NORADRENALINE: ACTIVE IN AROUSAL, BUT NOT FOR THE HIPPOCAMPAL-NEOCORTICAL DIALOGUE

SETARE TOHIDI
Bachelor of Science, Amirkabir University of Technology, 2020

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

MASTER OF SCIENCE

in

NEUROSCIENCE

Department of Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

NORADRENALINE: ACTIVE IN AROUSAL, BUT NOT FOR THE HIPPOCAMPAL-NEOCORTICAL DIALOGUE

SETARE TOHIDI

Date of Defense: December 7th, 2023

Dr. Majid Mohajerani	External Professor	Ph.D.
Dr. Ian Whishaw Thesis Co-Supervisors	Professor	Ph.D.
Dr. Bruce McNaughton Thesis Examination Committee Member	Professor	Ph.D.
Dr. Robert Sutherland Thesis Examination Committee Member	Professor	Ph.D.
Dr. Artur Luczak Chair, Thesis Examination Committee	Associate Professor	Ph.D.

Abstract

Noradrenaline plays a role in modulating behaviors and memory. Memory can have short durations, or long durations. Long term memory, however, requires plastic changes for its permanent storage. Evidence suggests that neocortical-hippocampal dialogue during sharp wave ripples is essential for memory consolidation. Although studies suggest the importance of noradrenaline in memory formation and memory consolidation at cellular and synaptic level, less is known about the role of noradrenaline in system memory consolidation during neocortical-hippocampal dialogue. I conducted wide-field optical imaging of the mouse neocortical noradrenergic activity by recording fluorescent signal from GCaMP6s calcium sensors expressed in noradrenergic terminals of locus coeruleus in neocortex, combined with hippocampal electrophysiological recording. I found that neocortical noradrenaline deactivates around sleep and awake sharp wave ripples, with no significant differences between neocortical regions. These findings suggest either that noradrenaline is not necessary for hippocampal-neocortical memory transfer or that such transfer is only possible in the absence of noradrenaline. I also found that noradrenaline becomes active when the mice are aroused as indicated by pupil dilation. Parsimony suggests that noradrenaline is active in arousal but not in memory consolidation.

Acknowledgement

I would like to thank my supervisors, Dr. Majid Mohajerani and Dr. Ian Whishaw for their support and guidance throughout my journey. I would also like to thank my committee members, Dr. Bruce McNaughton, and Dr. Robert Sutherland for their constructive feedback.

I am deeply grateful for the opportunity of being in Dr. Mohajerani's laboratory and having the chance to learn from him and his lab members. Without their help, I could have not completed my master's degree.

Table of Contents

List of Figures

1. Introduction

Noradrenaline, also called norepinephrine, is both a neurotransmitter and a hormone which its release throughout the brain plays an essential role in modulating countless behaviors, such as sleep/awake regulation, attention and memory in cognitive tasks, and arousal and response to stress (Schwarz & Luo, 2015). Albeit its widespread role, its function is yet incompletely understood. In the following introduction I will first describe current knowledge about its function in memory consolidation and behavioral modulation. I will then present the theory and the hypothesis that would be investigated in this thesis.

1.1.Noradrenaline-Locus Coeruleus System

Cell bodies of noradrenergic neurons are located in seven distinct nuclei in pons and medulla oblongata which are labeled A1–A7, among which the area A6 is the largest nucleus, called locus coeruleus (Szabadi, 2013). Locus coeruleus is the main source of norepinephrine (NE) to the neocortex and hippocampus (Benarroch, 2018), areas that I have particularly looked at in this study.

1.1.1. Locus Coeruleus Anatomy

LC is located bilaterally in the brainstem, in the upper dorsolateral pontine tegmentum, under the cerebellum, lateral to the fourth ventricle, and medial to the mesencephalic trigeminal nucleus in the pons (Chandler, 2016). In the rats, it contains approximately 1600 neurons with morphologies of both multipolar and fusiform (Foote et al., 1983; Swanson, 1976) all of which produce noradrenaline (Schwarz & Luo, 2015; Swanson, 1976). Early studies have divided LC to

three subdivisions: anterior pole, containing mainly large multipolar cells, posterior pole, containing mostly fusiform neurons, and compact core which expands dorsoventrally as it progresses caudally, with dorsal portion comprising mainly densely packed fusiform neurons with smaller cell bodies, whereas ventral portion containing larger multipolar neurons (Loughlin et al., 1986).

Multiple peptides are found to be co-localized within LC neurons, including vasopressin, somatostatin, neuropeptide Y, enkephalin, neurotensin, corticotropin-releasing factor, and galanin (Aston-Jones & Waterhouse, 2016). LC-NE neurons, depending on their target destinations, occupy distinct locations within the LC structure. These locations exhibit bias either along the dorsal-ventral axis for projections to the hippocampus, cerebellum, and spinal cord or along the anterior-posterior axis for projections to the thalamus and hypothalamus. LC-NE neurons projecting to the cortex and amygdala, on the other hand, are distributed throughout the LC without a specific positional preference (Schwarz & Luo, 2015). Its small size and intricate structure pose a challenge when trying to accurately target it for studies involving ablation or inhibition, often inadvertently affecting neighboring structures. Furthermore, given its extensive projection pattern, any manipulations performed on the LC have widespread effects on norepinephrine signaling in numerous brain regions. Finally, long-term disruption of norepinephrine signaling, whether achieved through LC ablation or genetic modification of crucial norepinephrine synthesis genes, results in alterations in signaling across various neuromodulatory pathways. These combined obstacles have made it difficult to investigate whether specific variations in LC anatomy or activity may underlie its various roles in the brain (Schwarz & Luo, 2015).

1.1.2. Efferent Pathways

Multiple studies report that, cells within LC are spatially organized with respect to terminal field targets (Aston-Jones & Waterhouse, 2016). Efferent topography of LC could make differentiated modulation of diverse behaviors and cognitive functions possible (Poe et al., 2020). LC neurons projecting to the cortex are approximately 95% ipsilateral, while cells projecting to subcortical structures show a more evenly balanced bilateral distribution of their projections (Aston-Jones & Waterhouse, 2016). Generally, LC neurons projecting to forebrain regions, such as the hippocampus and septum, are primarily situated in the dorsal part of the LC. On the other hand, LC neurons projecting to the cerebellum are found in both dorsal and ventral, and cells projecting to spinal cord are predominantly found in the ventral region. Additionally, there is a distinct organizational pattern along the anterior-posterior axis. LC neurons that project to the hypothalamus are positioned anteriorly, those projecting to the thalamus are located more posteriorly, and LC neurons projecting to the cortex and amygdala are dispersed throughout the LC without a specific pattern (Schwarz & Luo, 2015). Recent findings also indicate that individual LC neurons tend to innervate functionally related, yet distinct components within an ascending sensory pathway. For instance, LC neurons projecting to the trigeminal somatosensory cortex are more likely to simultaneously innervate the trigeminal somatosensory thalamus, rather than non-somatosensory thalamic areas, such as the dorsal lateral geniculate nucleus. Consequently, individual LC neurons exhibit a preference for sending axon collaterals to multiple targets that process the same sensory information. These observations form the basis for the idea that the organization of the LC is influenced by the functional characteristics of its output destinations (Berridge & Waterhouse, 2003).

1.1.3. Afferent Pathways

Afferent pathways to LC have been a historically controversial issue (Sara & Bouret, 2012). In opposition to early studies suggesting limited inputs, arising primarily from the ventrolateral and dorsomedial rostral medulla as well as hypothalamus (Aston-jones et al., 1984), recent work using viral-genetic tracing methods confirmed that noradrenergic neurons in the locus coeruleus received inputs from many brain regions and displayed a significant diversity in their output projections (Schwarz & Luo, 2015). The microcircuitry of the locus coeruleus comprises a densely populated central 'core' region where noradrenergic cell bodies and processes are concentrated, and a surrounding 'shell' known as pericoeruleus (peri-LC), which houses dendrites from LC-NA neurons that extend far beyond the nucleus proper into pericoerulear regions (Aston-Jones & Waterhouse, 2016). Therefore, since pericoerulear regions receive inputs from many sources, including prefrontal cortex, amygdala, lateral hypothalamus, and dorsal raphe, we can conclude that LC-NA neurons receive input from various regions of both the brainstem and forebrain (Aston-Jones & Waterhouse, 2016). These inputs from diverse sources can lead to varying effects on LC-NA neuronal activity, both directly and indirectly, by selectively targeting either the core nucleus or the pericoerulear regions (Poe et al., 2020). This result suggests that the LC-NE system integrates and broadcasts information extensively to modulate various brain states (Schwarz & Luo, 2015).

1.1.4. Cellular Organization and Morphology

The evidence indicates that LC/NE neurons are heterogeneous in their anatomical projections, morpho-electric characteristics, and the distinct behavioral functions they are associated with (McKinney et al., 2023). Although all LC neurons contain norepinephrine, their

unique features contribute to their diverse functions (Schwarz & Luo, 2015). Loughlin et al., have categorized soma shape of LC/NE neurons into either fusiform, round, or pyramid (Loughlin et al., 1986). Cells characterized by a fusiform-shaped cell body display a more polarized dendritic branching pattern, which can be either bitufted or bipolar. In contrast, cells with a round or pyramid-like cell body exhibit a less polarized dendritic arborization. These distinctions have facilitated the categorization of LC neurons into two morphological cell types: multipolar cells (MP, approximately 35 mm in size) and fusiform cells (FF, approximately 20 mm) (McKinney et al., 2023; Swanson, 1976). In addition to their different soma shape, fusiform and multipolar cells have different local axonal arborization (McKinney et al., 2023). While both of these cell types are distributed throughout the LC, there is a noticeable bias in their distribution, with a greater concentration of fusiform cells in the dorsal edge and posterior horn of LC projecting to the neocortex and hippocampus and, and a higher presence of multipolar cells in the ventral and the anterior horn of LC projecting to the spinal cord, cerebellum, and hypothalamus (Loughlin et al., 1986; McKinney et al., 2023; Schwarz & Luo, 2015). In addition, fusiform cells and multipolar cells have different population sizes among the LC (McKinney et al., 2023), with fusiforms making about 38% of LC neurons and unequally distributed throughout the entirety of the LC (McKinney et al., 2023). All these differences emphasize the complexity of LC function and possible unknown underlying reasons when interpreting its behavior.

1.1.5. Electrophysiological Attributes of LC Neurons

Early studies considered LC/NE neurons homogeneous, with NE-containing neurons characterized electro-physiologically as slow (0-5 Hz), spontaneous discharge rates, broad (1-2 msec) action potential wave forms and burst discharges followed by a prolonged period of

quiescence or decreased firing (Berridge & Abercrombie, 1999), with synchronous pattern of discharge which is not mediated by transmitter release, rather are electrotonically coupled via interactions between dendrites outside the cell body region (Berridge & Abercrombie, 1999). With categorizing LC neurons, researchers have distinguished firing patterns of fusiform and multipolar cells as they have different membrane properties as well. (McKinney et al., 2023). Fusiform cells have a larger amplitude, while narrower spike, in comparison to multipolar cells, having a longer action potential with smaller amplitude (McKinney et al., 2023).

LC neurons display two distinct activity modes of tonic and phasic (Berridge & Abercrombie, 1999), and the transition between these modes regulates the different behavioral states (Benarroch, 2018). Tonic activity is characterized by a random pattern of discharges occurring at various, relatively low rates, typically falling within the range of 0.1 to 5.0 Hz (Devilbiss & Waterhouse, 2011). Sustained and regular discharge pattern (2–5 Hz) in the tonic baseline activity is state-dependent; the discharge rate is the highest during waking, as it is reported <2Hz during quiet waking and also >2Hz for active waking. Therefore, it is associated with alertness and wakefulness. This tonic LC discharge diminishes as arousal decreases, lowers during slow wave sleep to less than one hertz and it completely stops during REM sleep (Benarroch, 2018; Berridge & Abercrombie, 1999). During focused attention, LC neurons display phasic activity during which cells fire short (<300 msec), and spontaneous bursts of higher frequencies (10-15 Hz), superimposed upon tonic activity, and is often, but not always followed by a more prolonged period of suppression of discharge activity (Benarroch, 2018; Berridge & Waterhouse, 2003; Vazey et al., 2018). Phasic activity is in response to salient, task-related, or sensory stimuli. (Aston-Jones & Cohen, 2005). The phasic discharge facilitates precise task execution by filtering out

unimportant stimuli and is associated to accurate behavioral responses (Aston-Jones & Cohen, 2005).

1.2. Noradrenaline

Noradrenaline is synthesized from the amino acid tyrosine, by conversion of dopamine to norepinephrine by dopamine β -hydroxylase, and then transported to synaptic vesicles via vesicular monoamine transporter 2(VMAT2) (Benarroch, 2018). Norepinephrine is stored in vesicles in the nerve terminals till released upon stimulation. The effects of noradrenaline are terminated by reuptake back into nerve terminals via the presynaptic norepinephrine transporter (NET) (Benarroch, 2018). Enzymes and Monoamine oxidase in the mitochondria of the synaptic knob will metabolize and inactivate noradrenaline (Benarroch, 2018).

1.2.1. Noradrenaline Receptors

The effects of NE are conveyed by means of three families of G-protein coupled receptors, α_1, α_2 and β, each consisting of several subtypes (three β-receptor subtypes (β_{1-3}), three α subtypes (α_{1a}, α_{1b}, α_{1d}) and four α_2-receptor subtypes (α_{2A-D}) are recognized) (Berridge & Waterhouse, 2003). There is a differential distribution of adrenergic receptors within LC, as well as in the different targets of LC projections (Benarroch, 2018). For that reason, based on the level of their expression, similar input may lead to different responses from individual LC neurons (Schwarz & Luo, 2015). Also, the overall impact of noradrenaline release in a specific neural circuit relies on the presynaptic and postsynaptic complement of α-and β-adrenergic receptors and their subtypes in that area (Poe et al., 2020). α1 and β receptors are predominantly found in post-synaptic locations (Benarroch, 2018); The α1 receptors generally are located at post synaptic sites and

mediate excitatory effects by being coupled to the phospholipase C/inositol triphosphate/protein kinase C pathway. The β receptors are also located post synaptically and are positively coupled to adenylyl cyclase, increasing cyclic adenosine monophosphate (cAMP), directly and indirectly (via protein kinase A triggered cascades) affecting synaptic plasticity and excitability (Benarroch, 2018). Noradrenaline-released adrenoceptors are activated in both post-synaptic sites and on the noradrenergic neuron itself, known as "autoreceptors" (Szabadi, 2013). These autoreceptors belong to the α_2 subclass and have inhibitory functions. The α_2 receptors are found both pre-and post- synaptically. When located on the nerve terminal membrane, often referred to as "presynaptic receptors", they reduce neurotransmitter release by being negatively coupled to adenylyl cyclase, activating K+ currents leading to reducing neuronal excitability, and therefore inhibiting presynaptic calcium (Ca2+) channels, which leads to reduced neurotransmitter release (Benarroch, 2018). When located in the somato-dendritic region, they suppress neuronal firing (Szabadi, 2013). Notably, adrenoceptors that modulate presynaptic release are found not just on the axon terminals of noradrenergic neurons (autoreceptors) but also on the nerve terminals of other neurons (referred to as heteroreceptors which also belong to the α_2 subclass) (Szabadi, 2013). All three subtypes of α_1-adrenoceptor (α_{1A}, α_{1B} and α_{1D}) have been identified in the neocortex (Szabadi, 2013). Inhibitory α_2-adrenoceptors, even though in smaller numbers than α_1-adrenoceptors, have also been recognized with a more selective distribution, belonging mainly to the α_{2A} subtype (Szabadi, 2013). It has been suggested that these receptors could be located on inhibitory interneurons, and when activated, they might disinhibit cortical neurons, resulting in cortical stimulation (Szabadi, 2013). β -Adrenoceptors are also detectable in the neocortex (Szabadi, 2013).

1.2.2. Noradrenaline Release Within Neocortex

Early studies by Berridge et. al. showed that LC activation magnitude is followed by an increase in prefrontal cortical extracellular NE concentrations in a linear relation, while LC activation amplitude is increased up to 300%-400% of its basal discharge level, and beyond that, it doesn't evoke considerable increase in NE concentration (Berridge & Abercrombie, 1999). LC suppression is also followed by a decrease in extracellular NE concentrations (Berridge & Abercrombie, 1999). The extensive nature of the noradrenergic innervation of the neocortex suggests that the projection from the LC exerts a diffuse influence on this structure. Although anatomical evidence supports diffuse pattern of noradrenergic innervation (Loughlin et al., 1982), considerable topographic specificity has also been found (Foote et al., 1983). This diffuse pattern implies that activation of the LC results in release of NE throughout the cortex, equivalent to the activation (Agster et al., 2013). However, it's worth considering that, since density of noradrenergic fibers is relatively consistent and uniform across cortical regions in rat, differences in the distribution of axonal varicosities within regions, rather than differences in fiber density, might be involved in regulation and the release of noradrenaline (Agster et al., 2013). Axonal varicosities are enlarged, heterogeneous structures along axonal shafts that majority of them (70-90%) make no synaptic contacts (Seguela et al., 1989, Seguela et al., 1990). Axonal varicosities, rather than synaptic terminals, act as the major release and reuptake sites of neurotransmitter in LC axons (Agster et al., 2013; Swanson, 1976). Later studies have shown anatomical evidence for non-uniform NE release in functionally distinct cortical regions, with greater density of varicosities in the frontal cortex in comparison to the other cortical areas (Agster et al., 2013). This implies that after LC activation and then impulse activity transmission to LC terminal regions, release of

noradrenaline might be varying across the cortex, with the highest amounts of NE release within prefrontal cortex, and greater in superficial layers than in deep layers of cortex (Agster et al., 2013).

1.2.3. Noradrenaline and Synaptic Plasticity

Synaptic plasticity refers to the activity dependent modification of synaptic strength or efficacy over time, either by strengthening or weakening. These changes are the underlying mechanism for learning and memory (Hebb, 1949). Multiple forms of plasticity have been identified, ranging from short-term changes in release probability, such as augmentation and post-tetanic potentiation, to enduring forms of increased and decreased synaptic transmission, also known as long-term potentiation(LTP) and long-term depression (LTD), respectively (Collingridge et al., 2010; Kandel E. R., 2001). LTP is commonly defined as a synaptic enhancement or lasting increase of synaptic efficacy that follows brief, high-frequency electrical stimulation. LTD, in the contrary, is a synaptic weakening or lasting decrease of synaptic efficacy followed by a low rate (~1 Hz) stimulation for long periods. Most forms of plasticity are regulated by neuromodulators, including noradrenaline, by acting through metabotropic receptors and associated intracellular signals to modify synaptic function as well as neuronal excitability (Maity et al., 2022). During learning and memory events, the exertion of noradrenaline activates G protein-coupled receptors (GPCRs), which initiates multiple signaling cascades that leads to synaptic changes (Maity et al., 2022). In general, β-ARs are responsible for multiple forms of LTP, whereas α-1 and α-2-ARs contribute to synaptic potentiation and depression, correspondingly (Lemon et al., 2009; Maity et al., 2022). Studies suggest that activation of β-adrenoreceptors by noradrenaline could result in facilitation of synaptic transmission via increasing the intracellular cAMP concentration and new protein synthesis, contributing memory enhancement, and also stabilizing long-term potentiation

(LTP) in the hippocampus (Lemon et al., 2009; Nguyen & Connor, 2019; Raymond, n.d.). Noradrenaline has also been described to induce synaptic depression in CA3 region of hippocampus by acting on α1-adrenoceptors (Katsuki, 1997; Marzo et al., 2009) . Similar to the effects of α1-AR activation (Katsuki, 1997), α2-AR's effects on hippocampal plasticity are primarily presynaptic, inhibiting release of noradrenaline (Boehm S., 1999). Thus, α_2 adrenergic receptors have an indirect effect on synaptic plasticity, and possibly having neuroprotective of homeostatic roles (Nguyen & Connor, 2019).

1.2.4. Noradrenaline and Sleep Regulation

Early works investigating the role of the LC noradrenergic neurons revealed that during waking, noradrenergic neurons are activated, during slow wave sleep they decrease their firing rate and they become silent during rapid eye movement sleep (Aston-Jones & Bloom 1981; Hobson et. al, 1975). In addition, LC is crucial for triggering sleep-wake transitions and vigilance. Increased LC activity happens before the transition from sleep to awake (Aston-Jones& Bloom, 1981). This idea is supported by more recent experiments, showing optogenetic LC stimulation induces a sleep-wake transition (Carter et. al, 2010). While wake-promoting neural activity is inhibited during NREM sleep, sensory vigilance remains due to recurrent fluctuations noradrenaline. One recent study with a focus on sleep micro-structure posited that micro-arousals during NREM sleep are generated in a periodic pattern, riding on the peak of LC-generated infra-slow (~30) oscillations of extracellular noradrenaline, while descending phases of noradrenaline oscillations is associated with spindles (Kiaerby et. al, 2022). Altogether, both early and recent works agree that LC is involved in sleep/wake regulation, and it progressively reduces its firing rate from waking to REM sleep.

1.3. Noradrenaline and Memory Consolidation

Release of noradrenaline in the hippocampus and cortex is necessary for acquisition and consolidation of short-term to intermediate and to long-term memory (Gibbs et al., 2010). First proposed by Müller and Pilzecker, consolidation hypothesis states that newly formed memories are vulnerable to interference for a time after learning and may consolidate over time (Müller, 1900). This idea has initiated studies over the hormonal and neural influences involved in memory consolidation, as well as molecular and cellular mechanisms; Being involved in the first stated phase, memory formation, noradrenaline is a good candidate in this regard.

β-ARs have well-established roles in memory formation through noradrenaline release (I. N. Izquierdo et al., 1998; Ji et al., 2003; Kitchigina, 1997 ; Mello-Carpes et al., 2016; Straube et al., 2003). Even though β-ARs support both short- and long- term memories by mediating physiological and molecular events, researchers have mostly studied them for their role in long term memories (I. N. Izquierdo et al., 1998; Ji et al., 2003), based on which it can be stated that β-ARs have preferential influence in the late or enduring components of memory (Sara et al., 1999). Based on that, it can be concluded that noradrenaline should be involved in memory consolidation, to enhance the stabilization of newly formed memories; Conflicting results have been reported in this regard (I. Izquierdo & Medina, 1997; Mello-Carpes et al., 2016; Nguyen & Connor, 2019; Thomas & Palmiter, 1997) Being mostly focused on synaptic consolidation corresponding to late-phase long-term potentiation, less is known about noradrenergic involvement in systems consolidation, a process in which the hippocampus reorganizes the encoded, hippocampus-dependent memories into more stable long-term memories independent of hippocampus (Klinzing et al., 2019; Lechner et al., 1999; Müller, 1900). Based on the two-stage model of memory (Buzsáki G., 1989), during the acquisition, initial memories are formed when

theta and gamma waves activate a neuronal pathway for memory formation. Then, this pathway would get replayed by propagation of Sharp Wave Ripples to neocortex, resulting reinforcement of memories (Buzsáki G., 1989).

1.3.1. Sharp Wave Ripples and Memory Consolidation

Sharp waves are fast (40-100 msec) and large amplitude depolarizing events that can be recorded from hippocampus during NREM sleep. They are usually, but not always, superimposed by ripples, which are faster (100-300 Hz) short-lasting oscillatory activity. Together they form sharp wave-ripple (SPW-R) events (Bragin et al., 1999; Buzsáki G., 1986). Sharp waves originate from CA3 of the hippocampus when subcortical regions like medial septum-vertical limb of the diagonal band lift their suppressing control on the network of interconnected CA3 pyramidal cells (Brown et al., 2012). Highly recurrent excitatory collateral network of CA3 pyramidal neurons is an optimum underlying basis for the synchronized population bursts happening during sharp wave events (Buzsáki, 2015). Ripples originate from CA1 and are induced by the strong excitation coming from the CA3-generated sharp waves while the communication between Parvalbumin-expressing interneurons and pyramidal cells helps generate the ripple timing and support their spatial synchrony (Buzsáki, 2015).

There is evidence about memory performance impairment by interrupting sharp wave ripples during different stages, such as learning and post learning sleep (Jadhav et al., 2016). Also, coordinated activity between the hippocampus and neocortex, specifically during slow wave sleep, in the form of coupling between delta waves, spindles and sharp wave ripples have been reported in several studies (Abadchi et al., 2020; Abadchi et al., 2023; Battaglia et al., 2004a; Mölle et al., 2006; Pedrosa et al., 2022; Peyrache et al., 2011; Sirota et al., 2003). But studies looking at

noradrenergic neocortical-hippocampal dialogue have been rare. Even though there are studies looking at the effect of noradrenaline release on hippocampal sharp wave ripple generation, but studies with a focus on NE release in the neocortex at time of sharp wave ripples have been limited. Segal and Bloom have shown that the firing rate of most hippocampal cells (170 of 182) were depressed by noradrenaline application. Also, in anesthetized rats, by extracellularly monitoring the electrical activity of single pyramidal cells in the hippocampus during electrical stimulation, they have shown that LC stimulation has produced long-lasting inhibition of spontaneous activity of pyramidal cells (Segal et. Al, 1974); Berridge and Foote have also shown that, in the hippocampus, LC activation was followed by the appearance of almost pure theta activity (Berridge et. Al, 1991). While Logothetis et al. state that human SWRs are phase locked to hippocampal delta oscillations (Logothetis et. Al, 2012), we can conclude that NE release and SWRs generation do not occur at the same time. Accordingly, in one particular study, Novitskaya et al., 2016 have looked at LC stimulation during an "on-line" detection of sharp wave ripples, and have reported that high-frequency stimulation has caused a memory deficit by blocking generation of ripple-associated cortical spindles, interfering with hippocampal-cortical communication which emphasizes on its importance for off-line memory consolidation (Novitskaya et al., 2016; Peyrache et al., 2009a; Peyrache et al., 2011). All in all, little is known about role of noradrenaline hippocampal-neocortical dialogue and in system memory consolidation. Hence, a study of the relationships between SWR and noradrenergic neocortical activation is needed.

1.4. Noradrenaline and Behavioral States

Variations in brain activity as well as behavioral state are supported by broadly projecting neuromodulatory systems including the noradrenergic nucleus locus coeruleus (Collins et al., 2023. Sara & Bouret, 2012). There are several behavioral states hypothesized to be influenced by LC/NE activity, such as initiating or maintaining stages of the sleep-waking cycle (S-WC) (Aston-Jones & Bloom, 1981), and promoting arousal or vigilance (Berridge, 2008). These brain states are also accompanied by physiological changes such as pupil dilation/constriction, locomotion, and eye movements (Larsen & Waters, 2018). Therefore, pupil fluctuations have also been posited as being an indicator of noradrenaline activity (Joshi et al., 2016; McDougal & Gamlin, 2015). Moreover, recent works show that optogenetic activation of the LC induces pupil dilation and inhibition of the LC results in pupil constriction (Breton-Provencher & Sur, 2019; Reimer et. al, 2016). Considering noradrenergic involvement in behavioral states in this study I look at the spatiotemporal patterns of neocortical noradrenergic activity around behavioral hallmark of pupil dilation and pupil constriction.

Theory and Hypothesis

Theory. Standard consolidation theory

Standard consolidation theory states that, initially, (episodic and semantic) memories depend on the hippocampus, but hippocampal involvement is time limited. The structure is required to form necessary associations to create a coherent memory and to maintain it for a short period, and then eventually the memory reorganizes and become consolidated in their original forms in neocortical structures. After consolidation, memories can be retrieved without hippocampal involvement (Scoville & Milner, 1957; Squire, 1992; Squire & Alvarez, 1995; Squire & Zola, 1998).

***Hypothesis*.** Is noradrenaline involved in plasticity?

Learning causes persistent changes in synaptic transmission within experience-activated neural networks (Saar and Barkai 2003; Zhang and Linden 2003). Noradrenaline release fosters long-term synaptic plasticity (Marzo et al., 2009), which is the building block for learning (Hebb, 1949). As stated in Standard Consolidation Theory, memory consolidation is based on transferring information from hippocampus to neocortex, which requires changes in synaptic plasticity. Sharp wave ripples, indicative of experience-related neuronal sequence replay, provide time windows of increased cellular excitability (Buzsaki 1985), leading to stabilization of memory representations at the synaptic level, and therefore, synaptic changes. We assume that noradrenaline release, involved in synaptic changes, should be involved in transferring memory from hippocampus to neocortex and therefore should coincide with hippocampal sharp wave ripples, resulting in memory consolidation.

2. Materials and Methods

2.1. Animals

Six Ai162.Dbh-cre transgenic mice of both gender (male and female) were used for this experiment, expressing GCaMP in noradrenergic terminals of LC in the neocortex. We crossed TIGRE 2.0 Cre-dependent GCaMP6s (Ai162) reporter lines with Dbh-Cre mice. This resulted GCaMP6s expression in noradrenergic neurons of locus coeruleus, emitting fluorescence resulted from calcium activity, measuring both synaptic and non-synaptic (varicosities) noradrenaline release (Broussard et. al, 2018; Collins et. al, 2023). Mice were housed individually after head-plate/electrode implantation surgery. The animal protocols were approved by the University of Lethbridge Animal Care Committee and were in accordance with guidelines set forth by the Canadian Council for Animal Care.

2.2. Surgery

I first injected 0.5 gr/Kg buprenorphine subcutaneously half an hour before the beginning of surgery. Animals were then anesthetized with isoflurane (1–2% mixed in O2). Next, I removed the head skin, implanted an electrode in the CA1 region of hippocampus for recording LFP signal. The electrode was made from a Teflon-coated stainless-steel wire, bare diameter 50.8 micro meter, tip separation 0.5 mm. For that, I drilled a hole on the right hemisphere skull, tangent to the posterior side of the occipital suture with 33-degree angle relative to the vertical axis, and approximately 2.6 mm lateral to the midline. Then, I gradually lowered the tip of the bipolar electrode through the hole, visually and audibly monitoring the electrode signal with respect to the reference point (ground screws located on top of the cerebellum). I stopped the lowering as soon as I observed or heard a significant increase in the signal, if the electrode was near the calculated

coordination with depth of approximately 1.75 mm at the 33-degree angle, close to pyramidal layer of dorsal CA1. I then fixated the electrode on the skull using Krazy Glue and dental cement. Next, I implanted a head-plate, covered the skull with a thin and transparent layer of the metabond (Parkell, Inc), and at last covered the skull with a glass coverslip. Also, a bipolar multi-stranded stainless-steel wire (bare diameter 127 micro meter) electrode for EMG recording was implanted in the neck muscles using a 22-gauge needle. Animals were given enough time to recover, at least two weeks, before recordings started. At the end of the experiment, animals were perfused with PBS (1x) and PFA (4%) and their brains were extracted, sectioned, and mounted.

2.3. Habituation

After at least two weeks of recovery, mice were habituated to the recording setup. I put them one by one on the recording platform, initially they were free in the platform to explore and get used to the environment. Then I started head fixing them using two clamps, for a short period (5 min) in the first head fixing session, and gradually increasing the time (by steps of 5-10 minutes) to 1.5 hours over several habituation sessions. A night before recording, the animals were moved from their home cage colony room to another room, transferred the mice to a bigger cage containing a running wheel and several new objects, many food plates, as well as water container. I left the mice in the new cage in the same room overnight. The next day, the mice were transferred to the recording room early in the morning. I prevented them from falling asleep while they were kept in the cage by touching them using a cotton-tip stick whenever they showed being sleepy. The recording initiated at around 7:30 AM, and after the recording, mice were transferred to their own home cage and colony room and were allowed to sleep and recover at will. Between each recording

session of the same animal there was at least three days gap, to make sure that sleep restrictions don't change their sleep cycle.

2.4. Neocortical Imaging, Behavioral Imaging, and Hippocampal LFP Recording

The electrode signals were amplified (x 1,000) and filtered (0.1–10000 Hz) using a Grass A.C. pre-amplifier Model P511 (Artisan Technology Group, IL) and were sampled at 100 kHz using a data acquisition system (Axon Instruments). Using a behavioral V2 infrared pi camera, the animal's behavior and pupil movements were filmed at 25 fps. The animal's head was illuminated using a 940-nm infrared LED. For brain calcium imaging, image stacks were recorded at 30 fps, exciting the GCaMP6s indicators by two blue LEDs (470-nm center, Luxeon K2). Emitted fluorescence was filtered by passing through a 510-nm to 550-nm bandpass emission filter. Each imaging stack of spontaneous activity contained 170,000 frames.

2.5. Image Preprocessing

Raw calcium imaging of spontaneous activity were preprocessed in accordance with the following procedure: First, a baseline signal (F0) was calculated by averaging all the frames, and the fluorescence changes were quantified as $\left(\frac{F-F0}{F0}\right) * 100$, where $F - F0$ (or ΔF) was calculated using the locdetrend function in the Choronux toolbox (Abadchi et al., 2023; Nazari et al., 2023). To do so, a piecewise linear curve was fitted to the pixel time series using the local regression method (100s moving window, 70s step size). To reduce spatial noise, images were filtered by a Gaussian kernel (5 * 5 pixels, sigma = 1). Because most of the optical signal is concentrated in lower frequencies (Mohajerani et al., 2013), imaging data was also filtered using a zero-phase lowpass Chebyshev filter below 6 Hz which also eliminates the strong heartbeat artifact.

2.6. Pupil Diameter Detection

I used a thresholding based on intensity; an algorithm implemented in Facemap framework (https://github.com/MouseLand/facemap) to quantify pupil diameter based on pixel size.

2.7. Sharp Wave Ripple Detection

To detect hippocampal sharp wave ripples, the raw hippocampal LFP was first down sampled to 2 kHz. Then, it was band pass filtered using a 400-order band-pass FIR filter designed in MATLAB (MathWorks). Then, to gene rage the ripple power, the filtered signal was rectified and smoothed using a rectangular, 8-msec-length window. Then the output was thresholded and SWRs were identified when the ripple power signal passed the detection threshold defined by the mean plus a multiple of standard deviation (2 or 3, manually changed based on the signal) of the ripple power signals (Abadchi et al., 2020; Abadchi et al., 2023). Besides amplitude threshold, a duration threshold was also defined by a lower threshold (75% of the detection threshold) to identify the onset and offset of each SWR. Detected SWRs events were further monitored by applying a duration threshold and events shorter than the mean duration of all detected events were cut out. The center of the detected ripple was defined as the timestamp of the largest trough located between the onset and offset times. Moreover, events with centers less than 50ms apart were merged. To ensure that the peri-ripple neocortical activity was least affected by movement-related brain activity, the ripples with noticeable EMG activity within ±500 ms were excluded from the analyses (Abadchi et al., 2020; Abadchi et al., 2023).

2.8. Peri-SWR Averaging

For peri-SWR averaging of neocortical activity, first LFP signal was temporally aligned with concurrently recorded neocortical activity. Then for each detected sharp wave ripple, neocortical imaging frame corresponding to SWR center was detected and then averaged.

2.9. Neocortical Activation Peak / Deactivation Trough Detection of Optical Signal

Briefly, the calcium signal $\Delta F/F0$ captured from each neocortical region was thresholded for both amplitude and duration, similar to SWR detection.

The events with amplitudes smaller and durations longer than the corresponding defined thresholds were identified as peak deactivation in that neocortical region.

2.10. Z-Scoring Peri-Event Neocortical Activity

The z-scoring of peri-ripple traces was carried out against a null distribution which was obtained from traces/frames centered around random time stamps. These timestamps were not necessarily corresponded to those of ripple centers. To generate new, random timestamps, the inter-SWR intervals were randomly permuted. Afterwards, for each random time point, the imaging frames capturing ten seconds before to thirty seconds after, were temporally aligned, making a new image stack. The mean and standard deviation of the new image stack was calculated across all the randomly generated time points, which generated a mean and a standard deviation stack of frames (Abadchi et al., 2020). By using these two stacks, I z-scored all the individual peri-SWR stacks of neocortical activity.

2.11. Statistical Tests

All statistical tests for linear data were carried out using MATLAB built-in functions. Repeated-measure ANOVA with Greenhouse-Geisser correction for sphericity was performed for testing the hypothesis that there was a region-effect in amplitude of the peri-ripple traces and peri-trough hippocampal ripple power.

2.12. Sleep Scoring

Alertness stages of each animal were categorized as awake, NREM, and REM by thresholding EMG and hippocampal theta-to-delta ratio signals (Abadchi et al., 2020; Nazari et al., 2023). Awake periods were picked out by visual assessment of animal behavior and EMG signal. Periods with low EMG and theta-to-delta ratio (5–10 Hz for theta 0.5–4 Hz for delta) and lack of facial (around the chin) movement for at least 50s were categorized as NREM sleep. Also, NREM periods REM sleep periods were detected when EMG signals were the lowest due to muscular atonia, and high theta-to-delta ratios. Pupil size was also used to verify our sleep scoring results. Also, as the last criteria, only NREM periods followed by a REM sleep were included (Nazari et al., 2023). I succeeded to record sleep data from 5 animals out of 6 animals that I was recording.

2.13. Phase-Binning Neocortical Traces Neocortical Traces

Pupil diameter was filtered below 1 Hz. For registration to a standard dilation/constriction cycle, neocortical fluorescent signals at each time point was binned by the Hilbert phase of the filtered pupil trace (64 bins from $-\pi$ to $+\pi$). The periods of noticeable movement were excluded. Phase plots were smoothed by averaging adjacent bins.

3. RESULTS

Using in vivo wide-field of view mesoscale optical imaging (Bermudez-Contreras et al., 2018; Kyweriga et al., 2017) and genetically encoded GCaMP6s sensors, I sought to study the spatial changes in noradrenergic cortical neural activities during hippocampal sharp wave ripples and behavioral states, i.e., pupil dilation/constriction. To do so, I simultaneously recorded spontaneous neocortical activity dynamics from a large cranial window in the right hemisphere and ipsilateral hippocampal LFP from 6 mice to capture hippocampal SWRs. Due to the high level of synchrony in neocortical activity (Mohajerani et al., 2010) and hippocampal sharp wave ripple (Chrobak and Buzsaki, 1996) across hemisphere, we recorded unilaterally from hippocampus and neocortex. These activities were acquired in the absence of external sensory stimuli, using a custom-built experimental setup. Wide filed calcium images were captured with 30 Hz frame rate at which we were able to resolve multiple time signatures of ongoing activity. Spontaneous electrophysiological and imaging data were recorded in continuous 94- minute sessions. Performed craniotomy was large enough to have multiple cortical regions in the imaging window. These regions included: primary and secondary motor cortex (M1, M2), hindlimb area of the primary somatosensory cortex (HLS1), forelimb area of the primary somatosensory cortex (FLS1), primary and secondary barrel cortex (BCS1, BCS2), parietal association cortex (ptA), dorsal and lateral agranular of retrosplenial cortex (RSC1 , RSC2), primary and lateral visual cortex (V1, V2), primary auditory cortex (A1), posterior auditory cortex (AUDp), dorsal auditory cortex (AUDd), supplementary somatosensory area (SUP), and primary somatosensory area, mouth (ULPS1).

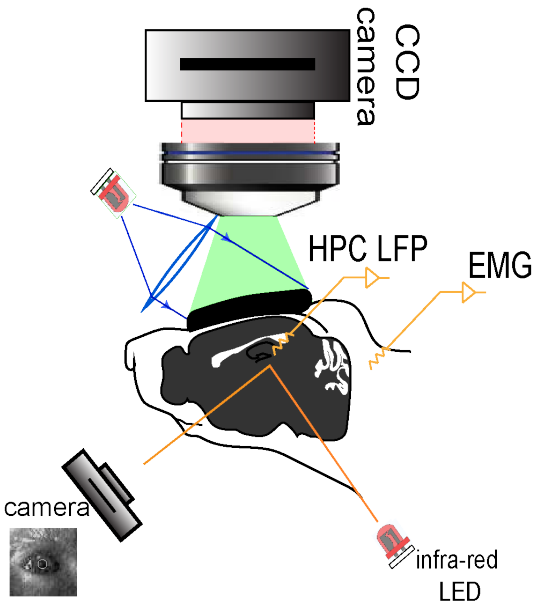

Figure 3.1., Schematic of the experimental setup for simultaneous wide-field optical imaging. A CCD camera detects reflected light coming from fluorescent indicators, in the superficial neocortical layers, which are excited by blue LEDs. An additional infra-red camera recorded pupil diameter. Hippocampal LFP recordings were used for SWR detection. An EMG electrode was also used to detect movement.

1 Retrosplenial, dorsal (RSC1)
2 Retrosplenial, lateral agranular (RSC2)
3 Secondary motor area (M2)
4 Primary motor area (M1)
5 Lower limb, HLS1
6 Upper limb, FLS1
7 Primary auditory (A1)
8 Posterior auditory (AUD p)
9 Dorsal auditory (ADUd)
10 Primary somatosensory area-mouth (ULPS1)
11 Secondary somatosensory area-barrel field (BCS1)
12 Primary somatosensory area-barrel field (BCS)
13 Supplamentary somatosensory area (SUP)
14 Parietal association cortex (PtA)
15 Primary visual area (V1)
16 Lateral visual area (V2)

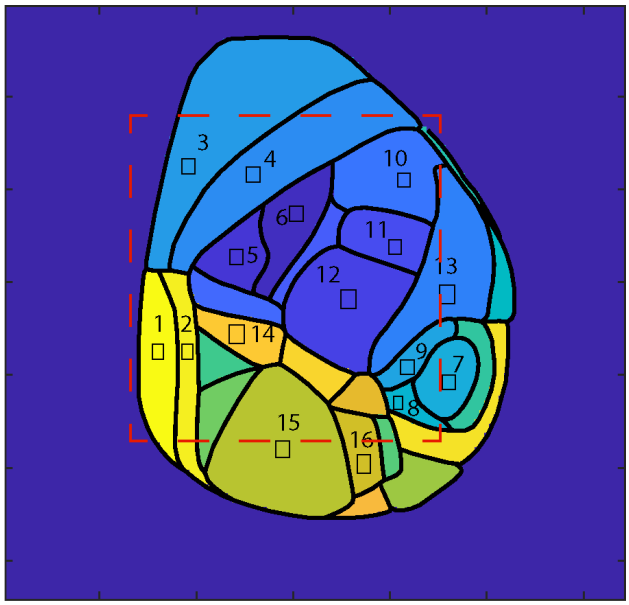

Figure 3.2., Schematic of a cranial window for wide-field optical imaging of neocortical activity. Cartoon of unilateral preparation registered on Allen institute Brain Atlas, showing the location of imaged cortical regions. Dashed rectangular shows the typical boundary of craniotomy.

3.1. Patterns of activity in noradrenergic neocortical activity are uniformly modulated around hippocampal sharp wave ripple

I began by studying the neocortical noradrenergic activity around hippocampal sharp wave ripples. To do so, I first separated SWRs occurring during NREM sleep, and during periods that animal was awake but with no movement, calling these periods "Quiet Wakefulness". Then I performed SWR-triggered averaging of neocortical noradrenergic activity for 10 seconds before and 30 seconds after of SWRs. For peri-SWR averaging of neocortical noradrenergic activity, for each detected sharp wave ripple, corresponding neocortical imaging frame was aligned to SWR centers and then averaged. Noradrenaline in almost all neocortical regions transiently deactivated after occurrence of sharp wave ripples, and spatiotemporal patterns of neocortical noradrenergic activity was similar around sharp wave ripples happening during NREM and Quiet Wakefulness. There was no significant difference between the amplitude of deactivation among regions (repeated measure ANOVA with Greenhouse-Geisser correction for sphericity) both in quiet wakefulness and NREM sleep. Also, even though amplitude of deactivation was less in NREM in comparison to quiet wakefulness, this difference was not significant comparing corresponding regions in NREM sleep and Quiet Wakefulness. The smaller deactivation amplitude could be due to decrease in NE level during NREM sleep (Aston-Jones& Bloom, 1981).

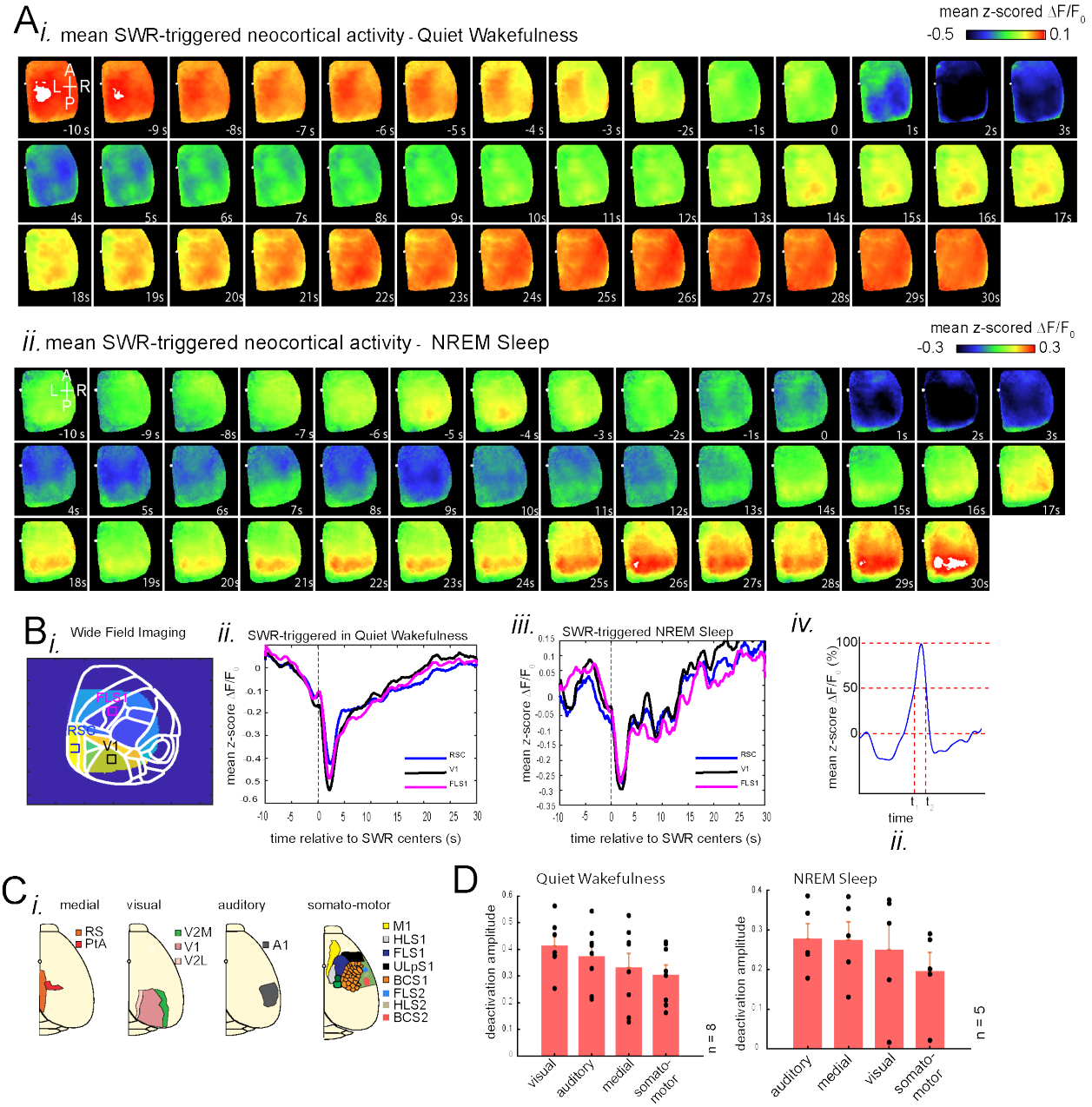

Figure 3.1.1. Neocortical noradrenergic activity is uniformly modulated around hippocampal SWRs**. (A)** Representative montage of mean peri-SWR neocortical noradrenergic activity during (i) quiet wakefulness and (ii) NREM sleep, respectively. 0s-time indicates SWR centers. Images have been z-scored and scaled to the depicted color bars. **(B)** (i) Cranial window registered on Allen Mouse Brain Reference Atlas based on anatomical landmarks (ii-iii) Example traces showing GCaMP6s fluorescent signals from selected regions in (i). Plots are the average of optical signals measured from 3×3 pixel boxes placed within retrosplenial (blue), visual (black), and forelimb somatosensory (magenta) cortices. (iv) Demonstration of how the deactivation amplitudes were quantified. the deactivation peaks were first rectified, then the deactivation amplitude was defined as the mean of the signal across full-width at half maximum (t_1 to t_2) **(C)** (i) Four major structurally defined neocortical subnetworks (medial, visual, auditory and somato-motor). **(D)**(i-ii) Grand average (n = 8 animals for quiet wakefulness and n=5 animals for NREM sleep) of

noradrenergic deactivation amplitudes across neocortical subnetworks, sorted in decreasing order. Please note that the deactivation peaks were rectified for group comparison. Each data point is the average of deactivation amplitudes of noradrenaline of all regions in a given subnetwork and in a given animal. A higher value of deactivation amplitude shows stronger deactivation. Bar graphs indicate mean ± SEM.

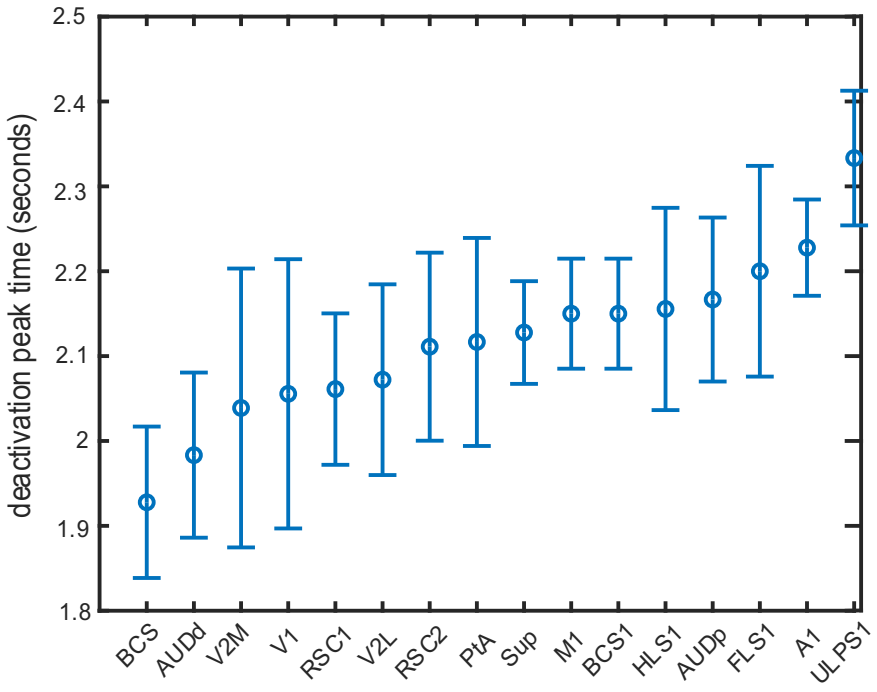

Figure 3.1.2. The peak time of mean peri-SWR noradrenergic deactivation across neocortical regions. Based on average of all animals (N=6), the regions on the horizonal axes are sorted in an increasing order in each region. Erro bars represent SEM.

3.2.Ripple power increases prior to troughs of neocortical activity in different neocortical subnetworks

After observing that noradrenaline in neocortical regions around SWRs uniformly deactivates, I investigated whether the reverse also applies, i.e., does ripple power increase when strong noradrenergic deactivation happens in a given neocortical region?

To investigate this question, first the calcium fluorescent signal recorded from each neocortical region was tresholded for both amplitude and duration to find peak noradrenaline deactivation in that region. Then the ripple power traces centered on peak deactivation in each region of interest were averaged and compared across neocortical subnetworks (Figure 3.1.2). Ripple power increased 1.5 secs before neocortical noradrenergic deactivation, and slightly decreased at time of neocortical noradrenergic troughs. These results were consistent among all the regions.

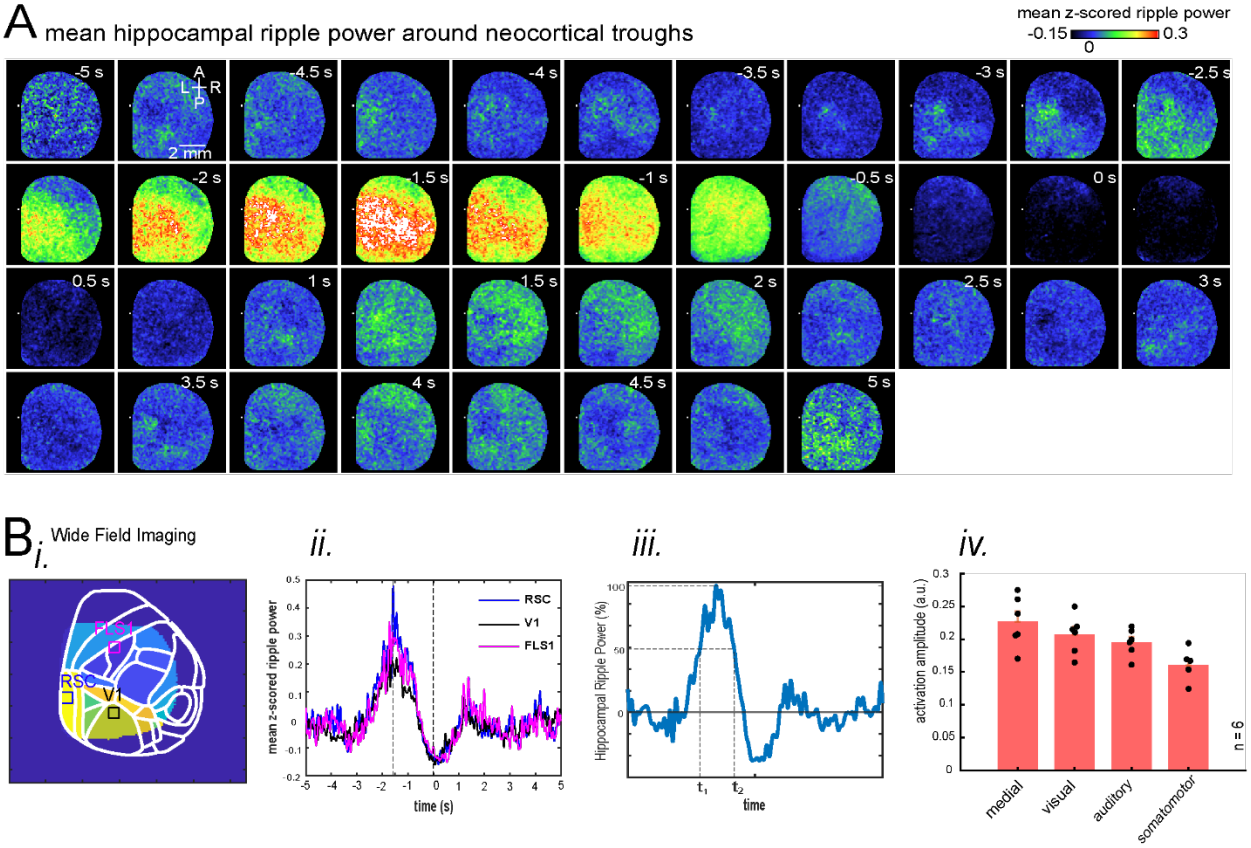

Figure 3.2.1. Ripple power increases prior to troughs of neocortical noradrenergic activity in different neocortical subnetworks. **(A)** Representative montage illustrating spatiotemporal pattern of mean hippocampal ripple power fluctuations around the peak of neocortical deactivations. Zero time shows peak deactivation in each neocortical pixel. **(B) (i)** All ROI coordinates were registered on Allen Mouse Brain Reference Atlas based on anatomical landmarks, the same shown in figure 3.1.1 Bi. Colored squares represent three regions of interest: retrosplenial (blue), visual (black) and forelimb (magenta) somatosensory cortices which their corresponding ripple power traces are displayed in (ii). **(ii)** Mean z-scored ripple power around neocortical troughs. Ripple power is increased ~1.5 seconds before neocortical peak deactivation, and slightly decreased at time of peak deactivation. This result is consistent among all the neocortical regions. 0-s time shows the trough of neocortical

activity in each region. **(iii)** Illustration of how the ripple power amplitude was quantified. Ripple power amplitude was defined as the mean ripple power signal across full with at half maximum (t_1 to t_2). **(iv)** Grand average (n=6) of mean hippocampal ripple power amplitudes across neocortical subnetworks shown in figure 3.1.1.1 C_i, sorted in decreasing order. Each data point represents the average of hippocampal ripple power amplitudes corresponding to all the regions in a given subnetwork and in a given animal. Bar graphs are mean ± SEM.

3.3.Neocortical noradrenergic activity is elevated prior to pupil dilation

It has been suggested that pupil dilation and constriction is linked by the activity of noradrenaline, therefore it's an indicator of release of noradrenaline. But till now, the dynamics of noradrenaline activity in different neocortical regions in mesoscale during this cycle has not been examined. Therefore, using wide-filed imaging, I addressed this question to find the dynamics of neocortical noradrenaline in a dilation/constriction cycle of pupil. To do so, I temporally aligned pupil diameter signal with neocortical activity and averaged frames corresponding to dilation onset. Noradrenergic activity was elevated prior to dilation onsets (preceding the peak of dilation) and was reduced during constriction. At the end of pupil constriction, when the pupil diameter was the smallest, there was another elevation of NE activity. Since in this analysis I have looked at average noradrenergic activity in a time window of three seconds before to three seconds after of pupil dilation onset, the second increase in noradrenergic activity might be due to contamination of next dilation/constriction cycles. In other words, since dilation/constriction cycles have different periods depending on animal's inner behavioral state, in a window of 6 seconds (three seconds before to three seconds after of pupil dilation onset) there might be several dilation/constriction cycles contaminating the average across all dilation onsets. To solve this possible problem, I conducted phase-binning analysis, in which neocortical fluorescent were aligned to cycles of dilation and constriction derived from the Hilbert transform (*Methods*) to avoid contamination.

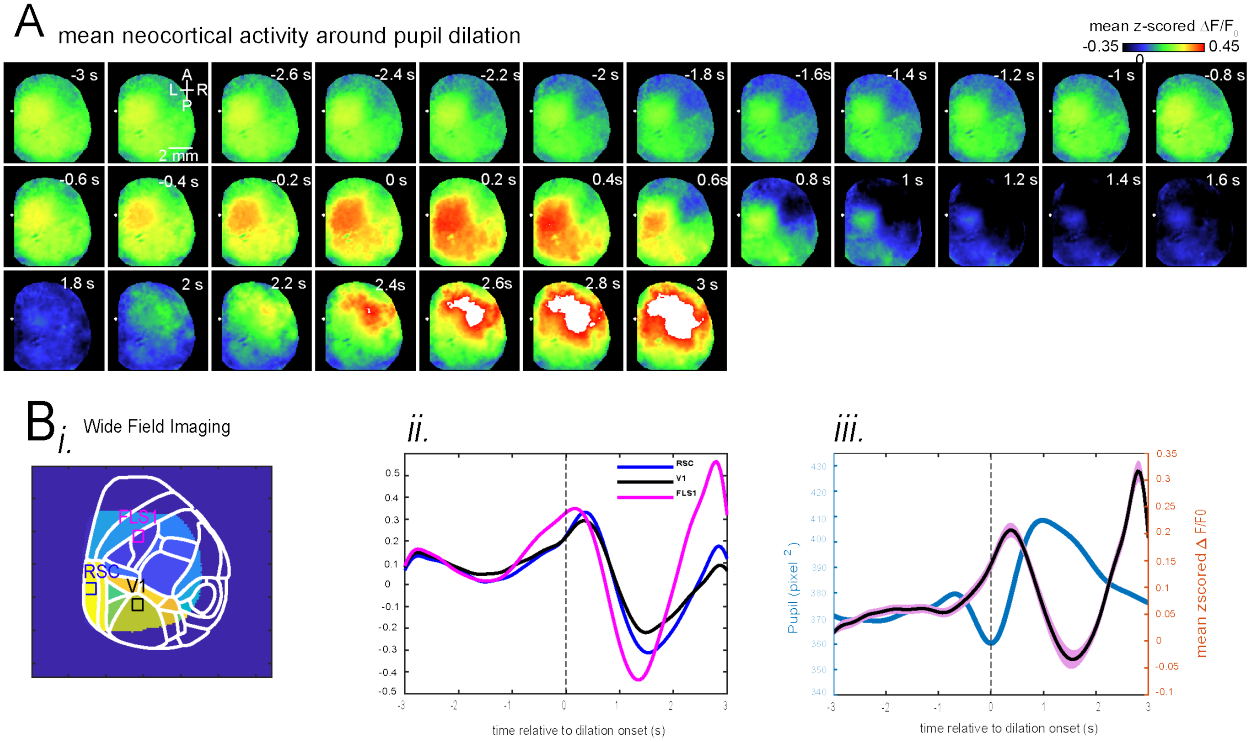

Figure 3.3.1. Neocortical noradrenergic activity is elevated prior to pupil dilation in peri-dilation averaging. (A) Representative montage illustrating spatiotemporal pattern of mean neocortical noradrenergic activity around onset of pupil dilation. Zero time shows dilation onset. **(B) (i)** All ROI coordinates were registered on Allen Mouse Brain Reference Atlas based on anatomical landmarks, the same shown in figure 3.1.1 Bi. Colored squares represent three regions of interest: retrosplenial (blue), visual (black) and forelimb (magenta) somatosensory cortices which their corresponding neocortical traces are displayed in (ii). (iii) Grand average of all neocortical regions in all animals (n=5) shown in magenta and a representative cycle of pupil dilation/constriction (blue). The neocortical trace shows mean ± SEM.

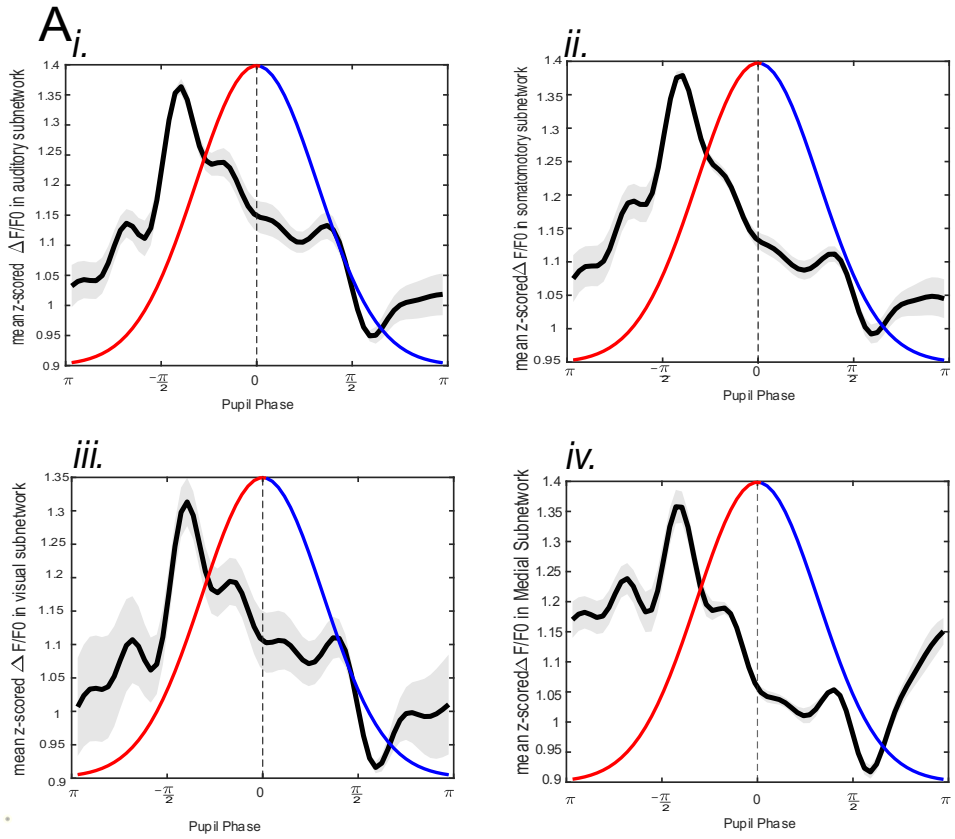

Figure 3.3.2. Neocortical noradrenergic activity is elevated prior to pupil dilation. Neocortical traces in each region were aligned to pupil dilation/constriction cycle; **(i-iv)** Grand average of all neocortical regions in all animals (n=5) divided to four major structurally defined subnetworks (auditory, medial, visual and somatomotory) the same as explained in Figure 3.1.1. Ci. The neocortical traces show mean ± SEM.

These results suggest that, in line with previous result, noradrenaline increases and then decreases in a pupil dilation/constriction cycle, and NE peaks prior to pupil dilation, when pupil phase is in – $\pi/2$ and decreases during pupil constriction, being minimum while pupil phase is $\pi/2$. Neocortical noradrenergic activity preceding the pupil dilation and constriction cycle by an increase and then decrease, respectively, is consistent among all the regions.

4. Discussion

The purpose of this research was to investigate the role of noradrenaline in memory transfer between the hippocampus and the neocortex. Noradrenaline was measured by GCaMP6s sensors expressed in noradrenergic terminals of locus coeruleus in neocortex. Memory transfer was measured by occurrence of sharp wave ripples in CA1 which are presumed to send information to the neocortex. The main funding in this study was that noradrenaline has started to decrease at just that time when memory transfer has been proposed to occur. During this study, it was noticed that the mice would periodically awaken as indicated by whisking and pupil dilation, and at that time noradrenaline was increased. Taken together, these findings suggest that noradrenaline plays a role in arousal but is not essential for memory transfer that might occur. These ideas will be discussed further in the following paragraphs.

In the first part of this study, I've looked at the interactions and timing between neocortical noradrenergic activity from LC terminals and hippocampal sharp wave ripples. Based on Standard Consolidation Theory, memory consolidation needs transferring information from hippocampus to neocortex, which requires changes in synaptic plasticity. Candidate time windows of synaptic plasticity are sharp wave ripples, which provide increased synaptic excitability. Since noradrenaline is also involved in changes in synaptic strength, therefore it should be present at time of ripples, provide neocortical-hippocampal dialogue at time of sharp wave ripples with more synaptic excitability, and therefore play a role in system memory consolidation. In other words, release and existence of noradrenaline throughout the neocortex at time of sharp wave ripples should be important for neocortical-hippocampal communication, and therefore in system memory consolidation (Buzsáki, 1989; Jadhav et al., 2016; Peyrache et al., 2009). Since noradrenaline is suggested to be involved in memory consolidation at cellular and synaptic level, it is important to

investigate the behavior of noradrenaline in system memory consolidation, i.e. in its wide-spread neocortical LC projections during neocortex-hippocampus dialogue, happening during sharp wave ripples. In this study, I addressed this gap by providing a mesoscale spatiotemporal map of peri-SWR dorsal neocortical noradrenergic activity using wide-filed imaging.

In the second part of the study, I've looked at the neocortical noradrenaline activity during pupil dilation and constriction. Wide-spread noradrenergic terminals have long been believed to drive changes in cortical network activity and therefore, modulating behavioral states, indicated by pupil changes (Collins et al., 2023.). Although previous studies have improved our understanding of relationship between release of noradrenaline in neocortex and pupil changes, their conclusions have been limited because of relatively spars spatial sampling due to their approaches. Therefore, this study fills this gap by using a wide-filed recording of neocortex alongside with tracking pupil changes.

4.1. Patterns of activity in noradrenergic neocortical activity are uniformly modulated around hippocampal sharp wave ripple

A deactivation around SWRs was observed across neocortical regions, with no significant difference between subnetworks. The four major structurally defined subnetworks that were accessible to us for imaging are subsets of larger brain-wide networks, especially retrosplenial and posterior parietal cortices are part of a medial subnetwork involved in multiple cognitive processes, most notably memory (Smith et al., 2018; Stafford et al., 2014). Therefore, these results suggest that even though noradrenaline is involved in memory formation (I. N. Izquierdo et al., 1998; Ji et al., 2003; Kitchigina, 1997; Mello-Carpes et al., 2016; Straube et al., 2003), and synaptic memory consolidation, but it decreases in neocortex during sharp wave ripples, and therefor is reduced for

neocortical-hippocampal dialogue in system memory consolidation. These results are not in line with our initial expectation of increase in noradrenaline release at time of sharp wave ripples, but are consistent with previous studies looking at the relationship between noradrenaline and hippocampal sharp wave ripple generation. For example, Segal and Bloom reported that application of noradrenaline leads to a suppression of most hippocampal cells (Segal & Bloom, 1974). Also, electrical LC stimulation has produced long-lasting inhibition of spontaneous activity of pyramidal cells (Segal & Bloom, 1974). Considering that, we can conclude that LC firing and SWR generation do not occur at the same time and LC stops firing at the time of sharp wave ripple generation.

Neocortical-hippocampal dialogue around sharp wave ripples have been studied with focus on other substances and in discrete neocortical regions due to lack of extensive spatial coverage of neocortical mantle. For example, previous studies have reported a neocortical modulation around hippocampal sharp wave ripples, (Abadchi et al., 2020; Pedrosa et al., 2022) with a differential level of activation and deactivation which was correlated with neocortical structural connectivity, i.e., regions with strong axonal interconnections were co-modulated to a similar degree around sharp wave ripples. Other than that, significant peri-SWR modulation of prefrontal cortex has been shown in several studies using electrophysiological methods (Battaglia et al., 2004; Mölle et al., 2006; Peyrache et al., 2009; Peyrache et al., 2011) as well as posterior primary somatosensory (Sirota et al., 2003) but these studies have not simultaneously characterized the differential patterns of activity across all the regions, and have not looked specifically at noradrenaline. Here, this study has expanded upon their findings by using high spatiotemporal resolution wide-filed optical imaging of GCaMP6s calcium sensors in neocortical LC terminals combined with electrophysiology.

One recent study that has focused on behavior of noradrenaline around hippocampal sharp wave ripples, (Novitskaya et al., 2016) has looked at the effects of ripple-associated LC activation on hippocampal and cortical activity in a spatial memory task; They have stimulated LC after an "on-line" detection of ripple while monitoring neural activity, and have reported no effect on spatial learning with a low-frequency (20 Hz) stimulation and a reference memory deficit while stimulating with higher-frequency (100 Hz) stimulations. The higher frequency train of electrical pulses (0.05 mA) have blocked generation of ripple-associated cortical spindles, interfering with hippocampal-cortical communication and thereby reducing the off-line memory consolidation efficiency; This study supports the idea that even though noradrenaline is involved in promoting synaptic consolidation at a cellular and synaptic level, but it is not involved in system memory consolidation, and also in line with our results regarding deactivation of LC around sharp wave ripples, it shows that LC activation interferes coordinated hippocampal-cortical activity. It is worth mentioning that, reported results by Novitskaya et al. could also be interpreted in other ways. The memory deficit is attributed to disruption of coupling between sharp wave ripples and neocortical spindles. But it could be effect of hippocampus stopping generation of sharp wave ripples, not the effect of decoupling.

4.2. Ripple power increases prior to troughs of neocortical activity in different neocortical subnetworks

Ripple power increases 1.5 second before neocortical deactivation and this increase diminishes while the neocortical trough happens; This result is consistent among all the regions of interest, and following the results of previous part mentioned above, it shows existence of a time lag between sharp wave ripples and LC activation/deactivation. This result shows that neocortical

noradrenergic activity decreases while ripple power increases, and this relationship is bidirectional. It is worth mentioning that this analysis does not indicate a causality relationship between neocortical noradrenergic activity and hippocampal sharp wave ripple generation, rather it is a report of observation. Underlying reason of this observation could be timing of noradrenaline release in hippocampus and neocortex, conducted by locus coeruleus, as it projects to both neocortex and hippocampus and is the main source providing both structures with noradrenaline. Also, the absolute value of reported time lag of 1.5 second does not represent a precise timing between LC activity and sharp wave ripple generation, as the GCaMP6s sensor used in this study is a slow sensor and its kinematics could account for the delay. To our knowledge, there is no other study looking specifically at the relationship between timing of noradrenaline release in neocortex and sharp wave ripple power. But using EEG, previous studies have reported an association between occurrence of hippocampal sharp wave ripples and peak activity of medial prefrontal cortex EEG (Mölle et al., 2006). Additionally, using genetically encoded sensor of extracellular glutamate (iGluSnFR) and voltage sensitive dye imaging, Abadchi et al., have found positive correlation between ripple power and peak activity in different neocortical subnetworks (Abadchi et al., 2020). Another analysis for future works that would help address this question would be analysis of ripple power around neocortical noradrenergic peak activations. Based on current results, we expect ripple power to decrease in relation to neocortical noradrenergic peak activation.

4.3. Neocortical noradrenergic activity is elevated prior to pupil dilation

A similar pattern to pupil dilation/constriction cycle was observed in neocortical noradrenaline activity, i.e., increasing and decreasing, but prior to pupil dilation and constriction. Reimer et. al have also reported that noradrenaline activity showed a large peak in cross-correlation with pupil,

and the time of the peak preceded pupil dilation (Reimer et. al, 2016). Previous studies have reported a rise in fluorescence of LC axons in visual cortex by eye pupil dilation (Larsen & Waters, 2018) suggesting pupil changes as an indicator of noradrenaline release (Collins et al., 2023). The mouse neocortex receives axonal projections from noradrenergic neuromodulatory neurons that drive changes in cortical network activity and therefore, accompany a shift in behavioral states, observable by pupil changes. Collins et al. have also reported that rapid variations in pupil size during both quiet wakefulness and locomotion are highly correlated with fluctuations in the activity of corticopetal cholinergic and noradrenergic projections (Collins et al., 2023.). They have also shown that, rapid pupil dilations are associated with phasic activity in noradrenergic axons. Other studies also support the idea that pupil size can be a reliable indicator of the locus coeruleus (LC) activity (Joshi et al., 2016; McDougal & Gamlin, 2015) and cortical state is controlled by the release of noradrenaline (Aston-Jones & Cohen, 2005; Berridge & Waterhouse, 2003).

In this study, peri-dilation neocortical activity was consistent with previous reports, but after pupil constriction we observed an elevation in noradrenergic activity. In this analysis, I have looked at the neocortical activity at the time window of three seconds before to three seconds after pupil dilation. In this time window, there might be several pupil dilation/constriction cycles depending on the animal's inner state. Therefore, this second increase in noradrenaline activity might be due to overlap with next cycles. A follow-up analysis of phase-binning neocortical traces solved this issue, while supporting the observation that neocortical noradrenergic peak activity occurs prior to peak pupil dilation. Considering this time lag, in contrast to previous studies suggesting pupil changes as an indicator of noradrenaline release (Collins et al., 2023), we can conclude that since pupil fluctuations are not controlled solely by noradrenaline, therefore it is not

a good indicator of noradrenaline release. Neuromodulatory systems underlying pupil fluctuations are yet to be understood.

McGinley et. al, (2015) have compared the pupil diameter to the rate of fast hippocampal ripples during spontaneous state changes in the absence of sensory stimulation (McGinley et. al, 2015) and have reported a strong inverse relationship of pupil diameter to the rate of fast ripple occurrences. This study links the gaps in present thesis in an indirect way, shining light on timing of noradrenaline activity, ripple occurrence and indirectly pupil fluctuation. But for the future works, replicating this analysis to investigate the relationship between pupil fluctuation and rate of ripples would be a matter of paramount importance.

5. Conclusion

Based on two-stage model of memory formation (Buzsaki 1989) neural representations of past experiences formed during acquisition reactivate in the hippocampus, mainly during sharp wave ripples. In other words, replay of recent hippocampal patterns is concentrated in sharp wave ripple bursts. This propagation of sharp wave ripples to neocortex could broadcast stored information to the downstream neocortical regions. Thus, a coordinated dynamic interaction between hippocampus and neocortex is predicted around sharp wave ripples. Noradrenaline, as a neuromodulator, is a great candidate for mediating memory (I. N. Izquierdo et al., 1998; Ji et al., 2003; Kitchigina, 1997; Mello-Carpes et al., 2016; Straube et al., 2003) especially during consolidation. Studies looking directly at noradrenaline involvement in memory consolidation related to neocortical-hippocampal dialogue have been limited.

Answer to this question was the main driver for the current thesis work. In this study, the dynamics of noradrenergic neocortical-hippocampal interactions around sharp wave ripples were examined. We used wide-filed calcium imaging of GCaMP6s sensors expressed in LC terminals in the neocortex to address the activity dynamics of noradrenaline in a large portion of the dorsal neocortex, while recording the hippocampal local filed potential (LFP) obtained from the dorsal CA1. This setup allowed us to address our question by providing a mesoscale peri-SWR spatiotemporal map of neocortical noradrenergic activity. SWR-triggered averaging revealed a uniform deactivation of noradrenaline among all regions of neocortex; Next, it was confirmed that this relationship between neocortical noradrenergic troughs and ripple power goes both ways, and neocortical noradrenergic peak deactivation averaging of hippocampal sharp wave ripples revealed an increase in ripple power prior to neocortical noradrenergic trough and a slight decrease at time of trough. These results suggest that sharp wave ripples and LC activation do not occur at the same

time, and LC is silent during ripple occurrence. How ever, since we looked indirectly at LC activity by recording its terminals in the neocortex, confirming this idea requires further investigation by directly recording LC activity to maintain a better temporal resolution. However, this idea is consistent with previous works reporting a depression in hippocampal cells firing rate with application of noradrenaline (Segal & Bloom, 1974) and also high frequency LC stimulation during "on-line" detection of sharp wave ripple has resulted in memory deficit by blocking generation of ripple-associated cortical spindles, interfering with hippocampal-cortical dialogue (Novitskaya et al., 2016). It remains unclear as to why the peri-SWR neocortical noradrenaline activity is suppressed during hippocampal sharp wave ripples, and how noradrenaline effects generation of sharp wave ripples. Our findings may be used to understand how noradrenaline is involved in synaptic and systems memory consolidation.

In the next part of the study, neocortical noradrenaline activity was examined during pupil fluctuation. We observed that noradrenaline activity precedes pupil dilation. This time lag between noradrenaline peak activity and peak pupil dilation suggests that pupil fluctuation might not be a good indicator of noradrenaline release, while previous studies suggest otherwise (Collins et al 2023.)

References

Abadchi, J. K., Nazari-Ahangarkolaee, M., Gattas, S., Bermudez-Contreras, E., Luczak, A., McNaughton, B. L., & Mohajerani, M. H. (2020). Spatiotemporal patterns of neocortical activity around hippocampal sharp-wave ripples. *ELife*, *9*. https://doi.org/10.7554/eLife.51972

Abadchi, J. K., Rezaei, Z., Knöpfel, T., McNaughton, B. L., & Mohajerani, M. H. (2023). Inhibition is a prevalent mode of activity in the neocortex around awake hippocampal ripples in mice. *eLife*, *12*, e79513. https://doi.org/10.7554/eLife.79513

Agster, K. L., Mejias-Aponte, C. A., Clark, B. D., & Waterhouse, B. D. (2013). Evidence for a regional specificity in the density and distribution of noradrenergic varicosities in rat cortex. *Journal of Comparative Neurology*, *521*(10), 2195–2207. https://doi.org/10.1002/cne.23270

Aston-Jones, G., & Bloom, F. E. (1981). ACTIVITY OF NOREPINEPHRINE-CONTAINING LOCUS COERULEUS NEURONS IN BEHAVING RATS ANTICIPATES FLUCTUATIONS IN THE SLEEP-WAKING CYCLE'. In *The Journal of Neuroscience Copyrtght 0 Society for Neuroscience Printed in U.S.A* (Vol. 1, Issue 8).

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: Adaptive gain and optimal performance. In *Annual Review of Neuroscience* (Vol. 28, pp. 403–450). https://doi.org/10.1146/annurev.neuro.28.061604.135709

Aston-jones, G., Ennis, Mat., Pieribone, V. A., Thompson Nickell, W., & Shipley, M. T. (1984). The Brain Nucleus Locus Coeruleus: Restricted Afferent Control of a Broad Efferent Network. In *Proc. Natl. Acad. Sci. U.S.A* (Vol. 36). www.sciencemag.org

Aston-Jones, G., & Waterhouse, B. (2016). Locus coeruleus: From global projection system to adaptive regulation of behavior. In *Brain Research* (Vol. 1645, pp. 75–78). Elsevier B.V. https://doi.org/10.1016/j.brainres.2016.03.001

Battaglia, F. P., Sutherland, G. R., & McNaughton, B. L. (2004a). Hippocampal sharp wave bursts coincide with neocortical "up-state" transitions. *Learning and Memory*, *11*(6), 697–704. https://doi.org/10.1101/lm.73504

Battaglia, F. P., Sutherland, G. R., & McNaughton, B. L. (2004b). Hippocampal sharp wave bursts coincide with neocortical "up-state" transitions. *Learning and Memory*, *11*(6), 697–704. https://doi.org/10.1101/lm.73504

Benarroch, E. E. (2018). Locus coeruleus. In *Cell and Tissue Research* (Vol. 373, Issue 1, pp. 221–232). Springer Verlag. https://doi.org/10.1007/s00441-017-2649-1

Bermudez-Contreras, E., Chekhov, S., Sun, J., Tarnowsky, J., McNaughton, B. L., & Mohajerani, M. H. (2018). High-performance, inexpensive setup for simultaneous multisite recording of

electrophysiological signals and mesoscale voltage imaging in the mouse cortex. *Neurophotonics*, *5*(2), 025005. https://doi.org/10.1117/1.NPh.5.2.025005

Berridge, C. W., & Foote, S. L. (1991). Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *11*(10), 3135–3145. https://doi.org/10.1523/JNEUROSCI.11-10-03135.1991

Berridge, C. W. (2008). Noradrenergic modulation of arousal. In *Brain Research Reviews* (Vol. 58, Issue 1, pp. 1–17). https://doi.org/10.1016/j.brainresrev.2007.10.013

Berridge, C. W., & Abercrombie, E. D. (1999). RELATIONSHIP BETWEEN LOCUS COERULEUS DISCHARGE RATES AND RATES OF NOREPINEPHRINE RELEASE WITHIN NEOCORTEX AS ASSESSED BY IN VIVO MICRODIALYSIS. In *Neuroscience* (Vol. 93, Issue 4).

Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. In *Brain Research Reviews* (Vol. 42, Issue 1, pp. 33–84). Elsevier. https://doi.org/10.1016/S0165-0173(03)00143-7

Boehm S. (1999). Presynaptic alpha2-adrenoceptors control excitatory, but not inhibitory, transmission at rat hippocampal synapses. *The Journal of Physiology*, *519 Pt 2*, 439–449.

Bragin, A., Engel, J., Wilson, C. L., Fried, I., & Buzsáki, G. (1999). High-frequency oscillations in human brain. *Hippocampus*, *9*(2), 137–142. https://doi.org/10.1002/(SICI)1098-1063(1999)9:2<137::AID-HIPO5>3.0.CO;2-0

Breton-Provencher, V., & Sur, M. (2019). Active control of arousal by a locus coeruleus GABAergic circuit. Nature neuroscience, 22(2), 218–228. https://doi.org/10.1038/s41593-018-0305-z

Broussard, G. J., Liang, Y., Fridman, M., Unger, E. K., Meng, G., Xiao, X., Ji, N., Petreanu, L., & Tian, L. (2018). In vivo measurement of afferent activity with axon-specific calcium imaging. Nature neuroscience, 21(9), 1272–1280. https://doi.org/10.1038/s41593-018-0211-4

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E., & McCarley, R. W. (2012). Control of sleep and wakefulness. In *Physiological Reviews* (Vol. 92, Issue 3, pp. 1087–1187). https://doi.org/10.1152/physrev.00032.2011

Buzsáki G. (1989). Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience*, *31*(3), 551–570.

Buzsáki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus*, *25*(10), 1073–1188. https://doi.org/10.1002/hipo.22488

Buzsáki G., G. (1986). *Hippocampal Sharp Waves: Their Origin and Significance*.

Carter, M. E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., Deisseroth, K., & de Lecea, L. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nature neuroscience, 13(12), 1526–1533. https://doi.org/10.1038/nn.2682

Chandler, D. J. (2016). Evidence for a specialized role of the locus coeruleus noradrenergic system in cortical circuitries and behavioral operations. In *Brain Research* (Vol. 1641, pp. 197–206). Elsevier B.V. https://doi.org/10.1016/j.brainres.2015.11.022

Collingridge, G. L., Peineau, S., Howland, J. G., & Wang, Y. T. (2010). Long-term depression in the CNS. In *Nature Reviews Neuroscience* (Vol. 11, Issue 7, pp. 459–473). https://doi.org/10.1038/nrn2867

Collins, L., Francis, J., Emanuel, B., & Mccormick, D. A. (n.d.). *Cholinergic and noradrenergic axonal activity contains a behavioral-state signal that is coordinated across the dorsal cortex*. https://doi.org/10.7554/eLife

Chrobak, J. J., & Buzsáki, G. (1996). High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *16*(9), 3056–3066. https://doi.org/10.1523/JNEUROSCI.16-09-03056.1996

Devilbiss, D. M., & Waterhouse, B. D. (2011). Phasic and tonic patterns of locus coeruleus output differentially modulate sensory network function in the awake rat. *Journal of Neurophysiology*, *105*(1), 69–87. https://doi.org/10.1152/jn.00445.2010

Foote, S. L., Bloom, F. E., Aston-Jones, G., & Vining, A. (1983). Nucleus Locus Ceruleus: New Evidence of Anatomical and Physiological Specificity. In *PHYSIOLOGICAL REVIEWS* (Vol. 63, Issue 3).

Gibbs, M. E., Hutchinson, D. S., & Summers, R. J. (2010). Noradrenaline release in the locus coeruleus modulates memory formation and consolidation; roles for α- and β-adrenergic receptors. Neuroscience, 170(4), 1209–1222. https://doi.org/10.1016/j.neuroscience.2010.07.052

Hebb, D. O. (1949). *The organization of behavior; a neuropsychological theory.* Wiley.

Hobson, J. A., McCarley, R. W., & Wyzinski, P. W. (1975). Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. Science (New York, N.Y.), 189(4196), 55–58. https://doi.org/10.1126/science.1094539

Izquierdo, I., & Medina, J. H. (1997). Memory Formation: The Sequence of Biochemical Events in the Hippocampus and Its Connection to Activity in Other Brain Structures. In *NEUROBIOLOGY OF LEARNING AND MEMORY* (Vol. 68).

Izquierdo, I. N., Medina, J. H., Izquierdo, L. A., Barros, D. M., De Souza, M. M., & Mello E Souza, T. (1998). RAPID COMMUNICATION Short-and Long-Term Memory Are Differentially Regulated by Monoaminergic Systems in the Rat Brain. In *NEUROBIOLOGY OF LEARNING AND MEMORY* (Vol. 69).

Jadhav, S. P. P., Rothschild, G., Roumis, D. K. K., & Frank, L. M. M. (2016). Coordinated Excitation and Inhibition of Prefrontal Ensembles during Awake Hippocampal Sharp-Wave Ripple Events. *Neuron*, *90*(1), 113–127. https://doi.org/10.1016/j.neuron.2016.02.010

Ji, J. Z., Zhang, X. H., & Li, B. M. (2003). Deficient Spatial Memory Induced by Blockade of Beta-Adrenoceptors in the Hippocampal CA1 Region. *Behavioral Neuroscience*, *117*(6), 1378–1384. https://doi.org/10.1037/0735-7044.117.6.1378

Joshi, S., Li, Y., Kalwani, R. M., & Gold, J. I. (2016). Relationships between Pupil Diameter and Neuronal Activity in the Locus Coeruleus, Colliculi, and Cingulate Cortex. *Neuron*, *89*(1), 221–234. https://doi.org/10.1016/j.neuron.2015.11.028

Kandel E. R. (2001). The molecular biology of memory storage: a dialogue between genes and synapses. *Science (New York, N.Y.)*, 1030–1038.

Katsuki, H. , I. Y. , & Z. C. F. (1997). Noradrenergic regulation of synaptic plasticity in the hippocampal CA1 region. *Journal of Neurophysiology77(6)*, 3013–3020.

Kitchigina, V. , V. A. , H. C. , & S. S. J. (1997). Novelty-elicited, noradrenaline-dependent enhancement of excitability in the dentate gyrus. *The European Journal of Neuroscience*, *9*(1), 41–47.

Kjaerby, C., Andersen, M., Hauglund, N., Untiet, V., Dall, C., Sigurdsson, B., Ding, F., Feng, J., Li, Y., Weikop, P., Hirase, H., & Nedergaard, M. (2022). Memory-enhancing properties of sleep depend on the oscillatory amplitude of norepinephrine. *Nature neuroscience*, *25*(8), 1059–1070. https://doi.org/10.1038/s41593-022-01102-9

Klinzing, J. G., Niethard, N., & Born, J. (2019). Mechanisms of systems memory consolidation during sleep. In *Nature Neuroscience* (Vol. 22, Issue 10, pp. 1598–1610). Nature Publishing Group. https://doi.org/10.1038/s41593-019-0467-3

Kyweriga, M., Sun, J., Wang, S., Kline, R., & Mohajerani, M. H. (2017). A Large Lateral Craniotomy Procedure for Mesoscale Wide-field Optical Imaging of Brain Activity. *Journal of visualized experiments : JoVE*, (123), 52642. https://doi.org/10.3791/52642

Larsen, R. S., & Waters, J. (2018). Neuromodulatory correlates of pupil dilation. In *Frontiers in Neural Circuits* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fncir.2018.00021

Lechner, H. A., Squire, L. R., Byrne, J. H., & Keck, W. M. (1999). *00 Years of Consolidation-Remembering Müller and Pilzecker*.

Lemon, N., Aydin-Abidin, S., Funke, K., & Manahan-Vaughan, D. (2009). Locus coeruleus activation facilitates memory encoding and induces hippocampal LTD that depends on β-Adrenergic receptor activation. *Cerebral Cortex*, *19*(12), 2827–2837. https://doi.org/10.1093/cercor/bhp065

Logothetis, N. K., Eschenko, O., Murayama, Y., Augath, M., Steudel, T., Evrard, H. C., Besserve, M., & Oeltermann, A. (2012). Hippocampal-cortical interaction during periods of subcortical silence. *Nature*, *491*(7425), 547–553. https://doi.org/10.1038/nature11618

Loughlin, S. E., Foora, S. L., & Grzanna~, R. (1986). EFFERENT PROJECTIONS OF NUCLEUS LOCUS COERULEUS: MORPHOLOGIC SUBPOPULATIONS HAVE DIFFERENT EFFERENT TARGETS. In *Neuroscience* (Vol. 18, Issue 2).

Loughlin, S. E., Foote, S. L., Fallon, J. H., Foote, S. L., & FALLON Locus, J. H. (1982). Locus Coeruleus Projections to Cortex: Topography, Morphology and Collateralization. In *Brain Reseurrh Bulletin* (Vol. 9).

Maity, S., Abbaspour, R., Nahabedian, D., & Connor, S. A. (2022). Norepinephrine, beyond the Synapse: Coordinating Epigenetic Codes for Memory. In *International Journal of Molecular Sciences* (Vol. 23, Issue 17). MDPI. https://doi.org/10.3390/ijms23179916

Marzo, A., Bai, J., & Otani, S. (2009). Neuroplasticity Regulation by Noradrenaline in Mammalian Brain. In *Current Neuropharmacology* (Vol. 7).

McDougal, D. H., & Gamlin, P. D. (2015). Autonomic control of the eye. *Comprehensive Physiology*, *5*(1), 439–473. https://doi.org/10.1002/cphy.c140014

McGinley, M. J., Vinck, M., Reimer, J., Batista-Brito, R., Zagha, E., Cadwell, C. R., Tolias, A. S., Cardin, J. A., & McCormick, D. A. (2015). Waking State: Rapid Variations Modulate Neural and Behavioral Responses. Neuron, 87(6), 1143–1161. https://doi.org/10.1016/j.neuron.2015.09.012

McKinney, A., Hu, M., Hoskins, A., Mohammadyar, A., Naeem, N., Jing, J., Patel, S. S., Sheth, B. R., & Jiang, X. (2023). Cellular composition and circuit organization of the locus coeruleus of adult mice. *ELife*, *12*. https://doi.org/10.7554/eLife.80100

Mello-Carpes, P. B., da Silva de Vargas, L., Gayer, M. C., Roehrs, R., & Izquierdo, I. (2016). Hippocampal noradrenergic activation is necessary for object recognition memory

consolidation and can promote BDNF increase and memory persistence. *Neurobiology of Learning and Memory*, *127*, 84–92. https://doi.org/10.1016/j.nlm.2015.11.014

Mohajerani, M. H., McVea, D. A., Fingas, M., & Murphy, T. H. (2010). Mirrored bilateral slow-wave cortical activity within local circuits revealed by fast bihemispheric voltage-sensitive dye imaging in anesthetized and awake mice. The Journal of neuroscience: the official journal of the Society for Neuroscience, 30(10), 3745–3751. https://doi.org/10.1523/JNEUROSCI.6437-09.2010

Mohajerani, M. H., Chan, A. W., Mohsenvand, M., LeDue, J., Liu, R., McVea, D. A., Boyd, J. D., Wang, Y. T., Reimers, M., & Murphy, T. H. (2013). Spontaneous cortical activity alternates between motifs defined by regional axonal projections. Nature neuroscience, 16(10), 1426–1435. https://doi.org/10.1038/nn.3499

Mölle, M., Yeshenko, O., Marshall, L., Sara, S. J., & Born, J. (2006). Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. *Journal of Neurophysiology*, *96*(1), 62–70. https://doi.org/10.1152/jn.00014.2006

Müller, G. E. & P. A. (1900). *Experimentelle Beiträge Zur Lehre Vom Gedächtniss*.

Nazari, M., Karimi Abadchi, J., Naghizadeh, M., Bermudez-Contreras, E. J., McNaughton, B. L., Tatsuno, M., & Mohajerani, M. H. (2023). Regional variation in cholinergic terminal activity determines the non-uniform occurrence of cortical slow waves during REM sleep in mice. *Cell reports*, *42*(5), 112450. https://doi.org/10.1016/j.celrep.2023.112450

Nguyen, P. V., & Connor, S. A. (2019). Noradrenergic Regulation of Hippocampus-Dependent Memory. *Central Nervous System Agents in Medicinal Chemistry*, *19*(3), 187–196. https://doi.org/10.2174/1871524919666190719163632

Novitskaya, Y., Sara, S. J., Logothetis, N. K., & Eschenko, O. (2016). Ripple-triggered stimulation of the locus coeruleus during post-learning sleep disrupts ripple/spindle coupling and impairs memory consolidation. *Learning and Memory*, *23*(5), 238–248. https://doi.org/10.1101/lm.040923.115

Pedrosa, R., Nazari, M., Mohajerani, M. H., Knöpfel, T., Stella, F., & Battaglia, F. P. (2022). Hippocampal gamma and sharp wave/ripples mediate bidirectional interactions with cortical networks during sleep. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(44), e2204959119. https://doi.org/10.1073/pnas.2204959119

Peyrache, A., Battaglia, F. P., & Destexhe, A. (2011). Inhibition recruitment in prefrontal cortex during sleep spindles and gating of hippocampal inputs. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(41), 17207–17212. https://doi.org/10.1073/pnas.1103612108

Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009a). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature Neuro-Science*, *12*(7). https://doi.org/10.1038/nn.2337ï

Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009b). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature Neuro-Science*, *12*(7). https://doi.org/10.1038/nn.2337ï

Poe, G. R., Foote, S., Eschenko, O., Johansen, J. P., Bouret, S., Aston-Jones, G., Harley, C. W., Manahan-Vaughan, D., Weinshenker, D., Valentino, R., Berridge, C., Chandler, D. J., Waterhouse, B., & Sara, S. J. (2020). Locus coeruleus: a new look at the blue spot. In *Nature Reviews Neuroscience* (Vol. 21, Issue 11, pp. 644–659). Nature Research. https://doi.org/10.1038/s41583-020-0360-9

Raymond, J. R. (n.d.). *Multiple mechanisms of receptor-G protein signaling specificity*.

Reimer, J., Froudarakis, E., Cadwell, C. R., Yatsenko, D., Denfield, G. H., & Tolias, A. S. (2014). Pupil fluctuations track fast switching of cortical states during quiet wakefulness. *Neuron*, *84*(2), 355–362. https://doi.org/10.1016/j.neuron.2014.09.033

Reimer, J., McGinley, M. J., Liu, Y., Rodenkirch, C., Wang, Q., McCormick, D. A., & Tolias, A. S. (2016). Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. Nature communications, 7, 13289. https://doi.org/10.1038/ncomms13289

Saar, D., & Barkai, E. (2003). Long-term modifications in intrinsic neuronal properties and rule learning in rats. *The European journal of neuroscience*, *17*(12), 2727–2734. https://doi.org/10.1046/j.1460-9568.2003.02699.x

Sara, S. J., & Bouret, S. (2012). Orienting and Reorienting: The Locus Coeruleus Mediates Cognition through Arousal. In *Neuron* (Vol. 76, Issue 1, pp. 130–141). https://doi.org/10.1016/j.neuron.2012.09.011

Sara, S. J., Roullet, P., & Przybyslawski, J. (1999). *Consolidation of Memory for Odor-Reward Association:-Adrenergic Receptor Involvement in the Late Phase*.

Schwarz, L. A., & Luo, L. (2015). Organization of the locus coeruleus-norepinephrine system. In *Current Biology* (Vol. 25, Issue 21, pp. R1051–R1056). Cell Press. https://doi.org/10.1016/j.cub.2015.09.039

Scoville, W., Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery, and Psychiatry, 20, 11–21.

Segal, M., & Bloom, F. E. (1976). The action of norepinephrine in the rat hippocampus. IV. The effects of locus coeruleus stimulation on evoked hippocampal unit activity. Brain research, 107(3), 513–525. https://doi.org/10.1016/0006-8993(76)90141-4

Séguéla, P., Watkins, K. C., & Descarries, L. (1989). Ultrastructural relationships of serotonin axon terminals in the cerebral cortex of the adult rat. The Journal of comparative neurology, 289(1), 129–142. https://doi.org/10.1002/cne.902890111

Séguéla, P., Watkins, K. C., Geffard, M., & Descarries, L. (1990). Noradrenaline axon terminals in adult rat neocortex: an immunocytochemical analysis in serial thin sections. Neuroscience, 35(2), 249–264. https://doi.org/10.1016/0306-4522(90)90079-j

Sirota, A., Csicsvari, J., Buhl, D., & Rgy Buzsá, G. (2003). *Communication between neocortex and hippocampus during sleep in rodents*. https://www.pnas.org

Smith, V., Mitchell, D. J., & Duncan, J. (2018). Role of the default mode network in cognitive transitions. *Cerebral Cortex*, *28*(10), 3685–3696. https://doi.org/10.1093/cercor/bhy167

Stafford, J. M., Jarrett, B. R., Miranda-Dominguez, O., Mills, B. D., Cain, N., Mihalas, S., Lahvis, G. P., Lattal, K. M., Mitchell, S. H., David, S. V., Fryer, J. D., Nigg, J. T., & Fair, D. A. (2014). Large-scale topology and the default mode network in the mouse connectome. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(52), 18745–18750. https://doi.org/10.1073/pnas.1404346111

Straube, T., Korz, V., Balschun, D., & Frey, J. U. (2003). Requirement of β-adrenergic receptor activation and protein synthesis for LTP-reinforcement by novelty in rat dentate gyrus. *Journal of Physiology*, *552*(3), 953–960. https://doi.org/10.1113/jphysiol.2003.049452

Squire, L.R. (1992). Memory and the hippocampal region: A synthesis of findings with rats, monkeys, and humans. Psychological Review, 99, 195–231.

Squire, L.R., Alvarez, P. (1995). Retrograde amnesia and memory consolidation: A neurobiological perspective. Current Opinion in Neurobiology, 5, 169–177.

Squire, L.R., Bayley, P.J. (2007). The neuroscience of remote memory. Current Opinion in Neurobiology, 17, 185–196.

Swanson, L. W. (1976). THE LOCUS COERULEUS: A CYTOARCH1TECTONIC, GOLGI AND IMMUNOHISTOCHEMICAL STUDY IN THE ALBINO RAT. In *Brain Research* (Vol. 110).

Szabadi, E. (2013). Functional neuroanatomy of the central noradrenergic system. In *Journal of Psychopharmacology* (Vol. 27, Issue 8, pp. 659–693). https://doi.org/10.1177/0269881113490326

Thomas, S. A., & Palmiter, R. D. (1997). Disruption of the Dopamine p-Hydroxylase Gene in Mice Suggests Roles for Norepinephrine in Motor Function, Learning, and Memory. In *Behavioral Neuroscience* (Vol. 3).

Vazey, E. M., Moorman, D. E., & Aston-Jones, G. (2018). Phasic locus coeruleus activity regulates cortical encoding of salience information. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(40), E9439–E9448. https://doi.org/10.1073/pnas.1803716115

Zhang, W., & Linden, D. J. (2003). The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nature reviews. Neuroscience*, *4*(11), 885–900. https://doi.org/10.1038/nrn1248