, enhancing fear memory recall when administered into the dHPC, and impairing it when administered into the vHPC (Kenney et al., 2012), which may be related to the opposing effects of nicotine on dorsal and ventral OLMα2 cells (Siwani et al., 2018). While our manipulations were done in the offline period, we still found a distinction between the roles of OLMα2 cells in the ventral and dorsal hippocampus, with dCA1 OLMα2 inhibition negatively affecting both spatial memory and

contextual fear memory, and vCA1 OLMα2 inhibition only negatively impacting spatial memory recall.

OLMα2 cells are hypothesized to act as a gate switch between the two main sources of glutamatergic input into the CA1, the intrahippocampal Schaffer collaterals from CA3 and the external temporoammonic pathway delivering information from the entorhinal cortex layer 3. When active, OLMα2 cells inhibit pyramidal cell distal dendrites that receive EC3 afferents and disinhibit the proximal dendrites that synapse with CA3 afferents, prioritizing the incoming information from the hippocampus (Leao et al., 2012). Inactivation of these cells would instead result in biasing CA1 processing towards EC3 input, possibly enhancing encoding novel information. This may be why brief optogenetic inactivation of OLMα2 cells during task acquisition, as in Siwani et al., 2018, results in improved learning of novel object tasks. Our experiments, however, investigate how information is processed in offline states, during which EC3 input from the environment is not actively being encoded. Our results suggest that OLMα2 activity is important for maintaining the hippocampal theta rhythm during REM sleep, and that REM theta is necessary for sleep-dependent memory consolidation, which has been demonstrated through the manipulation of theta via the optogenetic inhibition of medial septum neurons in other studies (Boyce et al., 2016).

The results described here give rise to more questions regarding sleep-dependent memory consolidation and the role of OLM cells in facilitating it. Further studies are needed to more fully explore how OLM activity modulation affects memory consolidation. Due to the limitations of DREADD temporal resolution, our experiments do not let us determine specifically which stages of sleep and electrophysiological activity are crucial to these processes. While it seems likely that REM theta is a critical component affected by our manipulations, we cannot rule out the possibility that the reduction in REM theta seen in these experiments is correlative rather than causative. Other methods, such as optogenetic inhibition, will need to be used to determine the role of OLM cells with more specificity. The circuit-level mechanisms involved in

these processes have yet to be delineated as well. How hippocampal place cell firing is changed in response to OLM activity may provide answers to the role these cells play in learning and memory. For example, by redirecting the strength of inputs into the CA1, OLM activation or inhibition could disrupt the usual relationship between the hippocampal theta phase and cell firing. Disrupting the theta rhythm in the hippocampus overall could also change the plasticity of the hippocampal network on a larger scale. As offline consolidation periods are thought to be important periods of plasticity for strengthening memories of waking experiences, disrupting the rhythmic activity thought to generate this may result in aberrant memory consolidation.

Ultimately, our results support the idea that OLM interneurons play a necessary role in offline consolidation processes, possibly through facilitating the hippocampal theta rhythm and communication between dorsal and ventral hippocampal regions. The finding that disrupting OLM activity offline results in impaired learning and memory also lends support to the sleep and memory consolidation hypothesis more broadly. Given the role of interneurons in the pathogenesis of neurodegenerative diseases, epilepsy, and neuropsychiatric conditions, OLM cells may be a target for further study as a role in disease mechanisms. While the relationship between neurodegenerative and neuropsychiatric disorders and sleep disturbances has long been acknowledged, many unanswered questions remain regarding how the two are linked, and whether sleep disruption contributes to cognitive deficits seen in these and other disorders. The role of OLM interneurons in generating and maintaining the oscillatory activity needed for memory consolidation may be one link between circuit dysfunction and behavioral impairment seen in disease and disease models.

3.4 Supplementary Figures

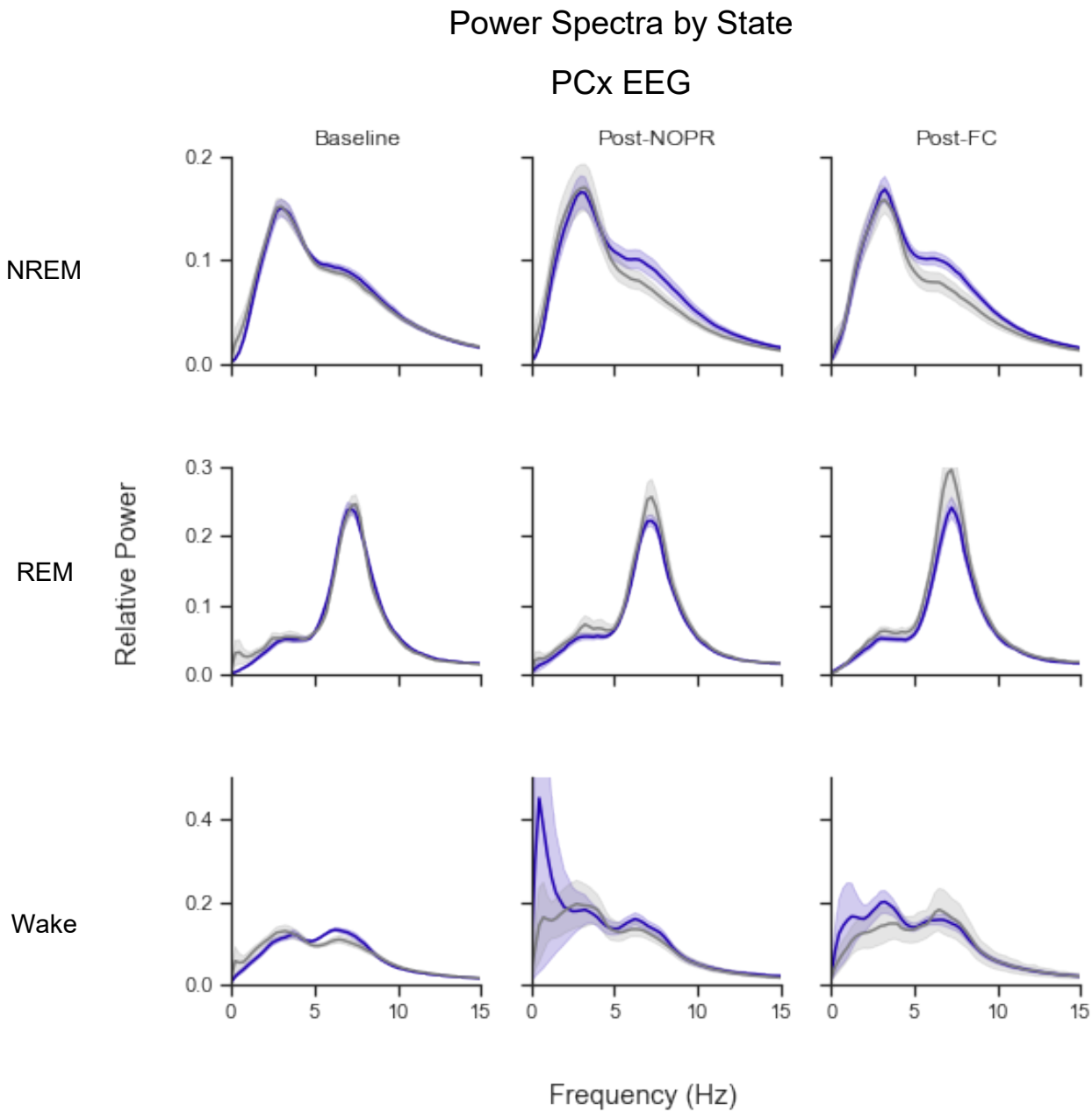


Figure S1: Chemogenetic inhibition of OLMα2 neurons did not significantly affect the spectral properties of each sleep state in the parietal cortex.

Using EEG recordings from the parietal screw, epochs from each sleep state were concatenated and the power spectral density was computed using the Welch's method with a 0.25 Hz frequency resolution. To compare between animals, the power spectra were normalized to the total baseline band power between 0-14 Hz. Each plot represents the group mean surrounded by the standard error.

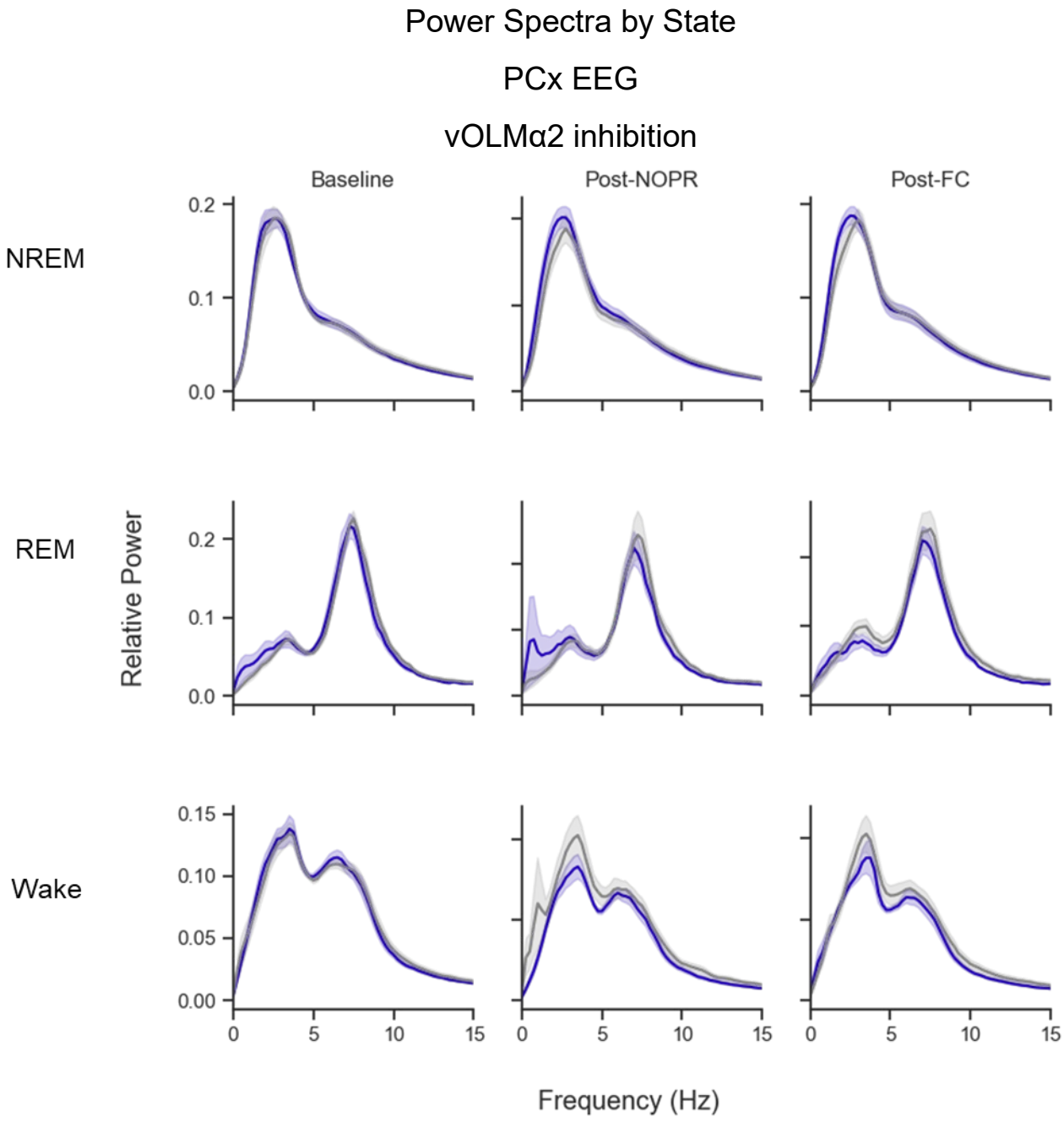


Figure S2: Chemogenetic inhibition of vOLMα2 neurons did not alter spectral properties in the parietal cortex

EEG recordings from the PCx taken during baseline and vOLMα2 inhibition did not show significant differences. Epochs from each sleep state were concatenated and the power spectral density was computed using the Welch's method with a 0.25 Hz frequency resolution. To compare between animals, the power spectra were normalized to the total baseline band power between 0-14 Hz. Each plot represents the group mean surrounded by the standard error.

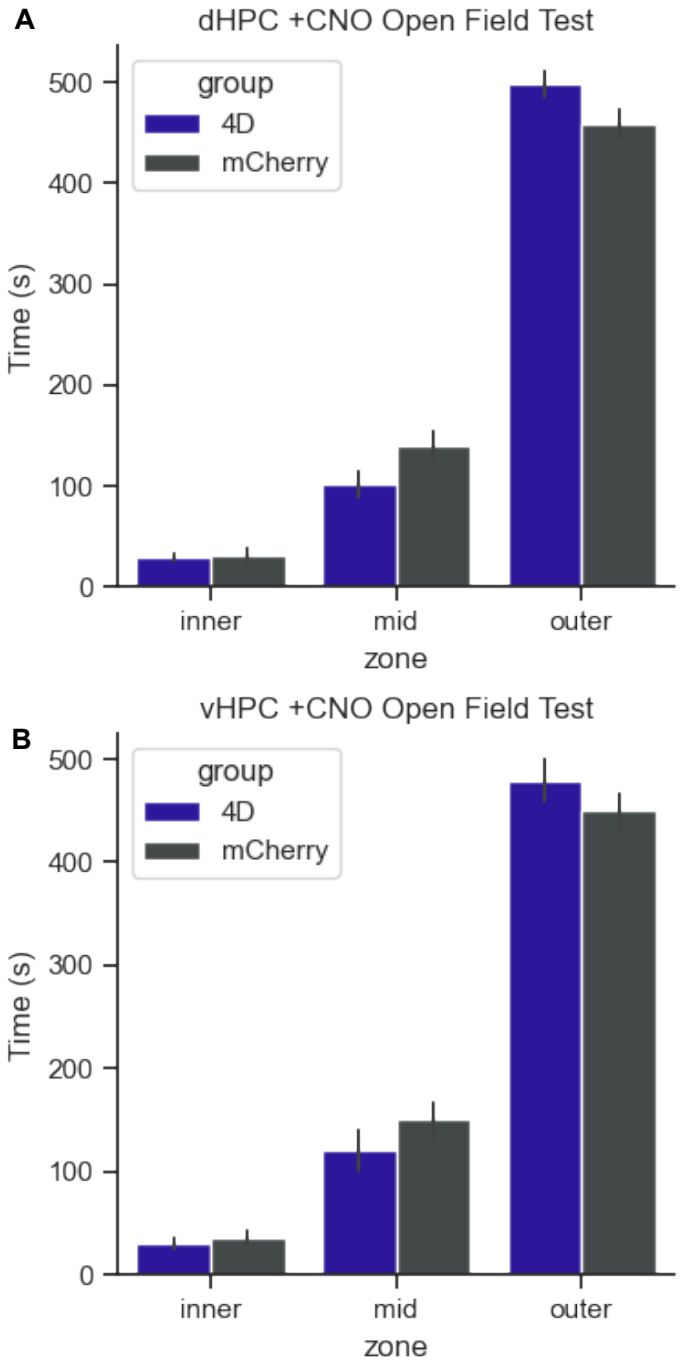


Figure S3: Inhibition of dorsal or ventral OLMα2 neurons during an open field task does not impair locomotion or increase anxiety behaviors.

To account for any effects of CNO on anxiety or locomotion, following completion of the hippocampal tasks, we gave mice a final CNO injection before an open field task in which they were placed in a large novel chamber and allowed to freely explore. The chamber was divided into outer, middle, and inner zones. We did not find a significant difference in the amount of time spent in each zone between 4D and mCherry groups with either dorsal **(A)** or ventral **(B)** hippocampus virus expression and presence of CNO (two-way ANOVA with significant effect of zone ($p<0.001$) but not zone x group ($p>0.05$)). This indicates that chemogenetic inhibition of these cells during waking does not impair locomotion or increase anxiety behaviors.

3.5 Materials and methods

Animals

Experimental protocols were approved by the Institutional Biosafety Committee and the Animal Research Committee of the University of California, Los Angeles, and conducted according to the National Institutes of Health guidelines for animal care and use.

Chrna2-cre C57Bl6 animals used were developed by the Kullander Lab at the University of Uppsala, as detailed in Leao et al., 2012. Briefly, the *cre* gene was inserted into the ATG region of the first coding region of the *Chrna2* gene, resulting in *Chrna2*-dependent CRE expression, localized specifically to OLM interneurons. Our breeding colony was maintained through crossing *Chrna2-cre* males to C57Bl6 females, and genotyping resulting litters for the presence of *cre*. Animals were allowed food and water ad libatum, and were maintained in a 12 hour light/dark cycle. Once experiments began, animals were moved to custom-built soundproof chambers for sleep recordings. All sleep recording was performed during the animals' light cycle.

CNO injections

For DREADD experiments, CNO was delivered via intraperotineal injection. 10 mg water-soluble CNO (Hello Bio) was suspended in 2 mL sterile water to create a 5 mg/mL stock solution, stored at -20℃. To prepare the CNO for injection, 100 uL of the stock solution was added to 1 mL of sterile water, making a 0.05 mg ml^{-1} solution, 100 uL of which is injected per 10g, resulting in a 5 mg kg^{-1} dose of CNO.

Stereotaxic surgeries

Prior to the beginning of surgery, male mice (P60-70, >20g) were induced using 5% isoflurane at 100% oxygen concentration until breathing slowed. They were then fixed in a stereotaxic

frame with a nose cone (Kopf) delivering isoflurane vapor (1.5 - 2%) for the duration of the surgery. Body temperature was maintained at 38℃ using a heating pad, and respiration rate and toe-pinch reflex was monitored throughout to assess the depth of anesthesia. The animals' eyes were covered with lubricating ointment to prevent drying during surgery, hair was removed using depilatory cream (Veet), and the scalp was disinfected using three washes of betadine followed by 70% ethanol. A small circular incision was made in the scalp, and the skull was cleaned with hydrogen peroxide. The skull was scored using a sterile scalpel to promote adhesion of the dental cement to the skull. A ground screw was placed in the bone above the cerebellum, and two screws served as EEG electrodes bilaterally above the parietal cortex. EEG screws were turned into the skull until they rested on the dura without making contact with the surface of the brain and stranded stainless steel wire (Cooner Wire) was placed in the nuchal muscles to record EMG activity. Following implantation of the screws, the skull was adjusted to ensure that the dorsal/ventral coordinates of bregma and lambda were within 50 microns.

For virus injections, small holes were drilled in the skull with a microburr (0.6 mm diameter), and mice were bilaterally injected with 500 nl per DV location with AAV1-hSyn-DIO-hM4D(Gi)-mCherry (Addgene, titer > $7x10^{12}$ vg/mL) in the dorsal hippocampus (-1.7 mm anteroposterior relative to bregma, +/- 1.0 mm mediolateral from bregma, and 1.5 mm below the skull) or ventral hippocampus (-3.2 mm anteroposterior relative to bregma, +/- 3.8 mm from bregma, and 2.5/3.0/3.6 mm below the skull). Injections were performed with a microinjection pump (WPI, UMP3) affixed with a Hamilton syringe (Neuros Syringe, 33G). Virus was infused at a rate of 100 nL min^{-1}, and the needle was held in place for 10 minutes following completion of the injection. Following virus injections, tungsten LFP probes (PlasticsOne) were lowered to either the dorsal (-1.7, +/- 1.0, -1.5) or ventral (-3.2,+/-3.8,-3.6) hippocampal coordinates, and secured in place with cyanoacrylate glue. An initial layer of Metabond dental acrylic (Parkell) was applied, and the free ends of each electrode wire were soldered on to a pre-tinned connector.

Layers of dental cement (Ortho-Jet, Lang Dental) were applied to seal the skull and hold the connector and wires in place, and VetBond (3M) was applied around the circular incision to seal the skin. The animal was then taken off of isoflurane and placed on a heating pad until it became ambulatory. Post-operative animals were singly-housed, and received subcutaneous carprofen injections (5mg kg^{-1}) during surgery and for 7 days following. Animals also received amoxicillin (0.25 mg ml^{-1}) in their drinking water for the 7 day recovery period.

Behavioral Protocol

Mice were allowed to recover for 7 days following surgery. After the recovery period, mice were handled and familiarized with being connected to the recording apparatus and tethered. For 3 days the animals were tethered to their preamplifier chip for 10 min sessions. Mice then were recorded from for 2 full 24 hour recording sessions to assess whether their sleep-wake cycles were normal. Baseline recordings were followed by novel object place recognition (NOPR) and fear conditioning (FC) tasks. All behavioral training took place within one hour of when the lights turned on in the animal chambers, or the ZT = 0 time, in order to maximize the animals' homeostatic sleep drive following completion of the task.

After the acquisition phase of each task, animals were given an intraperotineal injection of CNO (5 mg kg^{-1}), connected to the recording apparatus, and returned to their home cage. 30 min after CNO injection the 4 hr recording session was initiated.

Novel Object Place Recognition Task

On day 1 of NOPR, animals were brought into the testing room and allowed to acclimate for 20 mins prior to beginning the task. Warm-colored floor lamps were used to illuminate the room instead of overhead fluorescent lighting, to decrease anxiety in the mice and encourage exploration. The NOPR chamber is a 37 cm x 42 cm rectangular structure made out of

Plexiglass, with all walls covered by semi-translucent matte contact paper and containing distinct visual cues on each wall. For the habituation day, animals were placed into the empty chamber and allowed to explore for 10 mins, before being returned to their home cage. On the training day, animals were again brought to the testing room and allowed to acclimate for 20 mins before beginning the task. In this case, animals were placed in the chamber containing two identical objects, located in different quadrants of the chamber, and allowed to explore for 10 minutes before returning to their home cages. On the final day, animals were again placed in the NOPR chamber, but with one of the objects relocated to a different quadrant. Mice were again allowed to explore for 10 mins before being returned to their home cage. The locations of the moved and stationary objects were counterbalanced between groups, and the chamber and objects were sanitized with RESCue disinfectant and 70% ethanol in between animals. A USB camera mounted above the chamber recorded all trials, and AnyMaze software was used to determine distance traveled, animal speed, and the amount of time spent interacting with each object. An animal was deemed to be interacting with an object the animal was oriented facing the object and its nose was within a 2 cm radius. Periods in which the animal was rearing up on the object or sitting on top of the object were not counted as time spent investigating. The discrimination index (DI) of the objects was defined as $(T_{moved} - T_{stationary})/ (T_{moved} + T_{stationary}) X 100$.

Fear Conditioning

All fear conditioning training and testing took place in identical 25.4 X 25.4 X 33.02 cm chambers (Habitest Operant Cage, Coulbourn Instruments) placed in sound isolating boxes (Med Associates, St. Albans, VT). Each chamber had Plexiglass walls with unique visual cues. Speakers and lights were mounted on the sides of the box to provide visual and auditory cues.The chamber floors consisted of metal rods spaced 1 cm apart, and connected to a shock generator (Precision Animal Shocker, Coulbourn Instruments). The stimuli protocols were

generated using FreezeFrame Software (Actimetrics) running on a personal computer, and sent to the chambers via a digital interface (ACT-712, Actimetrics). All training and trial sessions were recorded from cameras located above the chamber.

The contextual conditioning protocol consisted of the following: 120 sec period of baseline, followed by a 30 sec 3000 Hz tone co-terminating with a 2 sec 0.6 mA footshock. Two more 30 sec tone-shock pairs occurred, separated by 120 sec intervals. To create a stronger contextual impression, small weighboats of scented mineral oil (Johnson&Johnson) were placed underneath the metal floor grid. Chambers were cleaned with 70% ethanol in between experiments.

To test for retention of the contextual fear memory, mice were placed back into the chambers 24 h after training. Video recordings of the test were processed offline using AnyMaze, in which a freezing period was defined as near-complete immobility for at least 1 second.

To test for cue retention, the conditioning chambers were altered so that the animals would consider them to be novel environments. Opaque white plastic was placed over the metal grid, and another thin plastic sheet was fit into the chamber creating semicircular walls. Additionally, anise seeds were placed under the chamber floor, to create a unique olfactory cue. Cued recall was tested at least 1 hr following the contextual recall test, with the animals placed in the novel chamber for 120 sec of baseline activity, followed by 3 presentations of the 30 sec 3000Hz tone cue without the co-terminating shock. Freezing was calculated during the tone presentation and compared with the 120 sec baseline activity.

In Vivo Electrophysiology

All recordings took place in the home cage of the animal, which were placed in soundproof and electrically-shielded chambers. A custom connector on the animal's head was connected to a 32-channel headstage (RHD2132, Intan Technologies) which digitized the signal and transferred it to an Open Ephys data acquisition system. Raw signals were filtered between 0.1

and 7500 Hz and digitized at 1 kHz. All recordings were referenced to a stainless steel ground screw above the cerebellum. Initial baseline recordings were a full 24 hrs, while subsequent recordings following testing were 4 hrs.

Data Analysis

Electrophysiology processing

Individual channel data was extracted from OpenEphys binary files and reformatted into a Matlab array. Parietal EEG and hippocampal LFP were bandpass filtered between 0.1 and 400 Hz, notch-filtered to remove 60 Hz noise and its harmonics, and detrended, using the EEGLAB Toolbox (Delorme and Makeig, 2004). EMG signals were similarly bandpassed between 30 and 300 Hz and detrended.

Sleep State Determination

For scoring sleep, processed parietal screw EEG and EMG recordings were binned into 10 second epochs. AccuSleep software (Berger et al., 2019) was used to automatically score sleep data, using a mixture z-scoring method that takes into account two sources of distributional shifts in order to accurately assign sleep state labels to epochs. This method requires a small sample of labeled data for each subject to standardized scoring, so AccuSleep also provides a means for visualization of the recordings by epoch and manual assignment of activity state. Additional custom code was added to this interface which calculated the delta (0 to 4 Hz), theta (5 to 10 Hz) and sigma (11 to 14 Hz) bandpower of the EEG signal for each epoch, as well as determining the delta/theta bandpower ratio. Epochs were sorted into non-REM (NREM), REM, transition to REM (TR), or active waking (AW). AW was defined primarily by a high-power EMG signal, indicating movement. NREM was characterized by low EMG activity and the

predominance delta band activity, as well as a delta/theta ratio above 1. TR was determined by a delta/theta ratio below 1 along with an increase in sigma bandpower, which was followed by a REM episode. REM epochs were determined by low delta/theta ratio and near zero EMG power, indicating the atonia seen in REM sleep. Once several epochs of each sleep state are scored, a calibration file is generated which calculates mixture z-scoring parameters to feed into the Sleep Scoring Artificial Neural Network (SS-ANN), a trained machine learning algorithm that automatically labels the unscored data. Once the automatic sleep scoring is complete, the output is reviewed by an experienced sleep scorer, to make sure that it is congruent with manual scoring.

Sleep Architecture

The sleep architecture of all recording sessions was evaluated using custom Matlab code. Recordings were broken down into 4 hr time bins, and the scored sleep states with their timestamps were used to determine the number of epochs of each state within the 4 hr intervals, the percentage of time spent in each state, and the average bout length of each state, along with the standard deviation of these values, to compare the sleep structure between animals and experimental conditions.

LFP power analysis by state

Power spectral density of each state was computed as previously described (Uygun et al., 2022). The MATLAB function pwelch was used with a 4-second Hanning window and 50% overlap. EEG or LFP power was normalized to total power of the recording. To compare between animals, power spectral density plots were Gaussian smoothed, normalized to the maximum baseline value and then averaged across all animals in a group. To calculate

bandpower, the area under the curve of each power spectral density plot was taken between the frequency bands of interest.

Phase-Amplitude Coupling (PAC) analysis

To investigate the coupling strength between theta and gamma oscillations, we used a previously described method to calculate a modulation index (MI) between two frequency ranges (Tort et al., 2008,2009,2010). In this method, a lower frequency oscillation is determined to be the "phase-modulating" rhythm that interacts with a higher-frequency "amplitude-modulated" rhythm. For our purposes, we investigated theta (5-12 Hz) phase modulation of various gamma band amplitudes. The steps to calculating the MI are described as follows:

1) The raw signal is filtered between the phase-modulating and amplitude modulated frequencies.
2) The Hilbert transform is applied to both the phase and amplitude filtered signals. The Hilbert transform of the phase results in the time series of phases, and the Hilber transform of the amplitude results in a time series of the amplitude envelope.
3) The time series described by the time series of the phases and amplitude is generated, giving the amplitude of the higher-frequency rhythm at each phase of the lower frequency.
4) The phases of the time series are binned, and the mean amplitude is generated for each phase bin.
5) The mean amplitude is normalized by the sum of the phase bins, which gives a probability density function of the amplitude.
6) The final MI value is derived by multiplying a constant by the Kullback-Leibler distance of the uniform amplitude distribution. This gives a value between 0 and 1 which describes how far away from a null distribution (i.e., no phase-amplitude coupling) the observed amplitude distribution is, resulting in a numerical value of coupling strength.

Histology

Upon the completion of the behavioral protocol, animals were deeply anesthetized using isoflurane, then transcardially perfused with phosphate buffered saline (PBS) followed by formalin. Brains were extracted and stored in formalin overnight to complete fixation, then moved to PBS and sectioned using a vibratome (Leica VT 1000S). 30 micron sections were made and mounted onto slides, covered with mounting media (Prolong Gold), and coverslipped. Slides were visualized and photographed at 20X using a Zeiss apotome and Zeiss Zen software, and analyzed for the presence of mCherry-positive OLM cells, indicating successful transfection. Electrode tip localization was also confirmed visually. Animals without adequate virus expression or proper electrode localization were eliminated from the data analysis.

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