ENHANCING MEMORY-RELATED SLEEP SPINDLES THROUGH LEARNING AND ELECTRICAL BRAIN STIMULATION

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ABSTRACT

Sleep has been strongly implicated in mediating memory consolidation through hippocampal-neocortical communication. Evidence suggests offline processing of encoded information in the brain during slow wave sleep (SWS), specifically during slow oscillations and spindles. In this work, we used active exploration and learning tasks to study post-experience sleep spindle density changes in rats. Experiences lead to subsequent changes in sleep spindles, but the strength and timing of the effect was task-dependent. Brain stimulation in humans and rats have been shown to enhance memory consolidation. However, the exact stimulation parameters which lead to the strongest memory enhancement have not been fully explored. We tested the efficacy of both cortical sinusoidal direct current stimulation and intracortical pulse stimulation to enhance slow oscillations and spindle density. Pulse stimulation reliably evoked state-dependent slow oscillation and spindles during SWS with increased hippocampal ripple-spindle coupling, demonstrating potential in memory enhancement.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC	Alternating current
ACC	Anterior cingulate cortex
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	Brain-derived Neurotrophic factor
CA	Cornu Ammonis
CS	Conditioned stimulus
CT	Central Thalamic
DBS	Deep Brain Stimulation
DC	Direct current
DLPFC	Dorsolateral prefrontal cortex
EC	Entorhinal cortex
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
EPSP	Excitatory post synaptic potential
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma aminobutyric acid
GC	Gluco-corticoid
HC	Home cage control
HF	High frequency
HPA	Hypothalamus-pituitary-adrenal
HS	Head-stage
IAF	Individual alpha frequency
IEG	Immediate early genes
IPSP	Inhibitory post synaptic potential
KC	k-complex
LFP	Local field potential
LIA	Large irregular activity
LTD	Long-term depression
LTM	Long-term memory
LTP	Long-term potentiation
LVFA	Low voltage fast activity
MQ	Memory quotient
MRI	Magnetic Resonance Imaging
MS-DBB	Medial septum/diagonal band of Broca
MTL	Medial temporal lobe
MTT	Multiple trace theory
NMDA	N-methyl-D-aspartate
NREM	Non rapid eye movement
NREMS	Non rapid eye movement sleep

OSRP	Orientation-specific response potentiation
PBS	Phosphate buffer saline
PCB	Printed circuit board
PET	Positron Emission Tomography
PFC	Prefrontal cortex
PGO	Ponto-Geniculo-Occipital
PKA	Protein kinase A
PKC	Protein kinase C
PSTH	Peristimulus time histograms
PTSD	Post-traumatic stress disorder
RA	Retrograde amnesia
REM	Rapid eye movement
REMS	Rapid eye movement sleep
RSA	Rhythmical slow activity
SC	Subcutaneous
SD	Standard deviation
SEM	Standard error of mean
SMSC	Standard model for systems consolidation
SO	Slow oscillations
SO-tDCs	Slow-oscillatory transcranial direct current stimulation
STDP	Spike timing-dependent plasticity
STM	Short term memory
STN	Sub-thalamic nucleus
STP	Short-term potentiation
SWA	Slow-wave activity
SWR	Sharp-wave ripples
SWS	Slow-wave sleep
tACs	Transcranial alternation current stimulation
tDCs	Transcranial direct current stimulation
tPNs	Transcranial pink noise stimulation
tRNs	Transcranial random noise stimulation
TBI	Traumatic brain injury
TC	Thalamocortical
TES	Transcranial electrical stimulation
TMR	Targeted memory reactivation
TMS	Transcranial Magnetic Stimulation
TRN	Reticular thalamic neurons
UDS	UP-DOWN State
US	Unconditioned stimulus
VSDI	Voltage-sensitive dye imaging

Chapter 1 General Introduction (Literature review)

1.1 Memory

Memory is one of the primordial aspects of life on this planet. From cellular and collective memory in bacteria, to sophisticated cognitive information-processing circuitry in animals including humans, memories are biologically important from an evolutionary perspective. The interaction of a living organism with the environment is based on the inputs through sensory perceptions leading to a behaviour, an expression of processed information. Fundamentally, memory is a function of time, meaning that information about past experiences is stored and can be recalled, a link between experiences and behaviour. Given the significance of memory, it is not surprising that it has caught the attention of philosophers and scientists alike:

> *"Memory is the scribe of the soul."*
> - Aristotle, Ancient Greek philosopher.

> *"You have to begin to lose your memory, if only in bits and pieces, to realize that memory is what makes our lives. Life without memory is no life at all, just as an intelligence without the possibility of expression is not really an intelligence. Our memory is our coherence, our reason, our feeling, even our action. Without it, we are nothing."*
> - Luis Buñuel, Spanish filmmaker (Originally published in France as Mon Dernier Soupir by Editions Robert Laffont 1982; Translation 'My Last Sigh' by Alfred A Knopf 1983; 'My Last Breath', translated by Abigail Israel, 1994).

> *"Has it ever struck you ... that life is all memory, except for the one present moment that goes by you so quick you hardly catch it going?"*
> - Tennessee Williams, American playwright and screenwriter, from the play 'The Milk Train Doesn't Stop Here Anymore'.

> *"One of the most remarkable aspects of an animal's behavior is the ability to modify that behavior by learning, an ability that reaches its highest form in human beings. For me, learning and memory have proven to be endlessly fascinating mental processes because they address one of the fundamental features of human activity: our ability to acquire new ideas from experience and to retain these ideas in memory."*
> - Eric Kandel, Neuroscientist; Nobel Prize Lecture, 2001 (E. R. Kandel, 2001a, 2001b).

For humans, memories are what makes us who we are. They define and guide our thoughts, actions and behaviour. They shape our understanding of the world through sensory inputs and help us predict as actions and behaviours. Memories define our personality, society, culture, civilization, and future. Our life encompasses a whole range of events, ranging from mundane, such as daily activities and navigation to memorable, such as significant experiences. Memories of these aspects of our lives are the internal records which underlie our thoughts, decisions, personalities, emotional reactions and behaviours. Memories being the very core of our identity, understanding how memories are formed, processed and stored helps us in getting a better understanding of who we are and in extension, our place in the universe.

1.2 Types of memories

Memories can be classified into different taxonomies which are not mutually exclusive. Different classification schemes take into account several factors like the duration of memory, tasks, processes involved, types of memory trace, memory systems and brain regions recruited.

The most commonly acknowledged broad distinction is between short-term memory (STM) and long-term memory (LTM) and this is based on the duration of the memory trace. STM is the ability to encode and recall information for a short period of time, usually for a few seconds to minutes. LTM stores information for long periods: hours, days, years or sometimes even an entire lifetime. Several factors like rehearsal, depth of processing and relevance of the encoded information can influence whether specific content gets eventually to long-term storage. In recent literature, some scientists clearly distinguish between STM and LTM based on temporal decay and chunk capacity limit (N. Cowan, 2008). STMs and LTMs can also be distinguished on the basis of their biological mechanisms: STM relies on existing networks and post-translational modifications,

while LTM recruits structural and functional changes of neural networks requiring *de novo* gene expression (Alberini, 2009; E. R. Kandel, 2001a). Several subregions of the prefrontal cortex (PFC) and parietal cortex seem to play a critical role in working memory (Bledowski, Rahm, & Rowe, 2009; Goldman-Radic, 1995). The brain regions involved in LTM depends on the type of memory and memory systems as discussed in the following section.

There seems to be a lot of debate over whether working memory is STM or a distinct class of memory. Working memory is retention and manipulation of small amounts of information in a readily accessible form, facilitating execution of cognitive tasks like planning, comprehension, reasoning, and problem-solving in in goal-directed behaviors (A. Baddeley, 2012; Chai, Abd Hamid, & Abdullah, 2018; N. Cowan, 2014). Short-term-memory is sometimes referred to as working memory in some theoretical frameworks, which holds and manipulates goal-relevant representations for several seconds to minutes and is highly vulnerable to interference (A. Baddeley, 2003). According to Baddeley, older theories using the term STM regarded it as a unitary store, while modern frameworks acknowledge the STM store as a collection of subsystems under working memory, serving essential cognitive tasks like learning, reasoning, comprehending and understanding (A. D. Baddeley, 1997). Other scientists point out that working memory is a category by itself, distinct from STM, in timescale, features and physiological plasticity mechanisms. The time scale of working memory is from milliseconds to minutes while that of STM ranges from minutes to even days (Tetzlaff, Kolodziejski, Markelic, & Wörgötter, 2012). According to Tetzlaff et al, while short-term plasticity and reverberant neural activity have been suggested to underlie working memory, certain long-term plasticity mechanisms are involved in STM, and structural plasticity mechanisms likely underlie LTM. According to Cowan, the distinction between short-term memory and working memory depends on the definition that one accepts, but this distinction could be a matter of semantics (N. Cowan, 2008). Cowan further suggests that attention-related

cognitive aptitude for storage and processing functions and rehearsal could distinguish between STM and working memory. Working memory correlates well with cognitive aptitudes to the extent of attentional control and capacity for storage and/or processing of working memory. Preventing covert verbal rehearsal and relying more on attention-demanding processing and/or storage to carry out the task may be necessary for a working memory procedure to correlate well with cognitive aptitudes. In addition, the efficiency and use of attentional system in working memory differs with individuals, improves in childhood and declines in old age.

In addition to the relatively rough division between STM and LTM, some scientists look at memory from a temporal dynamic processing point of view leading to more than two categories, mainly based on the duration and involvement of specialized brain regions in processing. According to Kesner and Hunsaker, three critical time periods have been distinguished for episodic memories: STM with a duration of seconds, intermediate-term episodic memory with a duration from minutes to hours, and LTM or remote episodic memory with a duration from days to years (Kesner & Hunsaker, 2010). Kesner and Hunsaker proposed that this distinction is also physiologically encoded in the brain distinguished by the specific brain regions involved in each: Episodic STM mediated by the CA3 subregion of the hippocampus, intermediate-term episodic memory mediated by the hippocampal CA1 subregion with help from CA3 subregion, LTM or remote episodic memory mediated by the CA1 subregion. The dentate gyrus is proposed to have a modulatory influence on the CA1 and CA3, thus influencing their role in short-term and intermediate-term episodic memory. According to Frankland & Bontempi, LTM can be subdivided into a recent and remote component (P. W. Frankland & Bontempi, 2005), as discussed elaborately in the following section.

1.3 Memory systems

Memory can also be divided into memory systems, which differ in the quality of information encoded, and can work independently from each other as they rely on distinct underlying networks. In contemporary usage, the generally accepted categorization of memory systems for neuroscientists are declarative (subject to conscious recall) and non-declarative (procedural and conditioned) memories. In early literature, the terms explicit and implicit memory referred to types of memory resulting from different learning modes stimulated by active attentional requirements to the stimulus facilitating learning. This difference did not account for the existence of different underlying memory systems, in contrast to the postulation of Larry Squire using the terms declarative and non-declarative memories (L. R. Squire, 2004) as described in Fig 1. Declarative or explicit memory refers to the memory for facts (semantic memory) and events (episodic memory), while non-declarative or implicit memory refers to procedural skills, habits and conditioned responses. However, in the literature, these terms are often used interchangeably.

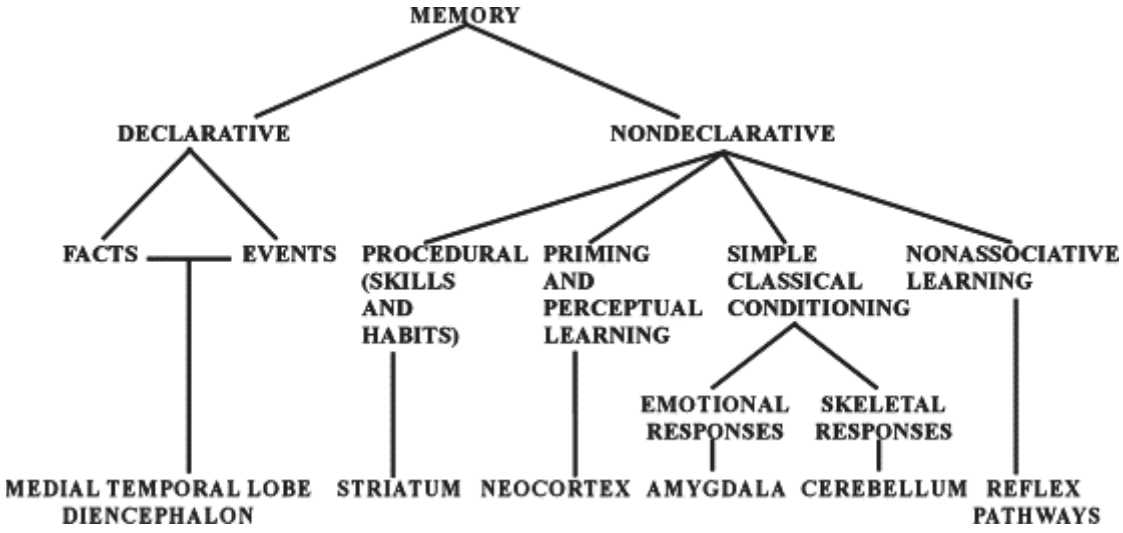


Figure 1 Taxonomy of mammalian long-term memory as proposed by Larry Squire

From (L. R. Squire, 2004): A taxonomy of mammalian long-term memory systems with the brain structures thought to be important for each of them.

Non-declarative memories rely on several brain regions depending on the type. Among these, procedural memories of skills and habits are processed mainly by the striatum, priming depends mostly on certain neocortical areas, conditioned emotional responses depend on the amygdala and skilled movements depend on the cerebellum (L. R. Squire, 2004). Cohen and Squire postulated that task learning in amnesiacs depends on the nature of information and not the extent of motor involvement. Rules or procedures govern non-declarative perceptual motor and pattern analysing skills, while data-based information is specific and declarative; and show divergent processing in the brain in terms of brain regions and circuits involved (Cohen & Squire, 1980). These classical divisions between memory systems and associated brain structures have been challenged by more recent findings. Memory is thought to be more flexible, and memory systems involve much more interactions between them than earlier hypothesized, based on relatively simplistic theoretical models (Henke, 2010). According to Henke, we need an updated model based on processing operations involved, rather than by attention or conscious learning because of evidence from both animals and humans that the hippocampus can mediate rapid associative encoding with and without conscious attention for both STM and LTM. However, the model depicted by Squire is still widely accepted as a framework for most investigations on memory. The division of memory systems into declarative/explicit and non-declarative/implicit, although generally applicable to all mammalian species, it is most empirically studied in humans where the quality of declarative memories is assessed by verbalized descriptions and reports requiring language, while the quality of procedural or perceptual memories are assessed with task-oriented approaches. Studies of hippocampal-dependent declarative memories in animals use a different approach with indirect behavioural measures of memory acquisition like spatial exploration with food or odours, maze exploration, spatial object recognition, navigational Morris water task and

contextual learning paradigms which have been demonstrated to depend on intact hippocampal functions (Vorhees & Williams, 2014).

Declarative memory is widely thought to require the MTL, but the extent of dependence varies and is based on the memory being episodic or semantic (Tulving, 1972). According to Tulving (Tulving, 1972), episodic memory referred to knowledge of temporal order of events, which he defined as "*about temporally dated episodes or events, and temporal-spatial relations among these events… it is always stored in terms of its autobiographical reference to the already existing contents of the episodic memory store*". Semantic memory corresponds to general knowledge of the world and was defined by him (Tulving, 1972) as "*memory necessary for the use of language. It is a mental thesaurus, organized knowledge a person possesses about words and other verbal symbols, their meaning, and referents, about relations among them, and about the rules, formulas, and algorithms for the manipulation of these symbols, concepts, and relations. Semantic memory does not register perceptible properties of inputs, but rather cognitive referents of input signals*". The above given definitions were originally proposed by Tulving (Tulving, 1972), and they were further discussed (Duff, Covington, Hilverman, & Cohen, 2020). Tulving's distinction of the two systems was based on the nature of information, subjective versus cognitive reference, retrieval conditions and vulnerability to interference. Although he did not go into details to distinguish between episodic and semantic components in terms of structural and functional features, he proposed the distinction as having utility in understanding the memory phenomena and experimental research of the time. Thus, Tulving suggested very early on that a distinction between episodic and semantic memory might be important in memory research because they differ in their properties and neural substrates (Tulving, 1972). Eventually, this classification turned out to be very significant and considerable research effort has gone into understanding the similarities and differences between these two forms of memory. Understanding these two memory systems

empirically and theoretically, was a significant catalyst in the study of multiple memory systems and this distinction can give insights into the most significant human abilities, namely memory and language.

The specific role and temporal involvement of the hippocampus in episodic and semantic memories has been a matter of considerable debate (Duff et al., 2020). Detailed studies of amnesiacs over several decades have led to the understanding that different types of memories are stored and processed in different brain regions/subregions and that the types of lost memories depend critically on the region of brain injury/trauma. Several lesion studies in animals as well as in patients with lesions have shown that the medial temporal lobe (MTL) structures (e.g., hippocampus, rhinal cortices) are of critical importance for declarative memory. The earliest and one of the most famous and impactful case studies is of patient H.M. (Scoville & Milner, 1957). H.M. underwent experimental bilateral MTL resection at the age of 27 to alleviate intractable epilepsy symptoms. Even though the surgery largely alleviated his seizures, the surgery also left him with profound memory deficits. He demonstrated a global deficit in forming new declarative memories, a phenomenon called anterograde amnesia (loss of the ability to create new memories), regardless of memory test, stimulus material and sensory modality. The deficit appeared to involve both episodic and semantic memories. But on the other hand, his language comprehension, acquisition of non-declarative visuomotor skill learning and visuo-perceptual learning were undisturbed (Corkin, 2002). This distinction supported the idea that different memory systems subserve different types of memories. The fact that some semantic memories could still be acquired while episodic memories were completely impaired suggested that semantic memories may be supported by extrahippocampal regions. One interpretation for this observation is that contextually-rich episodic memories rely more heavily on MTL than semantic memories, or less-contextually-rich versions of episodic memories. Alternatively, it may be that spared hippocampal tissue allowed

H.M. to retain a restricted ability to form new semantic memories (Corkin, 2002). In addition to anterograde amnesia, it was discovered that H.M. also suffered memory decline for events that happened prior to the surgery; memories for events that occurred within the 11 years preceding the surgery suffered significantly more loss than those that occurred before this period, a phenomenon called temporally-graded retrograde amnesia (loss of memory-access to events or information, before an injury or the onset of a disease with recent ones being more affected than remote memories in a temporal gradient). This temporal gradient in memory loss is an indication of the time-limited role of MTL structures in memory storage (Sagar, Cohen, Corkin, & Growdon, 1985). However, there is disagreement among scientists as to the extent of memory impairment in H.M. Some suggest a retrograde temporal gradient extending back a few years while others claim that H.M. suffered a complete loss of episodic memories, independent of their time of occurrence. Hence, our understanding of the memory processing mechanisms in the brain needs more research and in-depth study (Steinvorth, Levine, & Corkin, 2005).

Findings from another patient K.C. have added to our understanding of the extent of hippocampal involvement in detailed spatial content of episodic memories (Rosenbaum et al., 2005; Rosenbaum et al., 2000). He had extensive bilateral hippocampal damage and exhibited deficits in remote autobiographical and spatial memories. But he was able to recognize salient landmarks from remotely learned spatial contexts. His anterograde amnesia was comparable to H.M. However, studies on his memory recall revealed a dissociation between semantic and episodic memory through his use of knowledge and experiences from the past before his accident. His general of the world was preserved, but he was incapable of recollecting the details of any personally experienced events. In terms of his spatial memories, general aspects (allocentric spatial knowledge of the world and neighbourhood) were preserved, but his ability to recognize non-salient features was severely affected. This suggests a dissociative involvement of the hippocampus

in terms of spatial learning. While the hippocampus is critical for specific location details, regardless of when they were acquired, it is not crucial for retrieval of remotely acquired major spatial representations including landmarks, routes, distances and directions. This is more in line with the Multiple Trace Theory of memory consolidation (reviewed in section 1.5) which postulates that entire hippocampal-neocortical ensemble in constitutes the memory trace for an episode. While the neocortical component of an episodic memory trace encodes a generalized version, the original episodic details are encoded in the hippocampal component of the trace; in the absence of hippocampal input, episodic details will be lost, and the retrieved neocortical memory trace will be more semantizised and schematized.

In summary, neuropsychological and neuropathological studies in other amnesic patients revealed that while anterograde memory is fairly consistently severe, the length of the gradient and severity in temporal retrograde memory impairment seems to differ greatly, roughly correlating with the extent of damage within the hippocampal formation (Rempel-Clower, Zola, Squire, & Amaral, 1996). Bilateral damage primarily limited to the CA1 region of the hippocampal formation results in moderately severe anterograde memory impairment while bilateral damage beyond the CA but limited to the hippocampal formation manifests as more severe anterograde memory impairment. More extensive damage to the hippocampal formation results in extensive temporally graded retrograde amnesia covering 15 years or more depending on the extent of lesion. These studies substantiate the understanding that severity of memory impairment is largely dependent on the locus of damage to regions adjacent to the hippocampus, including entorhinal, perirhinal, and parahippocampal cortices, and extent of damage within the hippocampal formation (Rempel-Clower et al., 1996; L. R. Squire et al., 2020).

However, findings from several rodent experiments show no evidence of temporally graded retrograde amnesia following lesions and challenged the standard model of systems consolidation.

In these studies, remote and recent memories are equally affected. In fact, they report flat RA gradients after both partial and complete hippocampal lesions (R. J. Sutherland, Sparks, & Lehmann, 2010). Several other reports also show no evidence of temporally graded retrograde amnesia and predict hippocampal involvement for retrieval of remote episodic memories (Corkin, 2002; Lynn Nadel & Moscovitch, 1997; Ocampo, Squire, & Clark, 2017). These observations challenged the standard model for systems consolidation (reviewed in section 1.5) leading to the understanding that if an episodic memory ever depended on the hippocampus, then it always depends on the hippocampus, the memory is never really consolidated as per the standard model.

While other studies have shown hippocampal involvement in remote memory retrieval based on inhibition time of the hippocampus in their experimental manipulations. Longer inhibition of hippocampus abolished hippocampal-dependence of memory trace, leading to the speculation of compensatory mechanisms in the brain involving other brain regions (Goshen et al., 2011; Sawangjit et al., 2018; Yassa & Reagh, 2013). Some scientists argue that the hippocampus is critical to both episodic and semantic memories (Duff et al., 2020). Theoretical and empirical advances in the study of semantic memory (words, concepts, and meaning) and its neural bases have demonstrated that its depth and richness are similar to episodic memory (episodes and events) and that they are both highly flexible, (re)constructive, relational and multimodal systems reliant upon the properties of the hippocampus (Duff et al., 2020).

These observations call for harmonization of memory consolidation models away from bifurcation of hippocampal-dependence and hippocampal-independence in memories. Overall, it puts in question the temporal disengagement of hippocampus and the overall premise of standard systems consolidation. It is possible that episodic memories never become fully independent of the hippocampus, more in line with multiple trace theory and hippocampal indexing theory (reviewed in section 1.5). Taken together, the emerging evidence seems to support the view that hippocampal

dysfunction leaves old semantic knowledge relatively intact but disrupts all episodic memories and acquisition of both new episodic memory and semantic knowledge (Moscovitch, Cabeza, Winocur, & Nadel, 2016; Moscovitch et al., 2005; Winocur, Moscovitch, & Bontempi, 2010). Memory consolidation in the light of this understanding is the process of extraction of semantic knowledge from episodic memories.

Based on several empirical observations in humans and animal models, various memory consolidation theories have been formulated to explain why different types of memories are impaired to different extents by damage to the same brain structure while, on the other hand, different brain structures seem to be important for different types of memories (reviewed in a following section 1.5).

1.4 Stages of memory formation

The earliest indication of the reorganization of memory over time, eventually leading to the concept of memory consolidation, came from observations of the French psychologist, Théodule Ribot (1880), who made the observation that in patients with brain trauma, memories of recent events suffered more impairment than those for remote events(Théodule Ribot, 1891). This effect has been termed ‘Ribot’s Law’ and the temporal gradient of memory loss the ‘Ribot Gradient’. Based on his observations, he proposed that memories might need to undergo a stabilization process. The observed memory loss for recent events was likely a result of the trauma occurring before the memories had gone through the stabilization process. Eventually, in mid-twentieth century, series of seminal studies by Scoville, Penfield, and Milner provided the first neuroanatomical evidence for Ribot’s proposition by providing insights into the brain regions important for memory reorganization (Penfield & Milner, 1958; Scoville & Milner, 1957). Patients

with brain damage in the MTL (anatomically connected regions of the hippocampus, entorhinal, perirhinal and parahippocampal cortices) tended to show evidence of temporally graded retrograde amnesia (discussed in detail in the previous section 1.3). It was the first hint that the MTL might be particularly critical for processing new memories, and responsible for the memory gradient observed by Ribot.

Memory is the ability to encode, store and retrieve information which in turn facilitates adaptive behaviour. In neurobiological research, memory processing is divided into three main stages: encoding, consolidation and retrieval together contributing to the proper functioning of LTM. Encoding is the acquisition of information when memories are first created and specifically refers to the process of creating an engram that will represent the memory in the brain. Memories are eventually stabilized, strengthened, and stored during consolidation. Finally, during retrieval, stored memories can be recalled. According to several memory consolidation theories (discussed in the following section), a labile memory trace which is formed during encoding is subjected to strengthening through consolidation and if successful, the memory trace can be retrieved at a later time point. There is also evidence for a process called "reconsolidation" during which disruption of post-retrieval memory has been observed due to the memory trace being in a labile phase during retrieval processes (Dudai, 2006). This process has been demonstrated in fear memory retrieval, which has therapeutic implications in post-traumatic stress disorders, phobias and addictions (Dudai, 2006; Misanin, Miller, & Lewis, 1968; K. Nader, Schafe, & Le Doux, 2000; Tronson & Taylor, 2007).

The term 'consolidation' has different usages in contemporary literature: synaptic consolidation and systems consolidation (L. R. Squire, Genzel, Wixted, & Morris, 2015). Synaptic consolidation describes events at the synaptic/cellular level, which stabilize synaptic plasticity immediately following learning. Synaptic consolidation involves molecular processes with the

recruitment of RNA and neuronal protein synthesis along with intracellular signal transduction cascades and eventually causing long-term structural and reorganizational changes involving neuronal plasticity in synapses (Dudai, 2004). In contrast, systems consolidation, refers to gradual reorganization of the brain systems that support LTM. Models of systems memory consolidation postulate that memory consolidation is a gradual temporal process: the memory being initially encoded into a temporary store, and then transferred during the course of consolidation to a long-term store. Within this framework, information is encoded in the neocortex as well as in the hippocampus at the time of learning. Memories are initially dependent on the hippocampus, and with gradual reorganization, the hippocampus gradually becomes less important for storage and retrieval, and a more permanent memory develops in distributed regions of the neocortex, establishing stable memory by increasing the complexity, distribution, and connectivity among multiple cortical regions (L. R. Squire et al., 2015). In this concept of systems consolidation, defined by the degree of consolidation, recent memory refers to memories still dependent on the short-term store, while remote memories are fully consolidated exclusively in the long-term store (P. W. Frankland & Bontempi, 2005). The timescale and physiological and cellular mechanisms of memory consolidation have led to considerable speculation and several memory consolidation theories to explain experimental observations from all the studies (reviewed in the following section 1.5).

1.5 Memory Consolidation: Theories, timeline and evolution

Even though it is widely accepted that memories need to be consolidated for long term retrieval, the details of the mechanistic process are widely debated by consolidation theorists. Over the years, research is gradually unraveling the neural mechanisms of memory encoding and

consolidation, that are thought to depend on an interaction of cellular and systems-level mechanisms. There have been several theories presented which account for findings from normal and amnesic human and animal experiments. In this section, the main propositions of most theories, addressing aspects of the consolidation process from molecular/cellular to systems perspective, have been documented.

1.5.1 Memory consolidation

Even though the central role of memory in cognition is unequivocal, it is a challenge for neuroscientists to uncover the underlying neural mechanisms to encode, consolidate, store and retrieve information. In theoretical models of memory research, consolidation ("to make firm" in Latin), refers to the progressive organization, assimilation and stabilization of a learning experience for long-term memory storage and retrieval after acquisition, as well as to the memory phases during which such presumed stabilization takes place. The idea that memories undergo a time-dependent consolidation process to fix them for long-term storage and retrieval has a long history, while the understanding of the process, time duration and the interactional dynamics between different brain structures in memory consolidation has evolved over time. The quest for the neurobiological basis of consolidation has triggered wide-spread research and discussions on the mechanisms from cellular to systems level given the complex role of various brain regions involved in the process. These discussions are primarily dependent on the available data, based on which theories were formulated. Based on evidence to validate the main features proposed by the theories, all or part of the propositions were either supported or refuted. Even the definition of memory consolidation has evolved from the basic idea of fixing memories for long term retrieval, to incorporating the contribution and role of different brain regions, primarily the hippocampus and

neocortex at various stages in the memory process. Most influential theories of memory consolidation sought to understand the role and involvement of hippocampus in memory formation, consolidation and retrieval. Connectionist models of systems consolidation look at the process by assessing feasibility and constraints in neuronal ensembles in different brain regions. Systems consolidation also attempted to consider the instantiation of memory from a connectionist model perspective in networks of neuronal assemblies and their interactions between modules in the brain.

The precise time course of consolidation is not clear; exactly when, how and where in the brain it takes place has been speculative. Consolidation could be a brief process of biochemical fixing or reorganization of synapses in hours following learning, or even gradual redistribution of long-term memories requiring changes in the critical anatomical substrates supporting memory expression over time.

1.5.2 Earlier theories of memory consolidation

Some of the earliest observations formed the basis of the idea of memory consolidation. The earliest evidence for the process of consolidation traces back to the pioneering work of experimental psychologists, Georg Müller and Alfons Pilzecker (Müller & Pilzecker, 1900). In their seminal monograph, they proposed a role for consolidation, which was based on 40 experiments on human subjects designed to identify the laws that govern memory formation and retrieval. They proposed that learning does not induce instantaneous, permanent memories and that memory takes time to be fixed (or consolidated). According to their **Preservation theory**, disrupting the preservation process would interfere with the formation of associative learning and that retroactive interference compromises the integrity of recent (and not-yet-consolidated) memories. Consequently, **Interference theory** proposed that memories acquired close together in

time compete for representational space, thereby interfering with each other in a time- and content-dependent manner; consolidation was assumed to render a memory trace more resistant to interference, similar to the time course of forgetting proposed by Ebbinghaus (Ebbinghaus, 1885). This property is also highlighted in the power law of forgetting (J. R. Anderson & Schooler, 1991; Wixted & Carpenter, 2007; Wixted & Ebbesen, 1991), and Jost's law of forgetting (Jost, 1897; Wixted, 2004a). A possible explanation is that the continuous reduction in the rate of forgetting of a memory trace with time, is a reflection of the increased resistance to interference as it undergoes a slow process of consolidation (W. A. Wickelgren, 1974; Wixted, 2004a, 2004b).

Building up on earlier theories, the following theories were built on the distinction between STM and LTM from a mechanistic and temporal perspective:

The **Dual trace theory of memory** proposed by Donald Hebb distinguished between short term memory (STM) and long term memory (LTM). STM is a transient trace in the form of reverberatory activity within local neural circuits and LTM is the stabilization of the reverberation to induce structural changes at the synaptic level in the reverberating network. Thus, during consolidation, the transient, dynamic trace is converted into a permanent structural trace (Hebb, 1949). This was further elaborated by Wickelgren and Berian (W. A. Wickelgren & Berian, 1971), by defining total memory trace strength as the sum of short term and long-term components with the strength of each trace depending on acquisition, consolidation and decay functions.

The **Multi-store model of memory** of Atkinson and Shiffrin further added to this concept, by proposing a passive, one-way linear model with information flow through a system of sensory memory to STM mediated by attention and from STM to LTM mediated by rehearsal (Atkinson & Shiffrin, 1968). Baddeley and Hitch further elaborated on this model by proposing the working memory model: working memory is a central executive which is in control of a phonological loop (spoken and written material) and a visuo-spatial sketchpad (visual or spatial form) between

sensory memory and LTM (A. D. Baddeley & Hitch, 1974). This was later updated to include the episodic buffer component as a mediator between STM and LTM (A. Baddeley, 2000). The episodic buffer is defined as the temporary multimodal store component of working memory that integrates information from the phonological loop, visualspatial sketchpad, and LTM to create a unified memory of an event, combined with information about time, such that detailed representation of experiences are encoded as a coordinated sequence of events rather than as discrete segments. The episodic (holds integrated episodes or scenes) and buffer (provides a capacity-limited interface between systems using different representational codes) components together address the shortcomings of the original working memory model: process of chunking information (described in a following paragraph of 'Chunking and consolidation model') and linking distinct representational formats of the loop and sketchpad. Thus, the episodic buffer provides a mechanism for multiple simultaneous sources of information, creating a spatial and temporal representation of the experience that may be subject to later manipulation to solve problems and plan future behavior.

The memory **Consolidation theory of McGaugh** proposed three independent memory trace systems for immediate memory, STM and LTM (McGaugh, 1966). The long-lasting trace of an experience is not completely fixed, consolidated, or coded at the time of the experience and that consolidation requires time. Hence, if permanent memory traces consolidate slowly over time, then other processes must provide a temporary basis for memory while consolidation is occurring.

Lewis' **Active trace theory** proposed consolidation from STM to LTM as a parallel, rather than sequential process, with STM and LTM both being initiated at the onset of learning until STM drops out, while LTM continues. STM was proposed to be active, fragile and temporary, while LTM as inactive and permanent, with vulnerability as a function of age and state of memories (Lewis, 1979).

1.5.3 More recent theories of memory consolidation

More recently, theories addressed the functional role and interaction of different brain regions and the timing of memory consolidation. As mentioned previously, the concept of a temporal gradient of retrograde amnesia (RA) was noted by Ribot (Théodule Ribot, 1891; Thodule Ribot, 1896), but the central brain structures involved in the phenomenon were not known. As reviewed in section 1.3, the concept of memory consolidation in the mainstream of memory processes was revived by the profound case of temporally-graded retrograde amnesia and anterograde amnesia after the removal of medial temporal lobe structures reported in the famous H.M case, highlighting the importance of those structures in normal memory processes (Scoville & Milner, 1957; L. R. Squire, 2009). The role of hippocampus as an important medial temporal lobe structure in memory processes has since then been studied and a number of theories and models have been proposed for the mechanism and the extent of hippocampal involvement. The ideas that the hippocampus functions as a temporary repository until consolidation, that waking patterns of neural activity are reinstated or replayed during sleep, and that the cortex is important in extracting semantic knowledge structure, form the bases of several contemporary models of memory formation (D. Marr, 1971; L. R. Squire & Alvarez, 1995; W. Wickelgren, 1987).

David Marr proposed the first **Model for systems consolidation** with separable roles for the archicortex including hippocampus, and for the neocortex in memory (David Marr, 1970; D. Marr, 1971). According to this model, hippocampus and neocortex are separate processors. Hippocampus is conceptualized as being capable of rapid storage of new patterns, in a temporary memory store, but it cannot directly integrate them with the larger body of existing knowledge. It facilitates and directs the gradual transfer of these patterns to neocortical storage units, which would eventually reorganize and classify this information, incorporating it with existing knowledge to reduce interference. Hippocampus was conceptualized as an autoassociator network with

features that support internal recurrency, sparse encoding and plasticity that contribute to its functions in pattern storage, pattern separation, pattern completion and retrieval, which were subsequently validated (Bliss & Lømo, 1973; Kelso, Ganong, & Brown, 1986; B. McNaughton, 1991; B. L. McNaughton & Morris, 1987; Rolls, 1989; Treves & Rolls, 1992). This autoassociative network was implicated in sequence learning, spatial navigation and episodic memory consolidation. It was proposed that even with pattern separation and sparse coding, a pattern stored in the hippocampus would be short-lived before it is overwritten by storage of newer memories implying that memories stored in the hippocampus must be transferred elsewhere to survive for long periods (Gluck & Myers, 1997).

According to the **Chunking and consolidation model** proposed by Wickelgren (W. Wickelgren, 1987; W. A. Wickelgren, 1979), chunking is a process by which a set of nodes, representing components or attributes of a whole, comes to be associated with a new node. The hippocampus itself does not store any information, but controls chunking of neutrally encoded node in the cortex. The degree of activation or rate of firing above baseline spontaneous firing, creates a new chunk node to represent the information by strengthening of associations among the components. The neurons that are already associated to represent an idea are 'bound neurons', and those that are available to be encoded by selective strengthening of pre-existing weak connections are the 'free neurons'. During encoding, the hippocampus connects to free neurons in an excitatory manner and does not connect to bound neurons or connects to them in an inhibitory manner, thus helping in assigning free neurons to represent the new idea. During retrieval, a different arousal system primes the set of bound neurons at the expense of free neurons. According to this model, consolidation is the weakening of connections between the cortical cells and newly bound hippocampal neurons. This model would directly account for anterograde amnesia. Recent bound neuron chunks affected due to a chunking deficit in hippocampal damage would also explain co-

occurrence of AA and RA, and time course of RA, depending on whether a piece of information is in the process of being chunked or is already bound together.

One of the first models of memory consolidation, describing the interplay between the hippocampal complex and neocortical storage sites, and accounting for the phenomenon of temporally graded retrograde amnesia, is referred to in the literature as the "**Standard Model of Systems Consolidation**" (L. R. Squire, Cohen, & Nadel, 1984). Information is initially encoded in specialized primary and associative cortical areas. The hippocampus integrates features of information from distributed cortical modules into a coherent memory trace. Successive reactivation of this hippocampal-cortical network leads to progressive strengthening of cortico-cortical connections eventually allowing them to become independent of the hippocampus by gradual integration into pre-existing cortical memories. A key feature of this model is that changes in the strength of the connections between the hippocampal system and the different cortical areas are rapid and transient, whereas changes in the connections between the cortical areas are slow and long-lasting. It assumes a temporally-limited involvement of the hippocampal complex in the maintenance of declarative memories that have not yet been consolidated (Martijn Meeter & Murre, 2004; L. R. Squire & Alvarez, 1995). After the process of consolidation, memory is solely dependent on neocortical storage traces. According to this model, episodic and semantic memories are equally affected in retrograde amnesia, as they both depend on same medial temporal memory system, with recent memories being more susceptible than remote ones. As an extension of the standard model, it was later proposed that as memory becomes hippocampal-independent, the integrative role could be adopted by the PFC instead, to link cortical modules in the memory trace (P. W. Frankland & Bontempi, 2005; Paul W Frankland & Bontempi, 2006).

The **Multiple Trace theory** differentiated between consolidation of episodic and semantic memories to account for flat retrograde amnesia in episodic memories. It proposes the necessity of

the hippocampal complex for maintaining episodic or equally context-rich semantic memories, for as long as they exist (Fujii, Moscovitch, & Nadel, 2000; Lynn Nadel & Moscovitch, 1997). According to this theory (Figure 2), the 'hippocampal-neocortical complex' is the episodic memory trace, and that each re-activation encodes a new sparse, distributed trace in the network. Memories are strengthened with time by a process of memory trace multiplication within the hippocampal-neocortical structures, rather than by consolidation in the traditional sense. Vulnerability to memory loss would be related to multiplicity of trace representations. Semantic memories on the other hand, are consolidated with time, getting independent of the hippocampal complex. Thus, the model would predict graded RA for semantic memories and flat RA for episodic memories.

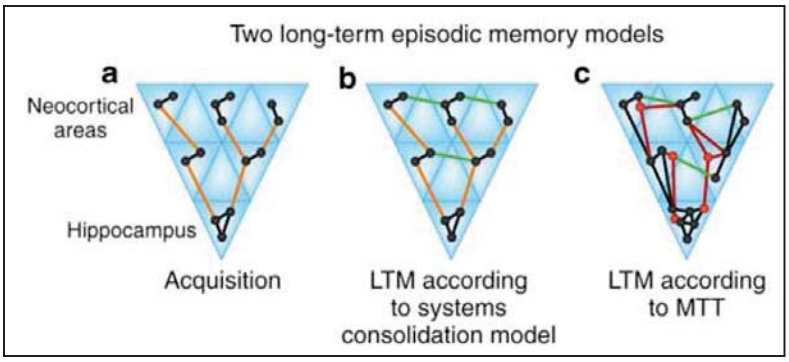


Figure 2: LTM according to Systems consolidation and Multiple trace theory.

Note: Adapted from (Lynn Nadel & Hardt, 2011)

(a) Acquisition: the hippocampus indirectly links brain regions involved in the processing and representation of memory trace. The standard systems consolidation and multiple trace theory propose differential temporal involvement of the hippocampus in memory.
(b) Standard systems consolidation: episodic memories are initially hippocampal-dependent. With the establishment of direct neocortical connections (green lines) between components of the memory trace, it becomes independent of the hippocampus over time.
(c) Multiple trace theory: Hippocampus is always involved contextually rich episodic memory retrieval. The hippocampal-neocortical complex is the episodic memory trace, and each reactivation encodes a new distributed trace in the network. Memories are strengthened with time by memory trace multiplication (new nodes and connections in red) within the hippocampal-neocortical structures.

Drawing on Marr's proposition of hippocampal-neocortical interactions, the **Complementary learning systems** paradigm predicted fast hippocampal storage of new experiences, followed by a slow process of neocortical consolidation during reactivation events during offline periods of rest or sleep by gradual adjustments of synaptic weights (McClelland, McNaughton, & O'Reilly, 1995). Initial fast storage of memories in the hippocampus can avoid interference, as the newly learned material is slowly and gradually interleaved in neocortical modular knowledge database system. The role of entorhinal cortex as compression/convergence zone, and parahippocampal & perirhinal cortices as intermediate layers for sophisticated compression-decompression, was also predicted for pattern compression for sparse coding in hippocampal network. Evidence to support the claims of this model come from: neocortical interconnectivity (Felleman & Van Essen, 1991), reciprocal connections between neocortex and hippocampus through entorhinal cortex, sparse coding in the hippocampus (Barnes, McNaughton, Mizumori, Leonard, & Lin, 1990; B. L. McNaughton & Morris, 1987; O'reilly & McClelland, 1994), and the phenomenon of LTP at hippocampal synapses (B. L. McNaughton & Morris, 1987; B. L. McNaughton & Nadel, 1990). The time-limited role of the hippocampus in this model would explain temporally graded RA. Replay during offline periods is a prediction of the model, supported by several lines of evidence suggesting memory reactivation in post-task sleep (discussed in detail in section 1.10.5.1) (Euston, Tatsuno, & McNaughton, 2007; Hoffman & Mcnaughton, 2002; Mc.Naughton et al., 2003; S. C. Mednick et al., 2002; Pavlides & Winson, 1989; Skaggs & McNaughton, 1996; Stickgold, James, & Hobson, 2000; G. R. Sutherland & McNaughton, 2000; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002; M. A. Wilson & McNaughton, 1994).

Based on the intrinsic organization, synaptic physiology and anatomical relationship of the hippocampus to other regions of the brain, '**Hippocampal memory indexing theory**' was originally proposed by Teyler and Discenna (Teyler & DiScenna, 1986), depicted in Figure 3.

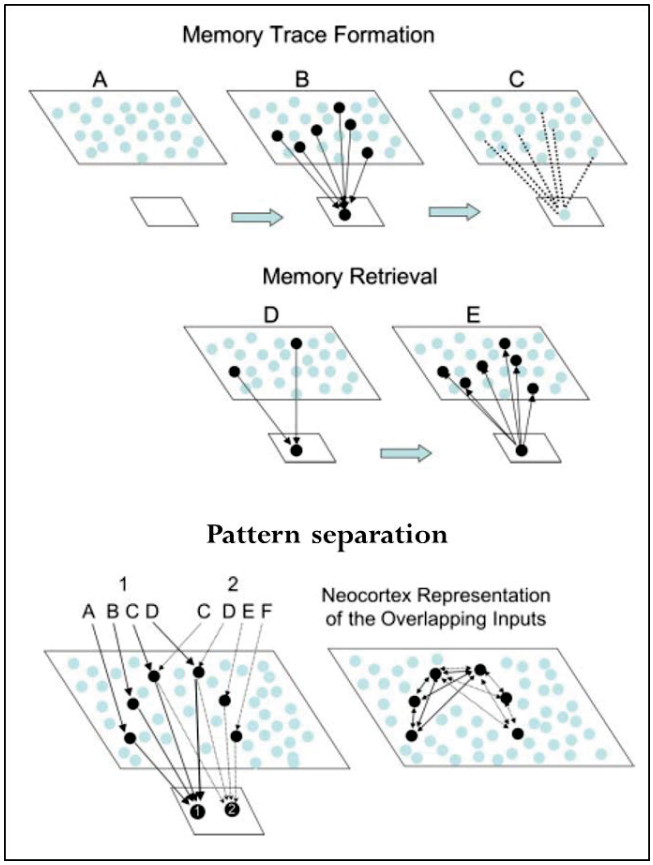


Figure 3: The Hippocampal Indexing theory

Note: Adapted from (Teyler & Rudy, 2007): Hippocampus stores an index to neocortical patterns.
Memory trace formation:
(A) The top layer: neocortical neurons; bottom layer: hippocampal neurons.
(B) A set of neocortical patterns activated by an event project to the hippocampal neurons.
(C) The memory for that experience is stored as strengthened hippocampal synaptic connections activated by the original input pattern.
Memory retrieval:
(D) A subset of the initial input pattern can activate the hippocampal representation.
(E) Output from the hippocampus projects back to activate the entire neocortical pattern for complete memory retrieval.
Pattern separation in the hippocampus: Creating separate indices to similar input patterns. Similar input patterns converge on different hippocampal representational units. The same two patterns would not be pattaren-separated in the neocortex.

They proposed that during episodic memory encoding, inputs from sensory cortical regions activate a small population of hippocampal synapses, which act as an index code representing the synaptic locations in the neocortex, where memory itself is stored. A modular, hierarchical neocortex with dense local connectivity and sparse intermodular connectivity could support random arbitrary associations in neocortex (O'kane & Treves, 1992). The higher level modules would serve to form an "index" (Mc.Naughton et al., 2003; O'kane & Treves, 1992; Paller, 1997) representing the corresponding low-level event, by generating a code unique to each distributed pattern (context) in the data modules. The hippocampal formation was thought of as the highest level of the cortical hierarchy to create the index codes in this top-down pattern of reciprocal connections between lower and higher level modules. In essence, based on the functional design and anatomical connectivity of the hippocampus, it could capture information about neocortical activity generated by the individual features of each behavioral episode. Further, because the hippocampus projects back to these neocortical regions, the stored information could serve as an 'index' to neocortical activity pattern produced by the episode. Repeated, spontaneous, retrieval of the index codes would provide opportunity for selection of the specific intermodular connections that are necessary to sustain the corresponding association, without the need for exhaustive intermodular connectivity (S.-H. Wang, Teixeira, Wheeler, & Frankland, 2009). Consequently, a partial cue that can activate the hippocampal 'index' could further activate the neocortical patterns associated with that 'index', and thus retrieve the memory of the episode within the set of low-level modules, which themselves are far too sparsely connected to accomplish such global completion directly. Thus, hippocampus could play a pivotal role in memory recall, pattern completion and pattern retrieval (Teyler & Rudy, 2007).

The hippocampal indexing theory is an extension of Marr's systems consolidation theory but differs from it in the temporal involvement of the hippocampus in retrieval of remote episodic

memories. Marr proposed the hippocampus as a temporary memory store that accurately stores events with the ability for pattern separation (orthogonal representations) and pattern completion (using its associative pathways to retrieve the original event from a partial cue). According to Indexing theory, hippocampal neuronal patterns store the index of memory for attributes that are actually stored in the neocortex. The hippocampal index is needed for the complete and reliable episodic memory retrieval with all its attributes. In other words, memory trace is the index, while content of the memory is in the neocortex. Thus, the hippocampus is not just a temporary memory store and may always be required to retrieve richly detailed episodic memories.

The **competitive trace theory** (Yassa & Reagh, 2013) acknowledged the indexing role of the hippocampus, but further proposes a modification to it. According to them, during every memory reactivation event, the hippocampus recontextualizes the memory trace by re-encoding a similar (but not identical) memory trace leading to memories becoming decontextualized over time by competitive interference among these similar multiple memory traces. According to this theory, memories are most episodic and veridical when first encoded, and with every subsequent reactivation, the memory can become less episodic, less accurate and more semantic. Central features of experiences are subject to simultaneous consolidation and decontextualization (loss of associated details) over time. It is different from MTT, in that memories are not stored in parallel, but compete for representation in neocortex. The role of the hippocampus during retrieval is hypothesized to be the recontextualization of memories during reactivation events to generate new competing traces since initial learning.

The idea of semantization of memories proposed by MTT, is similar to the **transformation hypothesis** (Winocur & Moscovitch, 2011), according to which, hippocampal-dependent episodic, or context-specific memories transform into semantic versions that are represented in extra-

hippocampal structures. To the extent that episodic memories are retained, they will continue to be hippocampal-dependent, while semantic memories are hippocampal-independent.

The **Trace-link model** (M Meeter & Murre, 2005; Murre, 1996) extrapolated on the previous theories and proposed three subsystems: a trace system (association areas of neocortex), a link system to connect the remote traces (hippocampus and adjacent regions) and a modulatory system for plasticity of the link system (basal forebrain, amygdala) which retrieves patterns in the trace system. It proposes the strengthening of inter-neocortical traces over time, making them independent of the hippocampal link system.

The **three-stage memory model** (Fiebig & Lansner, 2014) proposed a central role for the PFC: gradual organization from prefrontal STM to long-term neocortical memory with an intermediate sparse, pattern-separated hippocampal memory trace. During perception, feed-forward projections from neocortical input generate separate temporary prefrontal, cortical and hippocampal traces, which are associated to the cortical trace via Hebbian-learning. During the reflection phase, replay in PFC (active rehearsal) drives hippocampal reinstatements through parahippocampal cortices. During subsequent sleep, hippocampal-directed replay drives neocortical reinstatements, which facilitate long-term learning. During cued recall, the external neocortical activation generates corresponding cues in PFC and hippocampus, through feed-forward connections. Thus, the trace-link model and the three-stage neural network model proposed complementary and modulatory roles to other brain regions, in addition to the roles of hippocampus and neocortex.

The concept of **multiple memory systems** by Sherry and Schacter (Sherry & Schacter, 1987), proposed that different brain networks support different types of memories to serve distinct and functionally-incompatible declarative/episodic and non-declarative/procedural memory processes. Each system would encode, store, and retrieve information involving qualitatively

different representations. Three distinct studies suggested the existence of multiple memory systems with an important role for hippocampus in contextual retrieval of episodic memories, distinguishing them from hippocampus-independent semantic memories (Gaffan, 1974; Hirsh, 1974; L Nadel & O'keefe, 1974). The multiple memory systems concept has found expression in various theories (Lynn Nadel & Moscovitch, 1997; Sherry & Schacter, 1987; L. Squire & Zola, 1996).

At least three independent **parallel memory systems** were proposed to exist in rats from the experiments of Packard, Hirsh & White (Packard, Hirsh, & White, 1989) and by McDonald & White (R. J. McDonald & White, 1993), in which they could dissociate declarative, emotional and procedural memories, each being dependent on hippocampus, amygdala and the dorsal striatum respectively. The three information processing memory storage systems may operate simultaneously and in parallel, with some degree of independence and interdependence through cooperation and competition amongst them.

A **parallel dual store model** (Gulbrandsen, Sparks, & Sutherland, 2013; R. J. Sutherland et al., 2010) proposed that independent memory systems differ based upon learning rate parameters set by their intrinsic network properties and by modulatory interactions from other systems. Each learning episode independently establishes memories in both hippocampal and non-hippocampal networks according to intrinsic learning parameters.

The **distributed reinstatement theory** (Lehmann & McNamara, 2011; R. J. Sutherland et al., 2010) stated that at least for certain memories, systems consolidation process depends on a short period (on the time-scale of hours) of hippocampal-dependent cortical reiteration or replay outside of the context of original learning. The hippocampus rapidly acquires a useful context memory with one or few exposures, whereas non-hippocampal networks require more iterations to achieve similar associative strength i.e., it is the reactivation events rather than the passage of time

that makes a memory hippocampal-independent, distinguishing it from the standard model of systems consolidation.

To address the factors influencing temporal gradient and information content in systems consolidation, mechanisms of synaptic/cellular consolidation have been proposed. Synaptic consolidation describes events at the synaptic/cellular level, which stabilize synaptic plasticity immediately following learning. Synaptic remodeling of dendritic spines increases synaptic efficacy, promoting synaptic consolidation (E. R. Kandel, 2001a). Synaptic consolidation involves molecular processes with the recruitment of RNA and neuronal protein synthesis along with intracellular signal transduction cascades and eventually causing long-term structural and reorganizational changes involving neuronal plasticity in synapses (Dudai, 2004). The **unified theory for consolidation, C Theory** (Dash, Hebert, & Runyan, 2004) attempts to bring together the processes of cellular and systems consolidation. It proposes parallel long-term memory storage in the neocortex and in the hippocampus as a result of learning and that hippocampus serves the role of an initial coordinator for memory retrieval until extrahippocampal connectivity has developed the capacity to take over this role over time. The transport time for gene products to travel from the soma to tagged synaptic sites, through continued activity of the hippocampus to complete the consolidation process, defines the period of hippocampal dependency.

Memories had been proposed to be organized into mental structures called schema, as early as 1932 by Bartlett (Bartlett, 1932), to refer to pre-existing knowledge structures into which newly acquired information can be incorporated. This concept was incorporated into the **schema theory** of memory consolidation (Tse et al., 2007), which attempts to address the factors influencing the temporal component of information processing in systems consolidation. According to this theory, information is organized into schema and the rate of systems consolidation of new memories would depend on already existing associative schema i.e., the rate of systems consolidation in the

neocortex can be influenced by what is already known. Episodic memories may require more extended dialogue between the hippocampus and neocortex, while semantic memories can be incorporated into an associative "schema" very rapidly. Specific patterns of information representation, anatomical connectivity, and expression of synaptic plasticity affect memory consolidation. Acquisition of a neocortical schema takes time due to neuroanatomical growth processes that create an associative space for the trace.

1.5.4 Summary, debates and perspectives in the field of memory consolidation

Most models have been proposed based on evidence from experimental data. They then try to integrate the speculations with existing knowledge. The success of a model being weighed by the predictions it makes, current memory consolidation models have either failed in validations of some of their predictions at least for some forms of memories, others await their predictions to be tested over time. Based on the assumptions and predictions of these models, it is evident that they each attempt to explain a part of the process of memory consolidation. The characteristic pattern of temporally graded RA following damage to the hippocampus suggests that at least some forms of explicit memory undergo a transition from hippocampal-dependence for recent memories to hippocampal-independence for remote memories. The central issues in system consolidation are quality of the recalled memory at recent and remote time points and the interplay of hippocampal-neocortical systems. There is significant interest in studies to identify the extra-hippocampal substrates underlying hippocampal-independent remote memories. There are also tasks which can be totally hippocampal-independent right from learning to retrieval. In terms of the memory systems, it could be that multiple memory systems are at work simultaneously, independently or associatively. While independent brain circuits can mediate different forms of memory, the age of

memory can also be a significant contributing factor for the involvement of specific brain regions. Modulatory systems also have a role to play in strengthening or weakening memory traces in different compartments. While SMSC and MTT offer divergent predictions on the temporal role of hippocampus in memory, there exists speculative evidence for the permanent indexing role of hippocampus in the network model of long-term memory. In terms of how memories are consolidated in the neocortex, it could be in the form of schema representation. In terms of memory traces, it could be multiple and competitive. Long term consolidation would involve cellular consolidation as well with protein synthesis and transport of products to both pre-synaptic and postsynaptic locations. The role of reinforcement, reactivation or replay in ensembles of neurons activated during a particular learning task has attracted research for studying this as a mechanism for consolidation, which could also explain biochemical and molecular changes which it can confer for long term storage.

The debate in the field of memory consolidation focuses on fundamental issues concerning the nature of the memory trace with regards to its maturation, persistence, retrievability, and modifiability. Resolution of this debate would firstly depend on distinguishing episodic and semantic components of memories. The time course of memory consolidation of various forms of memory is not yet well defined. In lesion studies, it would be important to look at the extent of lesions, type of tasks employed to test memory performance and duration of amnesia. The molecular, cellular and systems-level mechanisms underlying memory process to explain phenomena observed from both lesion experiments and computation modeling is needed for a complete working model of memory consolidation.

After reviewing the contemporary theories of memory consolidation, it becomes clear that even though the fact that memories need to be consolidated is widely accepted, the mechanism of memory consolidation with the involvement and contribution of brain regions to the process is

widely debated, crediting the realization that the issue is complex. It warrants several factors to be taken into consideration to formulate a precise theory of consolidation, including discussion of different processes for different forms of memory. For a well-defined theory of memory consolidation, a multi-factorial approach may be necessary, including but not limited to human vs animal studies, the time frame for consolidation, the instantiation of memory traces, memory age vs. memory precision or generalization, cellular vs systems consolidation, performance vs resistance to interference, semantic vs episodic memories, role of reactivation or replay and the possibility of memory systems. Given the complexity of the phenomenon at several levels, it is unlikely that there will be a generalized one-theory explanation to account for all forms of memory consolidation.

1.6 Stress and memory consolidation

1.6.1 HPA axis, hormones and interactions

The evolutionarily preserved hypothalamus-pituitary-adrenal (HPA) axis forms an important part of adaptive behavioural coordination through activation of multiple neuroendocrine responses. In response to sensory inputs, neurons in the hypothalamic paraventricular nucleus (PVN) release corticotrophin releasing hormone into the pituitary portal circulation, which in turn increases the secretion of adrenocorticotrophic hormone from pituitary corticotrophs, which through further downstream endocrine signaling stimulates glucocorticoid (GC) secretion (cortisol in humans, corticosterone in rodents) from the adrenal cortex and epinephrine from adrenal medulla (S. M. Smith & Vale, 2006). GCs regulate stress response through complex molecular mechanisms of inhibitory feedback, delayed feedback, and rapid feedback inhibition. In addition to episodic stress-response related GC secretion, it is influenced by circadian and ultradian rhythms. HPA axis

is also indirectly controlled by frontal cortex, amygdala, and hippocampus, while GCs through neuropeptide- and neurotransmitter-mediated mechanisms, influence synaptic modulation by interacting with GC receptors by crossing the blood-brain barrier. GCs have been demonstrated to promote neuroplasticity in terms of modifications of neuron morphology, neuronal excitability and synaptic efficacy in the hippocampal formation, amygdala, and PFC, and thus can influence memory acquisition and consolidation, fear response and working memory through the respective structures (Sandi & Pinelo-Nava, 2007; Uchoa et al., 2014). Synergistic adrenergic-GC interactions and interactions with other hormones, neuromodulators and neurotransmitters, more specifically, noradrenergic- and GC-mediated involvement of basolateral amygdala and its interactions with several brain regions and systems, including the hippocampus and neocortex are involved in mediating stress effects on memory enhancement (McIntyre, McGaugh, & Williams, 2012; Roozendaal & McGaugh, 2011).

1.6.2 Effects of cortisol on memory

Most discussion of stress-induced cortisol effects on memory phases take place in the light of the Roozendaal's model (Roozendaal, 2002): Differential effects of stress-induced cortisol on distinct memory phases and an adaptive mechanism of enhancement of consolidation with impairment of retrieval performance. Accordingly, stress-induced cortisol blocks retrieval process in favor of salient survival-related consolidation of current memory encoding, by strengthening of synaptic connections involved in the memory formation of the events that led to their release, and impairment of long-term memory retrieval.

Stress-induced cortisol effects on consolidation memory performance seems to be a multi-factorial and complex process: depending on the timing, intensity, and duration of stress in relation to memory task, it can either enhance or impair memory.

Timing of stress is critically important in GC-mediated effects on memory. Memory enhancement effect of stress-induction right after encoding and at the beginning of consolidation is well documented. Typically, memory enhancement effect of stress-induction shortly before and during encoding is dependent on the exact timing and the emotional salience of the information. Stress-induced GC-mediated facilitation of delayed recall in declarative memory tasks has been documented correlatively and in GC blocking experiments. Behavioral and electrophysiological studies in animals support a dose-response inverted-U shaped curve for effect of GCs. Stress and direct cortisol administration before or after encoding selectively facilitates consolidation of emotional memories relative to neutral episodic memories (Payne, 2010), through amygdala-mediated mechanisms and tagging of emotional memory traces (Bennion, Mickley Steinmetz, Kensinger, & Payne, 2015). Endogenous cortisol levels in emotionally aroused individuals at encoding correlate with enhanced memory consolidation. These effects are consistent for consolidation of memories that is enhanced by the presence of an emotional response (O. T. Wolf, 2009). GC-mediated memory consolidation of emotionally arousing events is a generally accepted phenomenon demonstrated in several human and rodent studies (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006; Roozendaal, 2000; Sandi & Pinelo-Nava, 2007). Post-training injections of GCs produce dose- and time-dependent enhancement of memory through low-affinity GC receptor-mediated signaling. On the other hand, blockade of GC receptors, shortly before or immediately after training has shown to impair long-term memory (Roozendaal & McGaugh, 2011). Acute, but not chronic stress has shown enhancement of memory consolidation (Drexler & Wolf, 2017; Schwabe, Joëls, Roozendaal, Wolf, & Oitzl, 2012).

On the other hand, in certain situations, experimental stress induction and GC administration leads to impairments in episodic memory. Stress before memory retrieval leads to an impairment (D. de Quervain, Schwabe, & Roozendaal, 2017; Roozendaal, 2002; Sandi & Pinelo-Nava, 2007; Schwabe et al., 2012; Thomas & Karanian, 2019; O. Wolf, Atsak, De Quervain, Roozendaal, & Wingenfeld, 2016). Acute cortisol elevations during wakefulness can also impair performance on episodic memory (D. J. F. De Quervain et al., 2003) and at retrieval (Dominique, Roozendaal, Nitsch, McGaugh, & Hock, 2000). Elevated cortisol can impair memory by affecting neuronal structure and function, disrupting neurogenesis, and blocking the synaptic plasticity in the hippocampus (Payne, 2010).

1.7 Sleep, theories of sleep function and electrophysiological correlates of sleep stages

The physiology and brain states in majority living species oscillates between two distinct global activity states: wakefulness and sleep. The regulation and function of these two vigilance states can be investigated over a broad range of temporal scales and at different levels of physiological functional organization from molecular to the whole brain and organism levels.

Sleep is a complex behavioural and physiological process. As a behaviour, sleep or a sleep-like resting state of reduced sensory engagement has been characterized in many species ranging from physiologically complex to much simpler organisms. Sleep has been defined on the basis of several behavioural and electrophysiological criteria. The most tested in several species are the behavioural criteria: species-specific posture, behavioural quiescence, reversible upon stimulation (as opposed to hibernation or coma), elevated arousal threshold (decreased responsiveness to sensorial stimuli) and rebound after deprivation (homeostatic regulation) (Vassalli & Dijk, 2009). Based on these accepted criteria, sleep has been characterized in several species of mammals,

humans, reptiles, amphibians, birds, insects, fish (zebrafish, *Danio rerio*), flies (*Drosophila Melanogaster*) and worms (*Caenorhabditis Elegans*) (Allada & Siegel, 2008). There seems to be great amount of variability in the quality and intensity of sleep characterized in terms of these defined criteria in different species (Siegel, 2008). Some might not show the periods of greatly reduced awareness or the rebound after deprivation that defines sleep during certain periods of their lives for long periods of time, including migratory birds and some marine mammals. Still others appear able to greatly reduce the amount of sleep or even go without it. Sleep in most species is governed by the circadian rhythm. Humans typically sleep during the dark period while rodents are typically nocturnal, sleeping during the light period. Even though, sleep is fragmented and poly-phasic in both the species, rodents exhibit several shorter cycles interrupted by brief periods of wakefulness (Genzel, Kroes, Dresler, & Battaglia, 2014), as depicted in Figure 4. Short wake episodes during the inactive period and short sleep episodes during the active period of the night is also a characteristic of rodent sleep (Tobler, 1995).

Sleep might be perceived as an unproductive state maladaptive for procreation, survival and self-protection from predators. However, the fact that sleep occurs in a plethora of different organisms to some extent, indicates its physiological significance. For example, humans spend approximately one third of their lives in sleep; bats and opossums sleep for 18–20 hours a day, while elephants and giraffes sleep for as little as 3–4 hours a day (Siegel, 2005). The adaptive importance of sleep is illustrated by the consequences of sleep deprivation in terms of physiology, cognition and even survival. Summarizing from several studies in rats, it was found that total sleep deprivation is lethal within 2-3 weeks (Rechtschaffen, Bergmann, Everson, Kushida, & Gilliland, 1989). There were several other physiological consequences of sleep deprivation in animals within days: hyperphagia but weight loss, increased heart rate and energy expenditure, decrease in body temperature, progressive debilitation and emaciation, development of severe ulcerative

hyperkeratotic skin lesions, increase in plasma norepinephrine and decrease in plasma thyroxine. Effects of sleep deprivation in humans underscores the importance of sleep. Within 65 hours, sleep deprivation results in bradyphrenia (slowness of thought and information processing), impaired cognition, loss of memory, decreased contact with reality, ataxia, decrease in body temperature and loss of body weight (Kamphuisen et al., 1992). There are metabolic consequences of sleep deprivation as well, including alterations in glucose metabolism, upregulation of appetite and increased energy expenditure, among others (Knutson, Spiegel, Penev, & Van Cauter, 2007). Prolonged partial sleep loss or chronic sleep deprivation can have severe implications on health by affecting physiological and cognitive functions (Banks & Dinges, 2007). Physiological consequences include a range of neuroendocrine, cardiovascular, metabolic and immune responses including reduced glucose tolerance, increased blood pressure, and increased inflammatory markers. Cognitive consequences include cognitive dysfunction with state instability, reduced alertness and vigilant attention, slowed working memory, reduced cognitive throughput, depressed mood, and perseveration of thought. Chronic sleep deprivation has been correlated with obesity, cardiovascular morbidity, traffic accidents and death. In summary, sleep is not only critical for physiological homeostasis and recovery but also for several essential metabolic, psychological, cognitive and immune functions.

1.7.1 Theories related to the function of sleep

The vigilant wakefulness state offers an unequivocal evolutionary advantage, while the functional significance of sleep is less evident and is a matter of several theories. Over years of scientific research, several different theories have been proposed and tested for the function of sleep; some seem to be mutually exclusive while others have mutually inclusive components

(Mignot, 2008; Siegel, 2005). Theories of sleep have been reviewed by several authors (Assefa, Diaz-Abad, Wickwire, & Scharf, 2015; Frank & Heller, 2019; Zielinski, McKenna, & McCarley, 2016). Based on these reviews, some of the most prominent theories are presented below:

Evolutionary or adaptive theory: This theory (also called preservation or ethological theory) suggests that sleep inactivity has adaptive function to increase safety during vulnerable periods by avoiding predators and that sleep inactivity, daytime activity and circadian rhythms evolved through natural selection and are present in the brain neurochemistry. Some animals hunt during the day because they need the light. Others come out at night to avoid diurnal predators. In addition to this, comparative research of animal species has shown that animals like bears and lions that have fewer natural predators, sleep between 12 to 15 hours each day, while animals that have several natural predators have shorter periods of sleep, usually 4 or 5 hours.

Energy conservation hypothesis: Sleep conserves energy, conserving resources for competitive advantage during wakefulness. Caloric demand, energy metabolism and body temperature are reduced during sleep. Similarly, energy allocation theory proposes that behavioral strategies optimize utilization of energy by diverting it to and from different biological processes as demands vary across behavioural states.

Restoration of reversal damage theory: This theory basically states that the homeostatic state of the body is restored in sleep. Sleep provides the body the opportunity to rejuvenate and repair components that were disrupted during wakefulness. It encompasses several physiological parameters including but not limited to cellular restoration, tissue repair, growth hormones replenishment, anabolic processes like muscle growth and protein synthesis, synaptic recovery of nervous systems, neuronal network reorganization, brain energy stores of glycogen metabolism, oxidative stress recovery and the clearance of neurotoxic products of neuronal damage and amyloid clearance.

Synaptic homeostasis hypothesis: This theory was originally proposed by Tononi and Cirelli in 2003 (Tononi & Cirelli, 2003, 2006). The major propositions of the theory as stated in the original paper are: 1. Wakefulness is associated with synaptic potentiation in several cortical circuits; 2. Synaptic potentiation is tied to the homeostatic regulation of a sleep state known as slow-wave activity (SWA) 3. SWA is associated with synaptic downscaling (i.e., a reduction in the strength of certain synapses); 4. Synaptic downscaling is tied to the beneficial effects of sleep on performance. The major prediction, that synaptic strength in the brain should be biased towards net potentiation during wakefulness and towards net depression during sleep has been confirmed using molecular (AMPA receptor expression), ultrastructural (axon-spine interface) and electrophysiological (spontaneous miniature excitatory postsynaptic currents) measures of synaptic strength (Cirelli & Tononi, 2021).

Information-processing theory: According to this theory, which is based on cognitive research, sleep facilitates processing of information acquired during awake learning into memory traces through changes at levels ranging from molecular to systems level reorganization. This is reviewed in detail in sections 1.10 and 1.11 as this is the theoretical basis of the current thesis.

Given evidence for several of these theories, it is clear that not one unitary theory can explain the function of sleep. Several mutually non-exclusive explanations can come together to elucidate the function of sleep from an adaptive and physiological perspective.

1.7.2 Sleep architecture, sleep-wake transitions and neurophysiological characteristics of sleep stages

Sleep architecture is structure of sleep, while hypnogram is a method for visualizing and studying this structure. Sleep architecture refers to the organization of sleep stages, which

orchestrate changes in biochemical, neurotransmitters and neuro-modulatory differences. These stages are usually characterized using electrophysiological correlates specific to the stages and features of transition states between them. The study of sleep architecture is important in human and animal sleep research, sleep disorder research and shiftwork and jet-lag research.

In terms of the organization of sleep stages in most mammals, it is usually subdivided into at least two stages - rapid-eye movement (REM) sleep and non-REM (NREM) sleep (Tobler, 1995). This classification is found in all mammals studied so far (Allada & Siegel, 2008). A typical sleep hypnogram with its electrophysiological features is depicted in Figure 4.

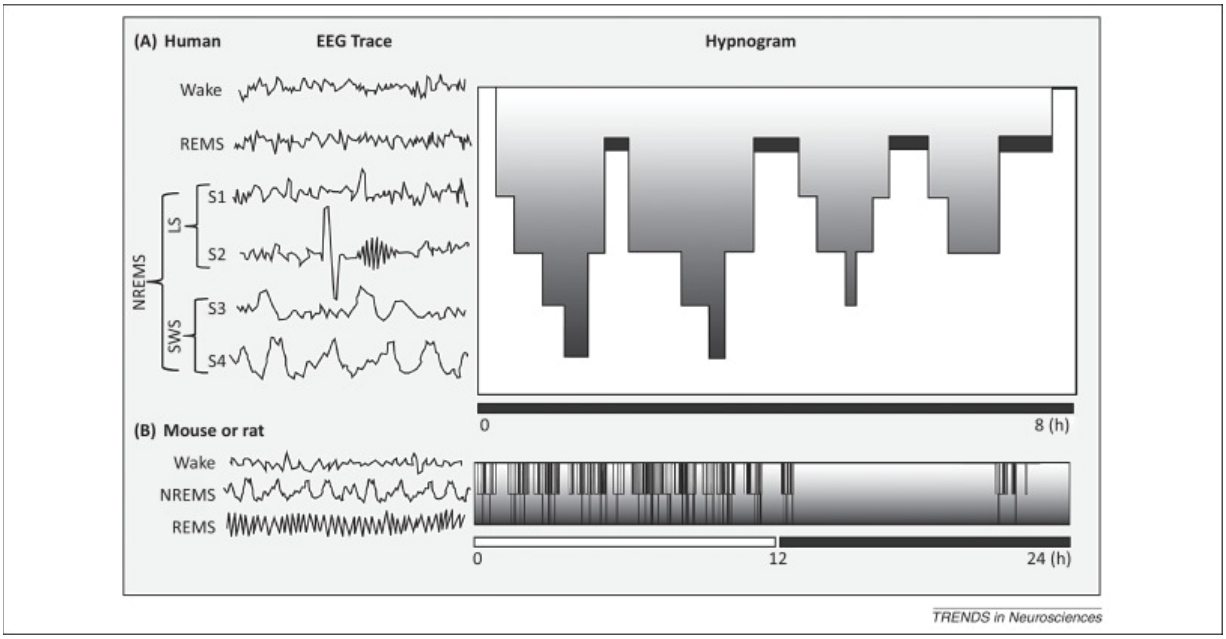


Figure 4: EEG traces of sleep stages with a representative hypnogram in humans and rodents

Note: Adapted from (Genzel et al., 2014): REMS and NREMS in mammalian sleep, which alternate in a cyclic fashion. **(A)** In humans, NREMS stages S1-4: light sleep (S1-2) and SWS (S3-4). SWS dominates the first part of sleep while REMS dominates later cycles. S2 light sleep seems to be the predominant sleep period. **(B)** Rats and mice are nocturnal and record polyphasic sleep with shorter NREM-REM cycles with interrupted waking. All NREMS is considered SWS.

The architecture of sleep, sleep-wake cycle, sleep-wake transitions and the transitions between the sleep stages is largely phylogenetically preserved (Tobler, 1995). The earliest work in

trying to find regions of the nervous system involved in state transitions was by Frederic Bremer (1935), who demonstrated that transection at the junction of the brainstem and spinal cord did not affect sleep-wake states and that spinal cord was not necessary for controlling state transitions. Electrical stimulation of the mesopontine reticular formation in anesthetized cats evoked desynchronized activity characteristic of wake and REMS (Moruzzi & Magoun, 1949) proposing an ascending reticular activating system in the dissection of state transitions in sleep-wake circuitry. The sleep-wake cycle and the transitions between the sleep stages are controlled by several brain regions, brain stem nuclei, cellular and molecular components, metabolites, neurochemicals and neuromodulators under homeostatic and circadian influence (Brown, Basheer, McKenna, Strecker, & McCarley, 2012; Datta & MacLean, 2007; Fuller, Gooley, & Saper, 2006; Saper, Fuller, Pedersen, Lu, & Scammell, 2010). Interactions between several brain regions (basal forebrain, locus coeruleus and hypothalamus), nuclei (ventrolateral preoptic nucleus (VLPO) of the anterior hypothalamus) and brain stem nuclei show activity patterns characteristics to promote stability and transition between the different states. Neuro-modulatory cholinergic, orexinergic and monoaminergic also contribute to the control of state changes. In general, sleep-wake and NREM-REM switches act together through complex reciprocal excitatory and inhibitory patterns. Studies suggest that brain mechanisms controlling waking and NREMS are strongly conserved throughout evolution (Brown et al., 2012).

The most global brain state changes are those observed across sleep-wake cycles as well as within various sleep stages, which are scored by their respective defining criteria. Most of the characterization of sleep stages heavily relies on specific patterns of electrical field potential oscillations obtained via electroencephalogram. In humans, skull surface electro-encephalogram (EEG), electro-oculogram (EOG) and electro-myogram (EMG) activity patterns are used as criteria for sleep-stage characterization in sleep clinics. In rats, the states of sleep are typically determined

by muscle activity levels in EMG and EEG activity patterns, distinguished by EMG and EEG power in particular frequency bands. Sleep scoring by EEG, EMG and EOG parameters in humans are governed by precise scoring manuals defined by strict criteria, updated as necessary (Iber, 2007). In rats, sleep scoring is not defined by standardized scoring criteria as in humans. Several scoring systems have been developed by rodent sleep scientists and computational experts using EEG and EMG, earlier through a time-intensive manual scoring and lately by several automated high-throughput sleep detection algorithms and even unsupervised real-time detection algorithms (Bastianini et al., 2015).

Wakefulness is characterized by high and variable EMG activity (indicating high muscle tone and sustained motor activity, especially in the neck muscles) and more importantly, brain cortical EEG activity measured as desynchronized high-frequency, low-amplitude waves with power concentrated in beta (14-30 Hz) and gamma (30-100 Hz) frequency ranges. During transition into sleep from wakefulness, a period of 'quiet rest' or 'quiet wakefulness' is characterized when eyes are closed, and cortical EEG activity displays prominent alpha wave oscillations (8-12 Hz). With the onset of NREMS, the waves show larger amplitude and slower frequency reflecting increased synchrony in firing of cortical neurons. These differences could reflect the timing of processing of cognitive, motor and perceptual functions (Fuller et al., 2006).

1.7.3 Sleep cycles

One of the characteristics of sleep architecture is the regular cyclic alternation of NREM and REM during a full night of sleep in an ultradian rhythm (recurrent rhythm in chronobiology ranging from fractions of hours to several hours throughout a circadian rhythm). This divides a sleep period into sleep cycles (alternation between NREMS and REMS): with light NREMS

followed by deep NREMS followed by REMS, with brief spontaneous awakening following most REM periods. The striking similarity of sleep and sleep stages in several mammalian species indicates some common evolutionarily preserved underlying mechanism of regulation (Tobler, 1995). Both human and rodent sleep alternates between NREMS and REMS, with some differences in the stability and length of sleep cycles. In healthy humans, a sleep cycle lasts for about 90 minutes with 4-5 cycles per night with the variation in cycle duration being related to NREM content. Sleep cycles in rats average about 10-13 mins with high variability (Trachsel, Tobler, Achermann, & Borbély, 1991).

The transition from wakefulness to sleep takes about 10 minutes in humans and seconds in rodents. After sleep onset, NREMS in humans progresses to predominant slow-wave activity (SWA) within 40 to 60 mins, while rodents enter NREMS within seconds after sleep onset and the transitions between NREMS and REMS approximately every 3-5 mins. NREMS is predominant during early part of sleep, with its duration and intensity reducing in later sleep, while REMS predominant in late sleep.

1.7.4 REM sleep (REMS)

REMS, also called active sleep or paradoxical sleep (PS), has characteristic electrophysiological signatures in brain and muscle activity, with physiological resemblance to the waking state in the terms of recorded brain activity. It is characterized by high frequency low amplitude desynchronized oscillatory activity (recorded from cortical EEG) and low EMG activity. In addition, rapid eye-movements (recorded by EOG, from where REMS gets its name), profound skeletal muscle atonia (except for extra-ocular and respiratory muscles) and vivid dreaming are the distinguishing characteristics of REMS (Hobson, Pace-Schott, & Stickgold, 2000; Saper et al.,

2010). Most importantly, electrically recorded neocortical low voltage fast activity (LVFA) and hippocampal rhythmical slow activity (RSA) and REM muscle tone are the defining electrophysiological criteria (S. M. Fogel, Smith, & Beninger, 2009; Gottesmann, 1992a). LVFA is composed of multiple frequencies and is similar to waking activity. RSA is high amplitude synchronous train of sinusoidal waves in the frequency range of 4 to 12 Hz (4-8 Hz or 6-10 Hz by different authors), also called theta activity (Stewart & Fox, 1990), being the hallmark of rodent REMS. In humans, scalp EEG do not record theta activity coming from the medial temporal hippocampus, but they have been detected as non-tonic activity during REMS (Fuller et al., 2006).The similarity in electrophysiological activity between wakefulness and REMS in terms of cortical LVFA and hippocampal theta is the reason why REMS has been categorized as active sleep or activated state.

In rodents, hippocampal theta oscillations are generated by cholinergic and GABAergic inputs from the pacemaker neurons of medial septum, which in turn receive and transmit tonic ascending input from the brainstem. A flip-flop switch model has been proposed for the mutual inhibitory interaction between REM-on (precoerulus area in pons, causing theta oscillations and sublaterodorsal area, causing atonia) and REM-off (periacqueductal gray and lateral pontine tegmentum) causing REM-NREM transitions. This switch, is in turn modulated by cholinergic inputs which promote REMS and noradrenergic and serotoninergic inputs which inhibit REMS (Fuller et al., 2006; Lu, Sherman, Devor, & Saper, 2006).

REMS has been subdivided into a tonic phenomenon (with a tonic and phasic component) and a phasic phenomenon (Robinson, Kramis, & Vanderwolf, 1977) with distinguishing characteristics:

Tonic REM: This continues throughout REMS with LVFA and RSA. Cholinergic input produces tonic RSA and LVFA during immobility, while non-cholinergic input produces RSA and LVFA

during phasic twitching of somatic musculature. Similar, but pharmacologically distinct neural system also produces these oscillations during wakefulness.

Phasic REM: This refers to brief and frequent bursts of REM occurrence during REMS. It is also characterized by somatic muscle twitches, ponto-geniculo-occipital spikes (intense burst of synchronized activity that propagates from the pontine brain stem to the lateral geniculate nucleus and visual cortex in animals) and presynaptic inhibition of sensory afferents.

1.7.5 NREM sleep (NREMS)

Like REMS, NREMS is also characterized by its distinguishing electrophysiological features. NREMS is characterized by low muscle tone in the EMG activity, absence or reduction of eye movement, synchronized large-amplitude slow oscillatory cortical EEG activity called slow-wave activity (SWA) with power concentrated in the delta (1–4Hz) frequency range, and fast large amplitude depolarizing event (40-100 msec) in the hippocampus. NREMS also has distinct physiological markers of decreased heart rate, respiration rate, blood pressure, and metabolic levels when compared to wakefulness (Siegel, 2009).

The classification of NREM sleep stages in humans has changed in sleep literature over time. NREMS was conventionally characterized into 3-4 successively occurring stages by different authors depending on different criteria. NREMS includes a deep slow-wave sleep (SWS, NREMS stages 3 and 4) and light sleep (NREMS stages 1 and 2) as successive stages, characterized as per the earlier classification of sleep (S. Diekelmann & Born, 2010; Gottesmann, 1992a). The first two stages (NREMS stages 1 & 2) are shallow, with easy awakening from sleep and the third stage (NREMS stage 3 & 4 combined) is deep sleep. The brain electrophysiological activity during each of the NREMS stages in humans is as follows (Fuller et al., 2006):

Stage 1: gradual disappearance of conscious awareness of the external environment, transition from alpha to slow 4-7 Hz theta oscillations in the EEG

Stage 2: complete loss of conscious awareness of the external environment, appearance of spindles transient (7-14 Hz/12-15Hz activity) and k-complex high-amplitude waveforms in EEG.

Stage 3 and 4: SWS or deep sleep with SWA or delta waves (1-3 Hz) in cortical EEG; increase in power, amplitude and incidence of slow waves defines increased sleep intensity during these stages. Neocortical autonomous SO are usually < 1 Hz, generally 0.5-2.0 Hz. Slow delta waves reflect synchronized oscillations as an output of thalamocortical circuit activity (Steriade, 2003a). Two sub-stages of SWS called low-amplitude sleep and high-amplitude sleep have also been characterized (B. M. Bergmann, Winter, Rosenberg, & Rechtschaffen, 1987).

American Academy of Sleep Medicine (AASM) changed its sleep stage classification in 2007, combining Stages 3 and 4 into a new stage N3, which begins when the brain achieves at least 20% delta waves and this was represented in later publications with N1, N2 and N3 as NREMS stages (B. Rasch & Born, 2013).

While, in rodents, all NREMS is commonly referred to as SWS (Genzel et al., 2014). It not separated into distinct stages as cortical activity transitions quickly from wakefulness to deactivated SWS. In rats, NREMS or SWS is characterized by slow cortical EEG activity, spindles, hippocampal SWR and low muscle tone.

Additionally, some authors have assigned a third and transitory sleep stage, not presented by human scalp EEG, called pre-REM. This short-lasting stage (for ~3.5 s) occurs mostly during the transition of NREMS to REMS. This has also been shown to occur during REMS to wake or REMS to NREMS transitions and is characterized by the intermingling of oscillations specific to both the stages: high amplitude spindles from the cortex superimposed on the nascent low frequency theta rhythm from the hippocampus and occipital cortex (Gottesmann, 1992a, 1996).

Multiple interacting neurotransmitter systems localized in the brain stem, hypothalamus, and basal forebrain converge onto common effector systems in the thalamus and cortex for sleep-wake transitions, specifically wake-NREM transitions. Genetic studies have suggested that the brain mechanisms controlling wakefulness and NREM sleep are strongly conserved throughout evolution, underscoring their enormous importance for brain function (Brown et al., 2012). Ventro-lateral preoptic area, median preoptic nucleus and basal forebrain contain a large number of GABA-ergic neurons which have sleep-on, wake-off firing patterns. Activity of VLPO neurons, which receive direct inputs from the retina and indirect projections from the suprachiasmatic nucleus via the dorsomedial hypothalamus, have been shown to directly correlate with NREM sleep time and EEG delta power. In the BF, caudally projecting, sleep-active, GABA-ergic neurons are interspersed with cortically projecting cholinergic, GABAergic, and glutamatergic neurons which show increased firing in correlation with cortical activation. Local, activity-dependent factors modulate the amplitude and frequency of cortical slow oscillations during NREM. A flip-flop model has been proposed for wake-NREM transitions. Mutually inhibitory interactions between VLPO neurons and neurons in the tuberomammillary nucleus (TMN), dorsal raphe nucleus (DRN) and locus coeruleus (LC) are thought to form a flip-flop switch, such that activation of VLPO leads to inactivity of TMN/DRN/LC neurons and promotes sleep, whereas activation of TMN-DRN-LC leads to inactivity of VLPO neurons and promotes wakefulness. Strong mutual inhibition between the two components of the switch creates a bi-stable state-dependent feedback loop, such that intermediate states of sleep and wakefulness are very brief.

1.8 Brain oscillations and Rhythmic oscillatory organization of neural networks

One of the characteristics of neurons is its organization into ensembles or networks with mutual interactions between a single unit and the network, affecting the activity of each other. One of the implications of this network organization is a collective and balanced activation-inhibition dynamics of population of cells generating rhythmicity of activity. Spatial and temporal dynamics of the activation of single neurons and activation pattern of neuronal populations across brain regions can be brought together mechanistically by neuronal coherence for effective neuronal communication for dynamic cognitive processing (Fries, 2005). Rhythmic fluctuations of excitation in the oscillating activation pattern of neuronal groups can provide temporal windows for effective communication. This phenomenon of coherence in oscillating neuronal groups opens simultaneous temporal interaction periods with synchronized input and output functions among them for optimal communication. Thus, cognitive flexibility can be a function of underlying flexible communication structures mediated by a flexible pattern of neuronal coherence.

Rhythmicity of activity generated by a collective and balanced activation-inhibition dynamics of population of cells in a network organization can be detected by measuring the extracellular potentials, as recorded with EEG with skull surface contact electrodes in humans or with depth electrodes measuring local electrical potentials called local field potentials (LFP) in animals (Buzsáki, Anastassiou, & Koch, 2012). Thus, brain oscillations, typically detected from EEG or LFP recordings from the extracellular space, represent neuronal population activity, which itself is a multi-factorial process, dependent on local structure and activity of neuronal processes, conductivity of extracellular medium, and electrode positioning.

The concept of brain oscillations started with the discovery of spontaneous alpha and beta waves in human EEG recording in 1929, by Hans Berger. This original discovery also pointed out to the state-dependent functional significance of brain oscillations as alpha wave was recorded

during relaxed wakeful state, and beta waves during attentive wakeful state. The fact that brain oscillations could be evoked comes from studies of Adrian in 1942, where he recorded gamma waves in response to stimulation. Thus, spontaneous brain oscillations reflect the current brain state while evoked potentials reflect brain response to cognitive stimulus (Karakaş, 2020; Karakaş & Barry, 2017).

Interest in the study of brain oscillations burgeoned in the following several decades with several recordings and studies from *in vitro* brain slices, *in vivo* brain tissue, EEG and ECoG recordings from humans as spontaneous and evoked response to stimuli, and LFP recordings from depth electrodes from animals and neurosurgical patients. Brain oscillations were studied in the context of cognitive and affective processes as an internal representation of stimulus-generated response in brain states in encoding and processing of various external stimuli at a cellular and population network level. This was further fuelled by changes in these oscillations in disease-states in several behavioural, neuro-cognitive and neuropsychiatric disorders implicating a prominent role for brain rhythms in complex cognitive brain operations (Karakaş & Barry, 2017).

The basic principles of brain oscillations have been elaborated as follows (Karakaş & Barry, 2017): (1) spontaneous EEG is composed of a set of oscillatory components of various frequency ranges, which can be separated by fast Fourier transform (FFT) power spectral analyses (2) the brain responds with oscillatory activity as a time-locked evoked response to sensory stimuli (3) post-stimulus oscillatory activity is a function of pre-stimulus activity, specifically amplitude and phase changes recorded as entrainment and phase-locking phenomenon (4) the temporal brain response results from superposition patterns of oscillatory components, reflecting information processing operations of the brain (5) every oscillation could have multiple functions and every brain function may have multiple underlying frequencies, (6) oscillations are spatially integrated,

reflecting intrinsic neural connectivity of sensory and cognitive functions through mechanisms of coherence and phase-locking.

The defining characteristics of oscillations are phase, frequency, amplitude and propagation velocity (Fell & Axmacher, 2011).

Phase of an oscillation is a population measure of average ensemble activity, reflecting neuronal excitability in the recorded population of cells at a temporal scale, determined by neuronal spiking. At a neuronal cell level, neurons can exhibit different patterns of activity across the phases. Phase synchronization reflects similarity in temporal coupling of neuronal spiking and hence excitability, which can strengthen neuronal connections at synaptic level (synaptic potentiation) based on Hebbian principles. Since field potentials are highly correlated with neuronal spikes, synaptic strengthening can be a result of phase synchronization, thereby leading to cross-regional communication. Phase synchronization can thus lead to gain of neuronal communication between brain regions, while phase desynchronization can lead to loss or reduction of communication between brain regions. Phase synchronization and desynchronization can define cognitive and hence behavioural outcomes through interactions between brain regions and underlying neuronal networks.

Frequency is the measure of number of oscillation cycles per unit time (typically per second or Hz). Frequency is determined by activity patterns of neurons and network properties. Different frequencies of oscillations can occur simultaneously within a given brain region. Frequency decomposition techniques identify oscillatory components of the recorded oscillatory waves and classify them into frequency ranges. Lower frequencies are slower oscillations lasting seconds, while higher frequencies are faster oscillations on a millisecond time scale. Mammalian forebrain activity is organized into oscillations spanning a wide range of frequencies between 0.05 Hz to 500 Hz, with experimentally defined frequencies having a linear progression logarithmic relationship

leading to the separation of frequency bands (G. Buzsaki & Draguhn, 2004). Conduction velocity limits of axons together with the frequency limits of extracellular matrix are the reasons why lower frequencies can travel longer and synchronize activity across distant brain regions allowing for computations at cross-regional network level, while higher frequencies travel shorter reflecting local circuit network processing and computations (Gyorgy Buzsaki, 2006). For e.g., SO occurring during SWS can synchronize several regions of the neocortex, while gamma rhythms coordinate local network processing.

Oscillatory synchronization is considered the most energetically efficient mechanism to maximize the probability of successful activation of downstream target neurons through spike-timing precision (G. Buzsaki & Draguhn, 2004). Oscillations within the same frequency band can interact across regions through phase-phase coupling while oscillations across frequency bands can interact by phase-amplitude coupling. Phase synchronization through cross-frequency phase coupling and phase-amplitude synchronization of different frequencies can form neural basis of high-level human cognitive processes like working memory, long-term episodic memory, language, and spatial cognition and reveal the functions of underlying neural circuits (J. Jacobs & Kahana, 2010). Phase-amplitude coupling can mediate consolidation of memory traces through neuronal synaptic plasticity mechanisms (Til O Bergmann & Born, 2018), specifically by local plasticity mechanisms mediated by global network oscillations (J. G. Maier et al., 2019). Cross-frequency coupling is the synchronization of oscillations of different frequencies, which can also be a functional mechanism for cross-regional communication and synaptic plasticity. Spindle-ripple coupling, spindle-SO coupling, ripple-SO coupling and SO-spindle-ripple coupling are important examples of cross-frequency coupling creating a platform for cortical-hippocampal communication (discussed in detail in sections 1.11.5-1.11.8).

Electrophysiological neural oscillations exhibit both periodic (center frequency, power, bandwidth) and aperiodic (broadband offset, exponent) properties (Donoghue et al., 2020). In addition to changes in true oscillatory power, broadband power and shifts in oscillation center frequency, changes in aperiodic exponent can also manifest as factor in observed narrowband frequency changes. Aperiodic activity has a 1/f-like distribution and exhibits exponentially decreasing power across increasing frequencies. Aperiodic exponent reflects the pattern of aperiodic power across the frequency range and is equivalent to the negative slope of the power spectrum. Similar to changes in oscillation bandwidth and oscillation center frequency observed across age and cognitive/behavioral states, aperiodic component also has putative physiological cognitive and perceptual interpretations. It has been observed to dynamically change with age, task demands and cognitive states. It reflects underlying physiological information as a factor of neuronal population spiking, blood-oxygen-level-dependent signal from fMRI, and integration of synaptic currents (Donoghue et al., 2020; Riddle & Frohlich, 2021).

The behaviour-dependence of oscillations, spatial and temporal dynamics of brain oscillations, synchronization or desynchronization at a temporal scale, brain connectivity at spatial scale, harmonic oscillators and the interactions between multiple frequencies of oscillations are the factors that establish the significance of brain oscillations as a cardinal player in local and global brain function. Several findings indicate that network oscillations perform a range of functions including input selection, temporally link neurons into cell assemblies at a network level, modulate synaptic plasticity at cellular level and facilitate information processing at a global brain level, combine and consolidate learned information, and can predict future probabilities through feed-forward and feed-back mechanisms (G. Buzsaki & Draguhn, 2004). Through serving these functions, these rhythmic oscillations seem to have functional relevance facilitating effective information encoding of external stimuli, information processing and information communication

across brain regions (G. Buzsaki & Draguhn, 2004). Functional relevance is also underscored by the phylogenetic evolutionary preservation of several brain oscillations. To further underscore the importance of brain oscillations, many neuro-psychiatric disorders including schizophrenia, bipolar disorder, depression, dementia, Alzheimer's, and eating disorders are associated with abnormality of brain oscillations, disrupted synchrony between oscillations and disruptions in sleep rhythms (Başar, 2013; Brown et al., 2012; Schnitzler & Gross, 2005).

1.9 Sleep Brain oscillations

The most prominent and well-characterized distinguishing electrical field potential oscillations during SWS are neocortical SO (0.8 Hz), delta waves (0.5-4 Hz), k-complex, thalamocortical spindles (10-15 Hz), and the hippocampal sharp wave-ripples (100-300 Hz). Hippocampal theta activity has been found to be functionally relevant during awake-stage information processing as well as in REMS. The properties, generation mechanisms, functional relevance and interactions of these oscillatory components are further elaborated in this section and in section 1.11.

1.9.1 Slow oscillations: UP and DOWN states

The largest global brain excitability changes detected as SO in the neocortical recordings is the cardinal rhythm during SWS and deep anesthesia. The first indication of global cortical oscillatory activity came from electrocorticography (ECoG) recordings during and after frontal topectomy of schizophrenia patients, which produced a significant reduction in electrical activity of the undercut cortex and the development of what the authors called "suppression bursts activity" alternating with quiet periods or sleep-like states, not directly apparent in EEG (Henry & Scoville,

1952). But it was not until 1993, that SO, as we know it, were characterized. Slow oscillations (SO) or Slow-wave activity (SWA) defining SWS, are in the ultra-low frequency band of < 1 Hz (0.5-1.5 in LFP in rodents and about 0.8 Hz in humans), originally described and characterized as depolarizing and hyperpolarizing components (S. Diekelmann & Born, 2010; Steriade, Nunez, & Amzica, 1993b). SOs have since then been characterized in cortical LFP recordings under anesthesia (Mircea Steriade, Angel Nunez, et al., 1993b), in hippocampus (Wolansky, Clement, Peters, Palczak, & Dickson, 2006), in neostriatum (C. J. Wilson & Kawaguchi, 1996), in basal ganglia structures (subthalamic nucleus and globus pallidus network) (Magill, Bolam, & Bevan, 2000), in basolateral amygdala (Crane, Windels, & Sah, 2009), in cerebellar cortex (Roš, Sachdev, Yu, Šestan, & McCormick, 2009), in neocortical slices (Sanchez-Vives & McCormick, 2000), in isolated cortical slabs (Timofeev, Grenier, Bazhenov, Sejnowski, & Steriade, 2000), *in vitro* thalamic neurons (Hughes, Cope, Blethyn, & Crunelli, 2002), during natural sleep and anesthesia (Sylvain Chauvette, Crochet, Volgushev, & Timofeev, 2011), quiet wakefulness (Petersen, Hahn, Mehta, Grinvald, & Sakmann, 2003) and in human EEG (Achermann & Borbely, 1997; Marcello Massimini, Huber, Ferrarelli, Hill, & Tononi, 2004).

SO is characterized by neocortical oscillations which reflect two distinguishing but synchronized alternating periods of active excitatory depolarized state and silent inhibitory hyperpolarized states as a cellular phenomenon tied to neuronal firing (Steriade, Contreras, Dossi, & Nunez, 1993; Steriade, Nunez, & Amzica, 1993a; Steriade, Timofeev, & Grenier, 2001). Bistable balanced network exhibiting non-rhythmic state transitions in a resting brain were observed from spontaneous population activity in somatosensory cortical neurons of urethane-anesthetized rats (Jercog et al., 2017). The prominent alternating feature of high neuronal activity followed by almost complete neuronal silence of excitatory cortical pyramidal cells and inhibitory

interneurons is unique to SWA, not recorded in wakefulness or during REMS (Steriade et al., 2001).

Neurons involved in the cortical SO have been observed to have bimodal, sub-threshold (subthreshold for action potential generation), and spontaneous fluctuations of cortical cellular membrane potential membrane potentials, which oscillate between a depolarized state with superimposed action potentials and a hyperpolarized state without action potentials. Fluctuations of membrane potentials which underlie SO are detected as depolarized (active/excitatory) or UP-state and silent/hyperpolarized as DOWN-state (silent/inhibitory) (C. J. Wilson & Kawaguchi, 1996) or also called UP-DOWN states (UDS). Membrane potential alternation between the two preferred states (hyperpolarized state from -61 to -94 mV and more depolarized state from -71 to -40 mV) define UDS at a cellular level (C. J. Wilson & Kawaguchi, 1996). The membrane potential of cortical neurons during SWS shows bimodal oscillation between -62mV mean depolarizing and -70mV mean hyperpolarizing states (Timofeev & Chauvette, 2011).

The cellular mechanisms of UDS have been investigated. DOWN-states at a cellular level could be a result of synaptic depression of active synapses through disfacilitation rather than by active inhibition (Diego Contreras, Timofeev, & Steriade, 1996; Timofeev, Grenier, & Steriade, 2001) involving slow inactivation of the persistent Na+ current, activation of Ca2+-dependent K+ and the Na+-dependent K+ leak currents which would displace the membrane potential of neurons from the active firing state and the entire network could possibly go to the hyperpolarized or silent state (Timofeev & Bazhenov, 2005). Recovery of active UP-state of depolarization could be the dynamics of some intrinsic currents in cortical neurons including the low-threshold hyperpolarization-activated cation current (T-type Ca2+ and Na+ currents) which can organize their oscillations in the delta frequency range and contribute to the maintenance of depolarizing membrane potential set up by post-synaptic generated potentials amplified by persistent Na+

current (Timofeev & Bazhenov, 2005). In addition, several other mechanisms have been described that could contribute to UP state initiation (Neske, 2016; Neske, Patrick, & Connors, 2015): including summation of excitatory post-synaptic potentials (mEPSPs) from spontaneous events (Sylvain Chauvette, Volgushev, & Timofeev, 2010), astrocytic glial cell regulation (Poskanzer & Yuste, 2011) and the fact that a subpopulation of layer 5 cortical pyramidal cells have intrinsic rhythmic low-frequency burst oscillatory properties that can maintain the SO (Lőrincz et al., 2015). Cortical layers L4/5 (Capone et al., 2019), L5 (Wester & Contreras, 2012) and L6 (Mattia et al., 2021) as possible origin layers of SO indicate its origin in deep cortex. Multiscale dynamics underlying neocortical SO) have functional significance in the initiation of UP-states.

1.9.1.1 Origin of UDS

Although SO generation has a predominant input from the cortical structures as demonstrated by the detection of SO *in vitro* cortical slices (Sanchez-Vives & McCormick, 2000) and isolated cortical slabs (Timofeev et al., 2000), and by the absence in decorticated thalamus (Timofeev & Steriade, 1996), thalamus has been shown to play a critical complex role in fine tuning the frequency of SO through a dynamic interplay between the regions for the full manifestation of SO (Crunelli & Hughes, 2010; David et al., 2013; Lemieux, Chen, Lonjers, Bazhenov, & Timofeev, 2014). Both cortical and thalamic contributions through the mutual interactions of neocortical neurons, reticular thalamic neurons (TRN) and thalamocortical (TC) neurons generate SO with the characteristic UDS in an intact brain (Timofeev & Bazhenov, 2005). The strong dynamic interaction of three cardinal oscillators in corticothalamic module: one predominantly synaptic based cortical oscillator and two intrinsic, conditional thalamic oscillators are equally and mutually

important for the emergence of UDS and manifestation of slow sleep oscillation with all its properties (Crunelli & Hughes, 2010).

Cellular mechanisms of the synchronous regular transitions and switching between the two stable UDS have been investigated in cortical and thalamic invitro slice preparations as follows:

Cellular mechanisms of the cortical UDS: Strong synaptic excitatory and inhibitory activity in the deeper cortical layers (V/VI) and not in superficial layers, reliably generates UDS. The UP state is terminated by the activity dependent K+ conductance causing hyperpolarization and a brief refractory period immediately following the end of an UP-state. K+ channels maintain the DOWN state (Sanchez-Vives & McCormick, 2000). The synaptic recruitment of increasing number of neurons in both the states generates the synchronous SO.

Cellular mechanisms of the thalamic UDS: Cortical input-driven excitatory glutamatergic activation of TC and TRN neurons triggers their intrinsic rhythmic oscillation of membrane potential at SO frequency (Crunelli & Hughes, 2010).

Thus, the high synchronicity of UDS is thought to be an emergent property of mutual thalamo-cortical interactions specifically between the three oscillators: cortical, TC and TRN cells. (Crunelli & Hughes, 2010).

1.9.1.2 Propagation of SO as a traveling wave

Early studies showed a homogenous pattern of cortical SO, but several later studies involving simultaneous high density EEG recordings over large cortical areas investigated the directionality and spatio-temporal dynamics of SO generation and subsequent propagation in both humans and animals. In anesthetized cats, neurons recorded simultaneously from differently located foci in the association, visual and motor cortices discharged synchronously during SO

(Amzica & Steriade, 1995b). A high density human EEG study demonstrated a traveling slow wave over large distances on a spatio-temporal scale with a distinct local focus point of origin, which can start at any cortical region and propagate to other cortical regions, but preferentially starts in prefrontal-orbitofrontal cortical regions and propagates across the neocortex in an anterior-posterior direction in adults (Marcello Massimini et al., 2004).

Several studies have investigated the origin and propagation dynamics of SO waves. Even though most recordings point to a frontal origin, the exact location of origin of SO and their propagation show slightly different patterns. Simultaneous scalp EEG, intracerebral EEG, and unit activity recordings from multiple brain regions of neurosurgical patients revealed that slow waves originate from medial PFC and propagate to the medial temporal lobe and hippocampus (Nir et al., 2011). Simultaneous scalp EEG and intracranial recordings in epileptic subjects showed that majority of the preferential frontal location of origin in the brain and had a tendency to travel to posterior and temporal regions (Botella-Soler, Valderrama, Crépon, Navarro, & Le Van Quyen, 2012). Simultaneous EEG-fMRI recordings to record electrophysiological slow waves and cortical-subcortical hemodynamic (BOLD) fluctuations showed a time-locked similar propagation of both measures from centro-frontal to inferior temporo-occipital cortices (Betta et al., 2021).

The evidence of SO as a propagating wave also comes from neuronal recordings. Extracellular unit recordings from layer 5 neurons showed that slow wave activates neurons and in particular, stereotyped sequences, in a unique spiking pattern regardless of the wave direction (Luczak, Barthó, Marguet, Buzsáki, & Harris, 2007) suggesting a systematic intrinsic drive upon slow wave activation. The neuronal activity patterns also showed a traveling-wave characteristic evident from sequential activity through simultaneous recordings from populations of cells in neocortical layer V of ketamine/xylazine-anesthetized rats (Luczak & Barthó, 2012).

Several different propagation patterns have been recorded in different studies. A focal origin of SWA from between cortical areas 5/7 with a variable spread in both anterior and posterior directions was observed (Volgushev, Chauvette, Mukovski, & Timofeev, 2006). A medio central propagation path along the frontal gyri, anterior and posterior cingulate and the precuneus was proposed for the spread of SO (M. Murphy et al., 2009). Spatio-temporal heterogeneity of cortical slow waves displaying diffused-focus peaks of activity, with complex local propagation patterns synchronized bilaterally across the hemispheres was observed with VSDI studies in mice (Mohajerani, McVea, Fingas, & Murphy, 2010). Different propagation patterns such as spiral waves (Huang et al., 2010), and non-linear complex reciprocal circular local patterns of SO propagation (Hangya et al., 2011) raise the possibility of complex propagation patterns emerging at a fine-tuned spatio-temporal scale.

Differences in the propagation of slow waves in terms of the components of UDS have also been speculated. It seems as though active UP-state onset starts focally in the neocortex and propagate to other regions in anterior-posterior direction (S Chauvette, Volgushev, Mukovski, & Timofeev, 2007; Sheroziya & Timofeev, 2014; Volgushev et al., 2006). The simultaneous onset of silent DOWN state in separated cortical regions suggests a global mechanism of synchronization (Sheroziya & Timofeev, 2014; Timofeev & Chauvette, 2011; Volgushev et al., 2006).

Human studies have also revealed an age-dependence on the origin and propagation of slow waves. The timing and location of the topography of SWA closely follows anatomical and behavioral developmental changes of cortical maturation (Kurth et al., 2010). Neuroimaging studies of regional assessment of gray matter development revealed a postero-anterior trajectory of cortical maturation from early childhood to late adolescence. Following the cortical development, a more posterior cortical origin of SO in early childhood which progressively moves to anterior frontal locations into adolescence and adulthood was observed. Another study showed a centro-

parietal origin of scalp SO in young children and frontal origin in adults propagating across the cortex in complex patterns (Timofeev et al., 2020).

1.9.2 Delta oscillation and slow oscillation

There seems to be an inconsistency in the use of terminologies - SO, delta wave or delta oscillations and even k-complex in the literature. Some have used these terms interchangeably, while others differentiate between them in terms of their electrophysiological characteristics. The discovery of SO warranted a redefinition of delta waves/oscillations. Early studies initially distinguished between SO and δ waves as separate neurophysiological low-frequency with different spatio-temporal properties. Classical delta wave occurs as indistinct peaks in the frequency band 0.5-4 Hz and SO between 0.5-1 Hz or <1 Hz oscillations (Florin Amzica & Mircea Steriade, 1997; Steriade, 2003a; Steriade, 2006; Mircea Steriade, Angel Nunez, et al., 1993b; Steriade & Timofeev, 2003). SO is a generally accepted term for <1Hz oscillations and delta between 1-4Hz. In fact, the term SWS is linked to the occurrence of these unique large amplitude delta wave patterns (1-4 Hz) as detected in deep cortical LFP and scalp EEG recordings (Sirota & Buzsáki, 2005; Steriade, 2006) and in immobility and deep anesthesia (Steriade, McCormick, & Sejnowski, 1993). The neocortex can also generate intrinsic SO (primarily in 0.5-1.0 Hz frequency range, but also in the 1.0-4.0 Hz frequency range) (Arrigoni & Fuller, 2012). Delta waves (1.0-4.0 Hz) are referred to as SWA in EEG during N3 deep sleep of NREM. Appearance of delta waves with increased power and amplitude in cortical EEG during NREMS is defined as a measure of increased sleep intensity reflecting synchronized oscillatory activity of thalamocortical circuit. However, according to Sirota and Buzsáki, slow waves and delta oscillations are characterized in different frequency bands, they are not separate patterns from each other and are tightly linked such

that delta wave/oscillations are equivalent to DOWN states of SO which correspond to transient periods of cortical silence (200–500 msec) tied to the cessation of synaptic and spiking activity of principal and interneuron firing in all cortical layers, followed immediately by periods of increased activity (0.3–1 sec) (Sirota & Buzsáki, 2005). Hence, SO have a delta wave component which can be used as a measure of SO to define electrophysiological NREMS. Both delta wave and SO are a result of complex thalamo-cortical interactions in intact brains, though primarily arising from neocortical networks. Rhythmic series of delta waves (DOWN states) at delta frequency during N3 SWS are a result of regular thalamic output to advance the phase of intrinsic neocortical SO (A. Kandel & Buzsáki, 1997). Hence, many studies use delta oscillation as an effective measure of SO, and further for defining NREMS periods (Sirota & Buzsáki, 2005).

The original definitions of the terms were as follows (Timofeev et al., 2020):

Slow Wave (SW): individual negative-positive wave in the scalp EEG or LFP deflection lasting several hundred milliseconds. Repeated slow waves measured with intracellular or intracranial EEG is SO.

Slow Oscillation (SO) or Slow Rhythm: cyclic cellular activity of <1 Hz as a periodic process (Hz), consisting of an alternation of active and silent states, measured with intracellular, depth electrodes or intracranial EEG from sleeping animals and humans.

Delta Waves or Slow Wave Activity (SWA): wave activity in scalp EEG or intracranial EEG (μV^2), in the delta frequency (0.5–4.5 Hz) expressed as EEG power density quantified from spectral analysis in the frequency range in the human scalp EEG.

Elaborating on these definitions, slow wave is a single wave manifesting the underlying SO (bistable periodic rhythm) measured as synchronous membrane potential transitions between DOWN and active UP states. In deep sleep, quantification of scalp-SW from scalp EEG in the delta frequency (0.5–4.5 Hz) band as an integrated signal is slow wave activity (SWA in μV^2) (Timofeev

et al., 2020). Morphology of the scalp-SW is determined by the degree of synchronization across cell populations. High amplitude, steep slope and more global propagation results from highly synchronized population SO activity, while low amplitude, flatter slope and local propagation indicates low SO synchronization among neuronal cell populations. Simultaneous SWs originate from different scalp locations and are recorded as overlapping SWs with multiple peaks (Timofeev et al., 2020). As such, slow (<1 Hz) and delta (1–4 Hz) activity is prominent in N2 NREMS. Slow and delta oscillations or isolated slow waves are referred to as SWA. Delta sleep or SWS or N3 NREMS is characterized by the high amounts of SWA. SWA is the predominant neocortical activity with the increased power density in 0.5–4.0 Hz frequency range (Y. F. Lee, Gerashchenko, Timofeev, Bacskai, & Kastanenka, 2020).

The frequency range of these oscillations used in scientific literature show some differences as well. The frequency of SO in animals under anesthesia ranges between 0.1 - 1 Hz (89% of them between 0.5 and 0.9 Hz) and in human natural sleep between 0.5-1.5 Hz (Achermann & Borbely, 1997; Florin Amzica & Mircea Steriade, 1997; Amzica & Steriade, 1998; Mölle, Marshall, Gais, & Born, 2002). Studies of SO in rats have used 1–4 Hz (Johnson, Euston, Tatsuno, & McNaughton, 2010; Mölle, Yeshenko, Marshall, Sara, & Born, 2006). The term 'slow wave' refers to both SO (<1 Hz) and delta oscillation (0.75–4.5 Hz) (Hubbard et al., 2020). In some studies, human slow EEG frequencies are divided into two components, slow waves (1–4 Hz) vs SO (<1 Hz) (Achermann & Borbely, 1997; Mölle et al., 2002). Delta waves could include two different components depending on site of cellular origin and mechanisms: thalamic component linked to thalamo-cortical intrinsic neuronal oscillations with cortical input-driven hyperpolarization-activated inward currents to < -65 to -70 mV and a cortical component independent of thalamic inputs (Steriade, 2003a).

Recent studies in mice and humans have characterized two distinct delta frequencies, δ1 and δ2 with different properties in recovery sleep, suggesting distinct neurophysiological substrates triggered by sleep deprivation (Hubbard et al., 2020):

δ1: slower δ (0.75–2 Hz) independent of sleep deprivation with a slow, linear decay

δ2: faster δ (2.5–4.5 Hz) dependent on sleep deprivation, increased power with fast, discontinuous decay during recovery sleep.

In addition to this classification, two distinct classes of slow waves or delta oscillations (1–4.5 Hz) during NREMS have been characterized across species (Bernardi, Siclari, Handjaras, Riedner, & Tononi, 2018; Genzel et al., 2014; Mölle et al., 2002; Siclari et al., 2014). They differ in amplitude, spread, sites of cortical origin, timing and distribution resulting from different synchronization processes(Siclari et al., 2014).

Type I slow waves: larger amplitude, global slow waves from a bottom-up subcortico-cortical synchronization process predominating in early sleep phase.

Type II slow waves: smaller amplitude, local slow waves from a horizontal corticocortical synchronization process that predominates in the late phases of sleep.

The type I slow (SO) and type II slow wave (δ waves) have been shown to have distinct and competing roles in determining the extent of memory consolidation during NREMS indicating that these two phenomena drive differential activity-dependent processing as an outcome of reactivation of neural ensembles during NREMS (J. Kim, Gulati, & Ganguly, 2019).

Another recently proposed classification of slow waves considered *transition frequency* of slow waves as a distinguishing feature and characterized slow switchers (slow waves with a slow transition) and fast switchers (slow waves with a fast transition) (Bouchard et al., 2021). Fast switchers were predominant in early sleep while slow switchers predominated later stages of sleep. The proportion of slow and fast switchers in normal sleep and recovery sleep, their pattern of

homeostatic decline and underlying brain functional connectivity indicates a possibility of their differential contribution to sleep-dependent memory consolidation processes and sleep-dependent cognitive processes.

1.9.3 k-complex (KC)

KC was first described in 1938 as an interesting large potential change with same amplitude and period as delta waves, evoked by auditory tone stimulation with a negative and positive component (positive-negative-positive waveform) (Loomis, Harvey, & Hobart III, 1938). They were not considered to be electrical or movement artifacts, could appear spontaneously, were most prominent in the top and least on the back of head, could spread over a large area and were followed by 8 to 14 oscillations. Loomis described the KC as a large triphasic deflection in surface EEG potential, with two distinct components, a slow component which we now refer to as the KC, and a fast component characterized by a high-frequency discharge, which was likely an alpha burst or sleep spindle. The features of KC were characterized as follows: major grapho-element unique to sleep; characteristics vary with sleep stages; usually follows auditory stimulation during sleep; auditory evoked but not with light or shock; evoked and spontaneous; quantifiable component structure with negative peak -50 to -100mV shortly after stimulating tone and positive +100mV peak with a 750ms delay from negative peak; higher voltage in frontal than posterior part of brain; refractory period of about 2-3 sec within a positive wave (Davis, Davis, Loomis, Harvey, & Hobart, 1939).

While some argue that the human KC is a unipolar EEG wave reflecting only the neuronal hyperpolarizing event (Cash et al., 2009), most literature supports a characterization of KC with three waves (positive-negative-positive in scalp EEG recordings) composed of a down-state

followed by an up-state and that, rhythmicity at variable degrees is a function of sleep depth (Amzica, 2010; Amzica & Steriade, 1995a). KC is now generally considered a large biphasic deflection, not including spindle oscillation and lasts 0.5 seconds or less. The KC is characterized by a large negative deflection of hyperpolarizing phase followed by a strong positive deflection of depolarizing phase but can also include a smaller depolarizing vertex sharp wave that precedes the early slow phase.

KC also formed the criteria for sleep stage classification as rhythmic KCs were identified during various sleep stages. The first stages of sleep, when SO appears less organized and synchronized, are characterized by less regular KC. In deep sleep, when SO appears more regular, synchronized and faster closer to 1Hz, KCs could be confounded with delta wave (Florin Amzica & Mircea Steriade, 1997; Steriade & Amzica, 1998). KC and delta-wave are also believed to be subcomponents of the SO; with the KC manifesting as a sharp down-to-upstate transition, and the delta wave as a slower oscillation encompassing the entire downstate (Steriade & Amzica, 1998).

Focal bipolar depth electrode recordings in human surgery patients showed that although KC are a universal cortical phenomenon, each KC may involve several local cortical regions variably with a variable directionality propagation dynamic (Latreille et al., 2020; Mak-McCully et al., 2015).

1.9.3.1 Mechanism of generation

The understanding of the cellular mechanisms of KC generation came from simultaneous recordings of intraneuronal activity, LFP and EEG. Sequences in membrane potential depolarization and hyperpolarization of cortical neurons are manifested as the two phases of KC in an EEG pattern, the initial surface positive wave and the subsequent surface negative wave

respectively. The shape and frequency of KC are modulated within the different sleep stages, due to changes in the cortico-thalamic network leading to the speculation that this periodic alternation could indicate different brain states. The depolarizing phase represents a cortical network that would be ready to operate in case of danger to wake up from sleep and the hyperpolarizing phase represents a brain state at rest (Amzica & Steriade, 2002).

Several studies from the Steriade group elucidated the production of KC and their cellular and network mechanisms in animal under anesthesia as a distinct phenomenon from SO and delta wave (Florin Amzica & Mircea Steriade, 1997). Emerging from cortically generated SO (<1-Hz), KC could be viewed as a single SO cycle followed by spindle activity. Human EEG and cat cellular and LFP recordings demonstrated that the shape of KC results from and correlates with periodic cortical neuronal excitation and inhibition in a synchronized cortical network manifested as peaks at 0.5 to 0.7 Hz (0.6–0.9 Hz). The spectral content in the 1-4 Hz delta band is partially influenced by the shape and duration of KC. SOs are related to KCs by the cortico-thalamic network activity. At a cellular level, rhythmic membrane depolarizations and hyperpolarizations of cortical neurons underlies the SO. Then first component of the SO is the positive wave and depolarizing phase of SO, expressed as KC and the second component of the SO is the hyperpolarizing phase. Taken together, SO is reflected in EEG recordings as rhythmic sequences of surface-negative waves of cellular membrane hyperpolarization and surface-positive (KC) cellular membrane activation through depolarization (F Amzica & M Steriade, 1997). The fact that cortical, but not subcortical volume contributes to K-complex amplitude is evidence of the importance of cortical input for KC generation (Colrain et al., 2011). KCs are also considered a sharp down-to-up state transition in the LFP, resulting from a shift from an absence of local neuronal activity to a fast synchronous discharge of local neurons (Johnson et al., 2010; Sirota & Buzsáki, 2005).

Some authors have proposed that SO and KC are different manifestations of the DOWN–UP state transitions of the same neuronal population event and that this transition of SO cycle takes the form of KC. Hence KC is not a separate entity but corresponds to the DOWN–UP transition in synchronous cortical neurons as reflected in the LFP as a fast transition (Sirota & Buzsáki, 2005). It is identifiable both in the LFP and as the sum of individual neuronal spiking at a population level (Johnson et al., 2010). It is speculated that SO can organize KC and spindles in defined temporal sequences, even though both can occur in isolation (Sirota & Buzsáki, 2005). KC can also be considered as a forerunner of delta waves based on variations of KC over the night and during transitions to SWS (De Gennaro, Ferrara, & Bertini, 2000).

Evoked KC is used as an index of delta and sometimes the terms ‘delta’ and ‘KC’ are used interchangeably (Colrain et al., 2010). Evoked KC in human EEG was shown to have a short positive voltage peak at about 200msec (P200), followed by a bistable cortical response: a large negative complex at around 550msec (N550) and a long-lasting positive peak at 900msec (P900), respectively reflecting down and up states of < 1 Hz SO, and eventually the potential ends with negative-positive complex after swings at 1500 and 1900 ms, respectively (Halász, 2005; Ujszászi & Halász, 1988). P200 could be a bottom-up propagating excitatory cortical response to a sensory stimulation, which could trigger the opening of K+ channels, which in turn causes cortical hyperpolarization triggering the N550/down state of electrical silence, while the P900 could be a result of recursive cortico-thalamo-cortical activities. Higher the level of excitatory activity sustaining P200, the higher the probability of triggering a down state and higher the local bistability (Laurino, Piarulli, Menicucci, & Gemignani, 2019).

1.9.3.2 KC in sleep-arousal decision and information processing

The large biphasic deflection of the KC is believed to be a transient and rapid processing of external stimuli, such as a knock or any other arousing stimuli, and if processed by the vulnerable sleeping brain to be non-threatening and unimportant, neural activity is suppressed to prevent the brain from activating arousal responses; as a form of sleep preservation (Colrain, 2005).

Evoked KC is considered to suppress brain arousal to external stimuli as an all or none phenomenon without a graded response related to stimulus intensity (Bastien & Campbell, 1992). Evoked KC has classically been associated with auditory (like the clap of someone's hands or snap of one's fingers) and somatosensory stimuli while recording local field potential activity under anaesthesia or sleep. The original observation by Oswald et al., in 1960, that significantly more KCs were elicited during sleep when the subject's name was called out as opposed to an insignificant neutral name, suggested that KCs may be important as stimulus-significance detectors and therefore important in sleep-related information processing (D. G. McDonald, Schicht, Frazier, Shallenberger, & Edwards, 1975; Oswald, Taylor, & Treisman, 1960). Brain responses to subject's own names and unfamiliar names uttered by an unfamiliar voice evoked stronger brain responses compared with a familiar voice (Blume, Del Giudice, Wislowska, Heib, & Schabus, 2018), and the processing of the difference in this information saliency was found to be manifested in the evoked KCs (Ameen, Heib, Blume, & Schabus, 2022). In addition, the decrease in the number of unfamiliar voice-evoked KCs in the second half of the night compared with the first half of the night was interpreted as being indicative of the sleeping brain continuing to learn new information during sleep (Ameen et al., 2022).

In addition to processing external salience of information, spontaneously generated KCs during sleep are thought to facilitate internal information processing. KCs could also be processing internal salient information related to recently encoded memory traces (Colrain, 2005).

Spontaneous KCs during natural sleep and anaesthesia tend to precede transitions from stage 2 NREMS to deep SWS. Additionally, the observation that KCs do not occur during transitions from stage 2 NREMS to paradoxical sleep reinforces its role in sleep preservation (Forget, Morin, & Bastien, 2011).

A few possibilities could be considered for the functional role of KCs in the sleep-arousal decision making. It is possible that salient information merely brings the sleeping brain closer to an arousal state closer to wakefulness and a large KC is generated to maintain sleep. KCs could thus be a manifestation of the brain's salience detector, indicating the salience of external stimuli processing by the sleeping brain. Such a mechanism would be important evolutionarily for survival during this vulnerable state. In short, KCs are speculated to be related to stimulus relevance, reflective of a conditioning process during sleep, indicative of an arousal or a sleep protective reaction to a potentially arousing stimulus. It could reflect a fine balance between arousal and sleep protection correlated with autonomic activation. This balance could be related to both spindles and delta waves being dominant activity during SWS, implicated to be involved in information processing, during which arousal thresholds are highest in humans (Colrain, 2005; Roth, Shaw, & Green, 1956). KC could be a key element linking the sensory systems and slow wave activity during NREMS, in turn regulating arousal-sleep decision at a network level. Thus, KCs could play a dual role in region-specific sleep-promoting and arousal promoting responses (Halász, 2016; Latreille et al., 2020).

Decrease in amplitude and frequency of KC is observed with aging (Wauquier, 1993). Evoked KC in comatose patients by auditory stimulation was an interesting observation (Chatrian, White Jr, & Daly, 1963) and this was eventually found to be predictive of a good recovery (Alster, Pratt, & Feinsod, 1993; Evans & Bartlett, 1995).

1.9.4 Sleep spindles

Sleep spindles, an important electrophysiological marker of NREMS N2-N3 stages in humans, is defined in the standardized scoring manual, The American Academy of Sleep Medicine Manual (AASM) for the Scoring of Sleep and Associated Events, as a train of waves 11–16 Hz in frequency and 0.5-3 seconds in duration (Iber, 2007). These transient, distinctive thalamocortical rhythms are detected in scalp EEG in humans and in LFP recordings in rodents during NREMS (De Gennaro & Ferrara, 2003; McCormick & Bal, 1997; Mircea Steriade, Angel Nunez, et al., 1993a). In rats, since NREMS is not subdivided into categories, spindles are detected during SWS (Genzel et al., 2014).

First described and named spindle in 1935 (Loomis, Harvey, & Hobart, 1935; Loomis et al., 1938), a SO or KC is often followed by a spindle during SWS, as recorded from EEG in humans or LFP in live animal brain, anesthetized animals (Diego Contreras, Destexhe, Sejnowski, & Steriade, 1997; De Gennaro & Ferrara, 2003; Loomis et al., 1935; Mircea Steriade, David A McCormick, et al., 1993; Mircea Steriade, Angel Nunez, et al., 1993a) as well as *in vitro* brain slices and at a cellular level as recorded from corticothalamic neurons (McCormick & Bal, 1997; Mircea Steriade, Angel Nunez, et al., 1993a). Sleep spindles have been recorded from several species including humans, mouse, rats, dogs, cats, non-human primates, rabbits and sheep (Iotchev & Kubinyi, 2021; Schneider, Vas, Nicol, & Morton, 2020). Spindle refers to its characteristic waxing and waning rhythmic oscillations of a fusiform shape, with maximum amplitude in the middle tapering off at either ends (Diego Contreras, Destexhe, Sejnowski, et al., 1997; Diego Contreras, Destexhe, & Steriade, 1997b; Schneider et al., 2020; Mircea Steriade, Angel Nunez, et al., 1993a).

The exact frequency range for spindles seem to differ depending on the authors and species studied. Typically, spindles are described in the sigma frequency band of 10 to 15 Hz and last for

a duration of 500ms to 3s (De Gennaro & Ferrara, 2003; Fernandez & Lüthi, 2020; Peyrache, Battaglia, & Destexhe, 2011; Schneider et al., 2020). A range of values have been proposed for spindle frequency (10-16 Hz with narrower bands spanning this range like 12-14 Hz, 11-15 Hz etc) and amplitude (8-25uV) depending on the species and the authors (De Gennaro & Ferrara, 2003; Nonclercq et al., 2013). Classical studies in cats have used spindle frequency range of 7-15 Hz (Diego Contreras, Destexhe, Sejnowski, et al., 1997; Diego Contreras, Destexhe, et al., 1997b). Spindle frequency in rats has varied from a narrower 12-15 Hz range (De Gennaro & Ferrara, 2003) to broad 5-15 Hz ranges (Terrier & Gottesmann, 1978), but typically 10–16 Hz frequency range (Andrillon et al., 2011).

1.9.4.1 Generation mechanisms

Bremer first proposed that spindles could be generated in the thalamus by showing impairment of cortical spindles by disconnecting the thalamus from the cortex. It was later shown that spontaneous spindle-like activity was generated in the thalamus independent of cortical connectivity. Thalamic generation of spindles was shown conclusively by Morison and Bassett in 1945 by recording thalamic region of cat stripped off any cortical or brain stem connectivity (Steriade & Llinás, 1988). While no spontaneous spindles occur in isolated cortex or athalamic cortex, slow waves (2-4 Hz) persist thalamectomy independent of thalamic inputs to cortex.

Several studies by Steriade and his group established the brain circuitry for spindle generation. Events beginning in the thalamus modulated by signals from the brainstem eventually activating intracortical circuitry form the spindle-generating circuitry. Brainstem reticular-activating system, including reciprocal excitatory-inhibitory synaptic connections between TC relay neurons, local circuit neurons and TRN neurons with TRN as a major intra-thalamic sleep

spindle pacemaker, form the primary spindle-generating circuitry (Lüthi, 2014; McCormick & Bal, 1997; Steriade, 2000; Steriade, 2003a; Steriade & Llinás, 1988; Steriade & Timofeev, 2003).

Spindle generation is made possible by complex reciprocal interactions between TC and TRN neurons involving intrinsic membrane properties, their anatomical interconnections, excitatory and inhibitory neurotransmitters and several channel types (Steriade, 2003a). SWS is favorable for spindle generation due to changes in inputs to TRN neurons from brain-stem nuclei (De Gennaro & Ferrara, 2003). TRN neurons normally spontaneously discharge at about 20Hz. At sleep onset, withdrawal of depolarizing monoaminergic inputs hyperpolarizes membrane potential from -50mV to about -65mV, thereby activating a range of a low-threshold voltage-gated Ca^{2+} channel, or T-channels, specifically $Ca_V3.2$ and $Ca_V3.3$, which depolarize TRN neurons to Na+ action potentials. Ca2+ spikes riding on Na+ action potentials are the key to TRN pacemaker capability. The rhythmic burst discharges are sustained by voltage-gated Ca^{2+} channels of the R-type and rapid K_V3 K^+ channels. Cortico-TRN excitatory postsynaptic potential synapses provides the initial triggers for neuronal bursting and in temporal coordination with SO. Inhibitory postsynaptic potentials involving synaptic $GABA_A$ receptors inhibition of TC cells from TRN cells provide the inhibitory bursts. This reciprocal excitation-inhibition cycle between TRN and TC neurons synchronize to provide the bursts in the spindle-generating network. The interplay of Ca2+ T-channel and Ca2+ dependent SK2 K+ channels generate repetitive burst sequences sustaining the rhythmic inhibition of TC neurons (Lüthi, 2014; McCormick & Bal, 1997; Steriade, 2003a).

Neocortex is not just a passive receiver of thalamic-generated spindles, but is critical in the generation, maintenance and amplification of the rhythmic spindle oscillation (Steriade, 2003a; Steriade, 2006). Intracortical circuitry from layer 5/6 recruiting pyramidal cells and interneurons further amplifies and entrains the thalamic rhythmic for the cortical propagation of spindles. Lateral GABAergic inhibition between TRN neurons, inhibition from basal ganglia, substantia nigra,

activation of the hyperpolarization-activated cation-nonselective (HCN) channels and Na^+-dependent K^+ channels act together to terminate spindles by attenuating TRN burst firing (Lüthi, 2014; Steriade, 2003a). Spindle frequency is the result of alternating TRN-induced inhibition (IPSP bursts) on TC neurons and rebound activation. The duration of IPSP bursts is a predictor of the exact frequency of spindles, which is itself predicted by overall brain inhibition levels. This could explain slight differences in spindle frequency band in different species (Steriade & Llinás, 1988).

1.9.4.2 Anterior and posterior spindles

Systematic characterization of spindles based on their location of recording in the cortex, frequency, amplitude and duration revealed three types of spindles in the rodent cortex: anterior spindles during SWS, anterior spindles during intermediate stages and preREMS and posterior spindles (Terrier & Gottesmann, 1978). Anterior spindles have a thalamic origin, similar to humans and cats, while posterior spindles have a different subcortical origin (E. Y. Sitnikova & van Luijtelaar, 2003). The properties of these spindles as characterized by (Terrier & Gottesmann, 1978) is as follows:

Properties of anterior spindles during SWS: more in anterior cortex, decreasing through parietal, limbic, occipital cortex and minimal in cerebellar cortex; anterior origin with lower amplitude and shorter duration in posterior areas with a time delay; 5-15 Hz frequency, but 90% between 9-13 Hz; 1-2 sec duration; 300-700uV amplitude.

Properties of anterior spindles during intermediate stage: more in anterior cortex, decreasing through parietal, limbic, occipital cortex and minimal in cerebellar cortex; anterior origin with lower amplitude and shorter duration in posterior areas with a time delay; 5-13 Hz frequency; 1-4 sec duration; 500-700uV amplitude.

Properties of posterior spindles: in posterior regions of cortex, reduced in parietal cortex and absent in frontal cortex; 9-18 Hz frequency; 0.4-0.8 sec duration; 200-500uV amplitude.

Anterior and posterior spindles, detected in both humans and rats, show distinctive topography, spectral features, pharmacological properties, time-evolution and circadian phases indicating distinctive generation mechanisms corresponding to specific frequency bands (E. Sitnikova & Van Luijtelaar, 2005). Spindles have also been shown to exhibit an anterior-posterior gradient as detected by EEG (Zhang, Campbell, Dhayagude, Espino, & Feinberg, 2021).

1.9.4.3 Slow and fast spindles

The observation that anterior and posterior spindles are of different frequencies, led to the more widely used classification of spindles as slow (low frequency <13 Hz) and fast (high frequency >13Hz) spindles (De Gennaro & Ferrara, 2003; Jankel & Niedermeyer, 1985; Lüthi, 2014; Mölle, Bergmann, Marshall, & Born, 2011; B. Rasch & Born, 2013). Slow spindles are predominant in the frontal cortical areas, while fast spindles in the parietal regions (Andrillon et al., 2011). They show some similarity in the activated regions like the thalamus, ACC, insula and superior temporal gyrus (Lüthi, 2014).

Differences between fast and slow spindles have been shown regarding their mechanisms of generation, genetic determinants, developmental trajectory, sex differences, response to sex hormones, spatiotemporal spread during NREMS, local appearance, pharmacological response, homeostatic regulation, circadian effects, age-related changes, hemodynamic correlates, roles in memory formation and electrophysiological temporal relations to neocortical SO and hippocampal ripples (De Gennaro & Ferrara, 2003; Fernandez & Lüthi, 2020). Fast spindles are more associated with increased hippocampal activation indicating a role in sleep-dependent memory consolidation

through phase-locking with hippocampal ripples (Mölle et al., 2011; B. Rasch & Born, 2013). Slow spindles have been thought to facilitate coupling in intra-cortical networks for local information processing, whereas fast spindles may be a result of thalamocortical coupling and cortical-subcortical communication (Doran, 2003).

1.9.4.4 High voltage and low voltage spindles

Additionally, two different kinds of sleep spindles can be differentiated based on their amplitude, within the broad spindle range of 6-20 Hz, as high-voltage spindles (hvs: 7-8Hz) and low-voltage spindles (lvs: 10-20Hz) (Johnson et al., 2010; A. Kandel & Buzsáki, 1997). Hvs has been studied for its role in epilepsy and parkinsonian tremors especially in rats (A. Kandel & Buzsáki, 1997; Perumal et al., 2020; C. Yang et al., 2015). In rats, fast, low voltage spindles following k-complexes are involved in hippocampal memory-reactivation processes, while slow high voltage spindles occur independent of k-complexes and could be negatively correlated in memory processing (Johnson et al., 2010).

1.9.4.5 Global and local spindles

Although spindles are generally considered to be synchronous and global at the cortical level, asynchronous spindles point to asynchronous generators modulated and regulated locally. Spindles can occur in isolation across different regions between hemispheres. This raises the possibility that global and local spindles could be mediated by differential mechanisms of generation and regulation (Nir et al., 2011).

The functional role of spindles in the broad field of memory consolidation through hippocampal-cortical coordination is discussed in a later section 1.11.3.

1.9.5 Hippocampal sharp wave ripples (SWRs)

Sharp-wave ripple complex represents the synchronized bursting of neuronal populations combining two characteristic components, sharp waves and ripples, with independent occurrence, physiological origins and mechanisms, which are often recorded as a compound oscillatory event occurring in sequence. Sharp waves are one if the important electrophysiological signatures of LFP recordings during the large irregular activity (LIA) from the hippocampus during behavioral immobility, non-exploratory consummatory behaviours and SWS. Sharp waves are transient, high amplitude (2mV) 50-100ms sharp polarity deflections and appear in conjunction with 120-250 Hz high frequency ripple oscillations. Ripples during LIA is prominent feature of SWS (Gyorgy Buzsaki, Horvath, Urioste, Hetke, & Wise, 1992; Buzsáki & Vanderwolf, 1983; Ylinen et al., 1995). They have been detected and described *in vivo* in several mammalian species including rats, mice, cats, bats, rabbits, nonhuman primate monkeys and humans, indicating a biologically conserved phenomenon (Joo & Frank, 2018). In vitro hippocampal slices studies elucidated the finer synaptic, cellular and ensemble mechanisms of SWRs (Butler & Paulsen, 2015).

1.9.5.1 Generation mechanisms

Strong parallel excitation of both CA1 pyramidal cells and interneurons by CA3 and their inherent interactions through cell activation and feedback inhibition in the pyramidal cell-interneuron loop circuit generates SWR. The hippocampal sharp wave component arises in CA3 and spreads to CA1 as a strong population depolarization of CA1 pyramidal cells, driven by CA3

and modulated by inputs from CA2 and dentate gyrus. Recurrent CA3 connectivity helps spread the depolarization through the region. The CA3 input parallelly also excites interneurons, driving a high-amplitude 150–250 Hz rhythmic excitation and inhibition of interneuron-coordinated pyramidal cell ensembles, which is manifested in the LFP as the co-occurring ripple event. It is also modulated by cortical and subcortical inputs. Inputs from Schaffer collaterals to CA1 pyramidal cells which are in turn recurrently connected to interneurons like parvalbumin positive basket cells (PV+ cells) through chemical and possibly gap junctions in a feed-forward and feed back inhibitory reciprocal interactions between the circuits generating the high frequency ripple activity as a reverberation or resonance effect. PV+ cells help synchronize ripples. Frequency-dependent depression of synaptic activity of these interneurons could eventually terminate the ripples. CA3 recurrent excitation can occur in low cholinergic states of quiet wakefulness and SWS when SWRs are observed, indicating a possibility of release from inhibition from cholinergic synaptic inputs (Buzsáki, 1986, 2015). Thus, they combine important spatio-temporal features of being large population events and highly synchronous activity of temporally coordinated bursting of pyramidal cell populations. The amplitude and temporal dynamics of sharp waves, modulated by inhibitory interneurons, is dependent on the number of cells recruited.

The functional role of ripples in the broad field of memory through hippocampal-cortical coordination is discussed in a later section 1.11.4.

1.9.6 Theta rhythm

Theta rhythms were first discovered in 1938 in rabbits as a 5-6 Hz oscillation (R. Jung & Kornmüller, 1938). Theta rhythm refers to a 4-8 Hz oscillation recorded at different levels of

neuronal organization from cellular level in pyramidal cells in rodents to synchronous activity in neuronal network activity such as hippocampal theta in animal deep LFP recordings and cortical theta in human scalp and cortical EEG recordings (Kahana, Seelig, & Madsen, 2001). It is predominant in an active hippocampus during alert motionless state, active exploration, locomotion, voluntary movements and REMS (Buzsáki, 2002; Sirota & Buzsáki, 2005). Human hippocampal theta has also been characterized and studied (Lega, Jacobs, & Kahana, 2012; Vass et al., 2016; Watrous et al., 2013). Human hippocampal theta rhythmicity is centered around 3 Hz while that of rats is centered around 8 Hz (Watrous et al., 2013).

The frequency range of theta rhythm depends on the species and brain state. Characterization of theta by Green and Arduini, showed that the hippocampal response was an expression of its physiological activity (systematically excluding the possibility of artifact), in wakefulness, sleep and arousal, characterized in rabbits, cats and monkeys, in resting animal (3-6 Hz), in awake alert animal (5-7 Hz) and as sensory stimulus evoked response (3-6 Hz) (Green & Arduini, 1954). The frequency range of theta has been reported between 4-12 Hz depending on the animal and region recorded: 6-12 Hz in mammalian brain, typically 5-9 Hz in hippocampus and entorhinal cortex, 4-7 Hz in human cortical EEG, 6-10 Hz in rat hippocampal and subcortical LFP, 6-7 Hz in alert motionless rat and 4-6 Hz in cats and rabbits.

Originally proposed in 1975 by Kramis et al (Kramis, Vanderwolf, & Bland, 1975), most scientists agree that two different types of hippocampal theta or rhymical slow activity (RSA) can be differentiated at the edges of traditional theta (4-8 Hz) based on pharmacology and behaviour: Type 1 atropine-resistant higher theta frequency (8 Hz) which occurs during locomotion, voluntary behaviour and REMS and a type 2 atropine-sensitive lower theta frequency (3–7 Hz range) which occurs during immobility and anesthesia (Colgin, 2013, 2016; Ferguson, Chatzikalymniou, & Skinner, 2017; Goutagny, Jackson, & Williams, 2009; Kramis et al., 1975). The high and low theta

have been associated with differential functional encoding and behaviours in rats and humans with phase synchrony between hippocampus and cortex, suggesting cortical-hippocampal communication through both oscillations (Lega et al., 2012; Tendler & Wagner, 2015). Type 2 theta appeared at around 10 days in neonatal rats during voluntary movements and during REMS and subsequently increased in amplitude and frequency, as recorded from adult rats (Konopacki, 1998).

1.9.6.1 Generation of theta

Theta rhythm is an outcome of interactions between several brain structures at a network level with the involvement of a multitude of theta oscillators, channels, currents and neuromodulators (Buzsáki, 2002; Colgin, 2013, 2016; Karakaş, 2020; Nuñez & Buño, 2021). The discovery that hippocampus could respond in the absence of cortex and showed a reverse pattern, hippocampal EEG being synchronous when that of the neocortex is desynchronized and vice versa was shown early on. Hippocampal-cortical interactions were also speculated as being mutually unexclusive, facilitative or controlling or through same afferent activation.

The theta rhythm is induced by septal connections, in the dorsal fornix, and conducted to the hypothalamus and thalamus through the fornix proper (Green & Arduini, 1954). Hypothalamic and brainstem inputs to the medial septal area and subsequent projections from the medial septal area are critical for hippocampal theta rhythm (Sirota & Buzsáki, 2005). Hippocampal theta depends strongly on cholinergic and GABA-ergic inputs from medial septum/diagonal band of Broca (MS-DBB) causing phase-dependent synaptic plasticity and spatial encoding during wakefulness (Kahana et al., 2001). Excitation of septo-hippocampal GABA neurons could trigger feed-forward disinhibition of CA1 pyramidal cells via hippocampal interneurons and cholinergic

activation of an intrahippocampal CA3 theta oscillator for the generation of theta (Buzsáki, 2002; Sirota & Buzsáki, 2005; Wu, Shanabrough, Leranth, & Alreja, 2000). Hippocampal-septal feedback by long-range GABAergic interneurons is crucial for producing widespread synchrony. Current source in CA1 pyramidal layer and current sink in the CA1 stratum radiatum are produced by the intrahippocampal and septal pathway interactions in a cholinergic network, independent of EC inputs (Sirota & Buzsáki, 2005). In addition to the MS-DBB, the hippocampus receives rhythmic modulatory subcortical inputs from several sources like the supramammillary nucleus and mammillary body (Kocsis & Vertes, 1994), brain stem-diencephalon theta-synchronizing inputs from nucleus reticularis pontis oralis and theta-desynchronizing inputs from median raphe nucleus (Vertes & Kocsis, 1997). Contrary to septal pacemaker hypothesis of theta generation in hippocampus, there exists evidence for theta generation and sustenance in local circuits of the hippocampus without input from septal stimulation or agonist stimulation in a whole-hippocampus *in vitro* preparation with properties similar to *in vivo* and living animal theta. Multiple weakly coupled oscillators along the septotemporal axis of the hippocampus provide additional evidence for a multi-generator hypothesis. The hippocampal inputs may then be involved in synchronizing these intrahippocampal oscillators at different septotemporal levels or may be a parallel mechanism of hippocampal theta generation (Colgin & Moser, 2009; Goutagny et al., 2009).

Showing similarity to *in vivo* recordings, theta oscillations have also been recorded invitro from hippocampal slices and EC slice preparations involving a GABAergic/cholinergic interactive mechanism for the production of theta-like activity. In vitro slice preparations allowed for the characterization of the intrahippocampal multi-generator hypothesis for independent theta generation from oscillators in CA1 stratum oriens neurons, stratum moleculare of the dorsal blade of dentate gyrus, and CA3c pyramidal cells (Konopacki, 1998). These oscillators are

interdependent, such that the timing of CA1 pyramidal cell action potentials during theta cycle is determined by cooperative CA3 neurons and entorhinal inputs (Kocsis, Bragin, & Buzsáki, 1999).

Several lines of evidence suggest an extrahippocampal theta oscillator in the entorhinal cortex: current dipoles from perforant path input to CA1 and CA3 pyramidal cells and dentate granule cells, entorhinal cortex phase-locking to the hippocampal theta, termination of theta by urethane anesthesia and lesions of the entorhinal cortex. In addition to the hippocampus and entorhinal cortex, theta-modulated neurons have been characterized in several limbic structures: perirhinal cortex, cingulate cortex, PFC, amygdala, anterior thalamus, mammillary bodies and the supramammillary nucleus, and the subiculum (Sirota & Buzsáki, 2005). These brain regions, including the neocortex are capable of generating their own theta fields or theta activity rhythms. Theta in subdural recordings in rats and in human EEG and intracranial recordings are most probably generated from subcortical oscillators (Gottesmann, 1992b; Kahana et al., 2001; Sirota & Buzsáki, 2005).

1.9.6.2 Functions of theta rhythm

The involvement of brain theta network in neurocognitive functions includes sensory perceptual processing, attentional processing, executive functions, working memory, executive functions, navigation and episodic memory, memory encoding consolidation and retrieval, sensory-motor integration, voluntary movement, eating, grooming, sleep, selective attention and sensory stimulation during sleep (Karakaş, 2020). Communication between hippocampal and the neocortical networks mediated by their dominant oscillatory rhythms are associated with memory processing and spatial navigation during active exploration and memory processing during REMS. Hippocampal theta across brain regions facilitates information processing and packaging through

several mechanisms, including information chunking, cross-frequency coupling, phase-locking of neuronal activity, phase-precession, synaptic plasticity mechanisms, coordination of cell assemblies and theta sequences (for detailed reviews see (Buzsáki, 2002; Colgin, 2013, 2016; Karakaş, 2020; Nuñez & Buño, 2021)).

1.9.6.2.1 Theta Rhythms in behaviour

Theta oscillations in the hippocampus during awake states have the capacity to support cognitive processing involved in locomotion, sensory stimuli processing, encoding during learning, memory retrieval, spatial decision making, directionality, reward processing, and future planning in both rats and humans (for detailed reviews see (Buzsáki, 2002; Colgin, 2013, 2016; Karakaş, 2020; Nuñez & Buño, 2021). Cortico-hippocampal connections underlie cognitive-affective correlates of the hippocampus through a relationship between cognitive functions and theta rhythm in the development of conditioned reflexes, homeostatic regulation of motivation instrumental conditioning, delayed conditional reflexes, orientation reaction, appetitive and aversive conditioning (Karakaş, 2020). Theta oscillation has also been investigated in the hippocampus for its involvement in processing time where transient increase was observed in theta power during time discrimination periods in a rat temporal discrimination task (Nakazono, Sano, Takahashi, & Sakurai, 2015).

Several of these functional processes have been related to influences on cell assemblies and theta sequences at a cellular and network level of organization. Theta oscillation can associate cell assemblies on a temporal scale through modulation of synaptic plasticity mechanisms linking cellular and network level mechanisms for information processing (Buzsáki & Moser, 2013). Organization and behaviour of cell assemblies in a hierarchical order may be considered as a neural

syntax with dynamic synaptic weights modulating their output and thus could form the underlying mechanism for operations of the brain, from encoding and processing information to memory and rational thinking (Buzsáki, 2010). Theta sequences are temporally ordered sequences of place field firing of hippocampal principal neurons during theta, reflecting their behavioural order and theta phase precession is predictive of theta sequences with precision implying their possible functional role at a cellular assembly level in navigational encoding and learning (Foster & Wilson, 2007).

1.9.6.2.2 Cross-frequency coupling

Cross-frequency coupling through interactions between different brain rhythms can be a mechanism for generating neuronal sequences for temporal ordering of events to store and retrieve information on a temporal scale for memories for long-term consolidation and future planning (G. Buzsaki & Draguhn, 2004). Most studies have evaluated the functional role of theta-gamma coupling. Cross-frequency coupling in the entorhinal-hippocampal network occurs by theta phase-modulation of gamma power (Colgin et al., 2009). Studies in both rats (Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009) and humans (Axmacher et al., 2010; Canolty et al., 2006) show correlations of theta phase-modulation of gamma with memory performance. Hippocampal theta is found to support learning through two inter-connected processes: gamma phase-locking at the trough of theta strengthens encoding of novel information, while gamma phase-locking at the peak of theta guides exploration based on prior experience (Kragel et al., 2020). A mechanism of theta (4–10 Hz) - low gamma (30–50 Hz) comodulation has been proposed as a predictor of memory retrieval in single-trial spatial memory task in rats with prominent comodulation in successful retrieval and weak comodulation in failed memory retrieval. (Shirvalkar, Rapp, & Shapiro, 2010).

In addition to theta-gamma association, theta-delta, theta-alpha coherence and their functional relevance has also been demonstrated (Karakaş, 2020).

1.9.6.2.3 Theta phase coupling between regions

Inter-regional theta coupling is thought to facilitate the transfer of information between brain regions during sensory information processing. Behaviour-dependent theta coupling has been demonstrated in mPFC (spatial memory processing), striatum (decision making), lateral amygdala (fear memory retrieval), olfactory bulb (odor discrimination) (Colgin, 2013). Strong theta phase coupling between frontal and posterior cortical areas and the hippocampus during rearing and exploratory behaviour and uncoupling during immobility or grooming could reflect cortico-hippocampal functional interaction units supporting differential behavioural demands (Young & McNaughton, 2009).

1.9.6.2.4 Theta phase coupling of neuronal firing: Hippocampal–neocortical interactions through theta

Phase-locked neuronal activity to hippocampal theta rhythm has been demonstrated in several cortical and sub-cortical regions: hippocampus, mPFC, cingulate cortex, entorhinal cortex, perirhinal cortex, subicular complex, visual cortex, striatum, amygdala, dorsal raphe nucleus, and anterior thalamic nuclei, highlighting functional cortico-hippocampal interactions to support behaviour (Buzsáki, 2002; Colgin, 2011, 2013, 2016; Karakaş, 2020; Nuñez & Buño, 2021). Local computations in the entorhinal-hippocampal circuitry can be temporally facilitated during EC neuronal spiking and theta synchrony (Mizuseki, Sirota, Pastalkova, & Buzsáki, 2009).

Phase locking between firing of mPFC neurons and hippocampal theta recorded as theta-entrained activity indicates hippocampal-prefrontal directionality and timing in freely behaving rats, and can underlie gating of information flow and information storage through plasticity across cortico-hippocampal networks (Siapas, Lubenov, & Wilson, 2005). Phase-locking of mPFC neuronal firing by hippocampal theta seems to be behaviour dependent: mPFC cells alternated between theta-entrained firing during behaviourally relevant task with directionality-dependent differential firing and non-phasic firing in less behaviourally relevant task (Hyman, Zilli, Paley, & Hasselmo, 2005). Phasic timing of prefrontal cortical neurons relative to hippocampal theta rhythm have been shown to be important for memory-guided action selection (Hasselmo, 2005). Increased synchronization in firing patterns of CA1 and mPFC neurons in rats with enhanced coupling in theta-frequency range during spatial working memory task could underlie the integration of hippocampal spatial information into decision-making cortico-hippocampal network through independent encoding and selective interaction based on behavioural demands (M. W. Jones & Wilson, 2005b). Hippocampus and medial prefrontal cortex (mPFC) show coherent oscillations during goal-directed behaviours and working memory tasks. Functionally relevant bursts of gamma-mediated cell assemblies in the mPFC phase-locked to hippocampal theta may facilitate hippocampal input during mnemonic tasks (Colgin, 2011). This could be of functional significance in terms of encoding, given the functional connectivity and interactions between the mPFC and hippocampus (Euston, Gruber, & McNaughton, 2012).

1.9.6.2.5 Theta phase precession

Theta precession is a temporal relationship between the activity of hippocampal neurons to the hippocampal LFP, such that neuronal spiking occurs at progressively earlier phases of the co-

occurring theta rhythm as measured in LFP during place field firing neuronal sequence. The robust observation of theta phase precession in dorsal CA1 pyramidal neurons, hippocampal interneurons and other areas of hippocampal formation, indicated its possible role in specifically mediating hippocampal-related behaviours like spatial navigation, action planning, rapid sequence encoding and episodic memories (Malhotra, Cross, & van der Meer, 2012).

Even though several studies show theta phase precession in hippocampal CA1, theta phase precession has been reported in other regions with anatomical and functional connectivity to the hippocampus, including dentate gyrus, CA3, the subiculum, the entorhinal cortex, and the ventral striatum. This cross-regional phase precession demonstrates the functional relevance of the hippocampal network interactions and a global role of phase-precession in rhythm-mediated coordination of neuronal activity across brain regions (Colgin, 2013). mPFC neuronal firing phase precession relative to the CA1 theta rhythm was shown to be associated with theta phase-locking of CA1 and mPFC as a function of spatial location of the rat (M. W. Jones & Wilson, 2005a). Phase precession in the ventral striatum with the firing of reward-anticipatory neurons has been shown to be important in linking place and reward circuitry, having implications in conditioned place preference and context-dependent reinstatement behaviours (Malhotra et al., 2012; Van Der Meer & Redish, 2011).

1.9.6.2.6 Theta rhythms during REMS

The functional significance of REM has been directly attributed to the theta rhythm, as this is the predominant and best characterized activity in electrophysiological recordings during REM. Studies by Born and Plihal have shown implicit, non-declarative, procedural memory consolidation during REMS (Born & Plihal, 2000). Mice optogenetic experiments have shown that medial septal

GABA neuronal activity specifically during REMS is required for contextual memory consolidation. Neuronal silencing selectively after learning and during REMS erased novel object place recognition memory and impaired fear-conditioned contextual memory, indicating a role of synaptic processes underlying REMS (Boyce, Glasgow, Williams, & Adamantidis, 2016). REM theta could be involved in emotional memory consolidation in humans (Nishida, Pearsall, Buckner, & Walker, 2009). Beta (15–35 Hz) - theta (4–8 Hz) coherence in reciprocally connected ACC and DLPFC regions could indicate their possible role in emotional regulation and in procedural motor and emotional memory consolidation (Vijayan, Lepage, Kopell, & Cash, 2017). The REM theta effects on memory consolidation could be indirectly influenced by retrosplenial cortical modulation of hippocampal activity (de Almeida-Filho et al., 2021).

Possible involvement of theta in memory consolidation during REMS could be mediated by theta-gamma coupling (Montgomery, Sirota, & Buzsáki, 2008) or REM replay (Louie & Wilson, 2001). Continuous hippocampal theta in rodents during REMS with gamma oscillation power associated with theta phase are thought to play a critical role in memory consolidation in rodents. But in humans, short bursts (~1 sec) of hippocampal theta waves (4-7 Hz) during REMS and during sleep-wake transitions were observed. This indicates phasic theta oscillation generation in human hippocampus during REMS. During REMS, there is no phase-locking between fluctuations in gamma oscillation power and REM theta bursts. Theta waves in basal temporal lobe and frontal cortex, incoherent with hippocampal theta oscillations, were recorded during sleep-wake transitions and in quiet wakefulness, but not in REMS, as an extension mechanism from hippocampus. This absence of functional coupling between neocortical and hippocampal theta indicates the possibility of multiple theta generators dynamically regulated by brain state (Cantero et al., 2003).

The idea of the involvement of REMS in memory consolidation has been challenged and seems to be controversial (Vertes, 2004). Even though, several early studies of memory consolidation during REMS, have shown correlations between REMS deprivation on lower memory performance, but this could also be due to decreased attention and mood changes.

1.10 Sleep and memory

1.10.1 Context of sleep in memory consolidation theories

Theory of systems consolidation of memory is based on the two-stage memory system, first proposed by Marr. It offers solutions to catastrophic interference (interference between new information to erase already existing information), stability-plasticity dilemma (encoding new patterns in existing neuronal networks) and capacity constraints (amount of information that can be stored in a neural network) (D. Marr, 1971; Willshaw, Dayan, & Morris, 2015). Active systems consolidation hypothesis adopts the two-stage memory system and hypothesizes initial encoding of memories in a fast-learning store, followed by gradual long-term storage. Hippocampus is proposed to be the quick learner to efficiently encode information through robust synaptic changes like LTP, even in one-trial learning like in episodic memories, although unstable and vulnerable to interference. Over time, this information can be gradually integrated and interleaved into neocortical synaptic circuit structures through cellular consolidation mechanisms during awake rehearsal and reinstatement or repeated reactivation and replay during offline periods like sleep. (S. Diekelmann & Born, 2010; Feld & Born, 2020; P. W. Frankland & Bontempi, 2005; McClelland et al., 1995; B. Rasch & Born, 2013).

The information-processing theory of the function of sleep hypothesizes that sleep offers a critical offline period in the two-stage memory system for systems memory consolidation

facilitating learning and memory through changes ranging from molecular to systems level reorganization. Reduced encoding of external information during sleep makes it a good candidate for network memory consolidation without interference (S. Diekelmann & Born, 2010; B. Rasch & Born, 2013).

Memory formation at the neuronal scale is brought about by changes in synaptic strengths in memory networks, including memory encoding through learning-induced synaptic plasticity namely, LTP or LTD and this reverberating activity in the network promotes synaptic and systems consolidation (Dudai, 2004). The reverberating activity in the newly encoded representation of the network stimulates a reorganization of neuronal representations in connected neuronal networks promoting systems consolidation of long-term memories (P. W. Frankland & Bontempi, 2005; B. Rasch & Born, 2013). Synaptic remodeling of dendritic spines increases synaptic efficacy, promoting synaptic consolidation (E. R. Kandel, 2001a). Synaptic plasticity is thought to occur through LTP mechanisms and systems consolidation occurs through reactivation and replay (S. Diekelmann & Born, 2010).

Taking into account the categorization of several memory systems, synaptic and systems memory consolidation, stages of sleep and testing of several diverse tasks to quantify learning, makes sleep-dependent memory consolidation a complex process to study. The relationship between sleep and memory consolidation needs to be addressed from the following perspectives: types of memories consolidated during sleep, functional role of specific sleep stages in consolidation and mechanisms of consolidation during sleep (Stickgold, 2013; Stickgold & Walker, 2005).

1.10.2 Sleep and memory research

Research in both humans and animals over the years indicate that one of the important functions of sleep is to offer an off-line time for memory consolidation of different memory systems (S. Diekelmann & Born, 2010; Stickgold, 2005; Walker & Stickgold, 2004). Evidence of the correlation came from two lines of research in animals and humans: memory improvement following sleep period after learning and memory impairment following sleep disruption after learning in natural and forced conditions, investigating the effects on several task-dependent memory recall measures. That sleep could be important for memory consolidation, is attributed to one of the earliest studies which showed a slower rate of decline of nonsense syllables memory in human subjects after a sleep period (Jenkins & Dallenbach, 1924). Early research focused on forgetting through decay and interference, and that sleep after learning reduced the amount of forgetting. Sleep was thought to passively protect memories from retroactive interference to prevent forgetting and protect memory traces from decay. Subsequent research confirmed the positive effect of sleep on memory retention from immediate sleep to about 6 days and inverse relation between learning and delay to sleep (S. Diekelmann & Born, 2010; B. Rasch & Born, 2013). Sleep-dependent post-training consolidation of human declarative, visual, auditory and motor skill learning procedural memories has been demonstrated in several studies (McClelland et al., 1995; Walker & Stickgold, 2004). Human research shows evidence for sleep-dependent consolidation of both declarative and procedural non-declarative learning, including perceptual and motor skill memory involving systems-level reorganization of memory within the brain (Susanne Diekelmann, Wilhelm, & Born, 2009; Stickgold, 2013; Stickgold & Walker, 2005; Walker & Stickgold, 2004). Memories important for future planning may be selectively consolidated during sleep (Born & Wilhelm, 2012).

Sleep can promote cellular or synaptic consolidation through learning-induced plasticity processes at the cellular and molecular level, as well as systems consolidation through memory storage at neuronal network level, transfer and transformation of memory trace from a short-term to long-term storage site in the brain (S. Diekelmann & Born, 2010; P. W. Frankland & Bontempi, 2005; D. Marr, 1971; McClelland et al., 1995). The function of sleep influencing plasticity, learning, and memory is evolutionarily preserved from drosophila to humans (S. Diekelmann & Born, 2010; Donlea, 2019).

Implications of sleep disturbances and sleep-dependent memory consolidation in several neuropsychiatric disorders including dementia, insomnia, phobias, anxiety, addiction, depression, schizophrenia, and post-traumatic stress disorder underscores the significance of sleep (Feld & Born, 2020; Goerke, Müller, & Cohrs, 2017).

1.10.3 Role of SWS and REMS in memory processing

The contribution of sleep stages to memory consolidation has been investigated in several human and animal studies. Information-processing theories of sleep hypothesize that either NREMS or REMS or both could be involved in consolidating memories. Consolidation seems to depend on the composition of sleep with differential results between the effects of REMS and NREMS stages and the type of memories and memory systems encoding them. Different forms of declarative memory are supported by different sleep stages, except for stage 1 NREM (Susanne Diekelmann et al., 2009; Stickgold, 2013; Stickgold & Walker, 2005; Walker & Stickgold, 2004). Perceptual and motor skills procedural learning show enhancement across periods of sleep and during specific sleep stages (Stickgold, 2005).

Some scientists believe that Tulving's model for characterization of memory systems into 4 categories of procedural memory, perceptual representation system, semantic and episodic memory is more useful in understanding the memory functions of sleep. They all benefit from NREMS or REMS or both. Semantic and episodic memories rely on different sleep stages for consolidation. Similarly, acquisition of various types of perceptual-motor, sensory-perceptual and cognitive skills and priming effects form different memory system facilitated by different stages of sleep (Rauchs, Desgranges, Foret, & Eustache, 2005). Thus, current models of sleep-stage dependencies of memory processing have been found to be inadequate based on the current experimental findings (Stickgold, 2013).

Most of the early research also focused on REMS as functions of REM deprivation protocols were well established and characterized (S. Diekelmann & Born, 2010; B. Rasch & Born, 2013). REMS deprivation impaired memory encoding in complex tasks compared to simple tasks. NREM stage 2 could be involved in motor procedural but not cognitive procedural learning (S. Diekelmann & Born, 2010; Susanne Diekelmann et al., 2009; B. Rasch & Born, 2013; C. Smith, 2001, 2011; Stickgold, 2013; Stickgold & Walker, 2007). Natural and pharmacological enhancement of REMS after learning increased memory. Animal studies showed increase in REMS activation following learning in several classic, aversive and appetitive conditioning procedures. Elevated periods of REMS occurred starting 2 hours after stressful Morris water task to 4 hours in appetitive tasks and 9 hours in avoidance tasks and lasted for up to 4-6 days after learning in some cases. Ponto-Geniculo-Occipital (PGO) waves, characteristic of REMS in animals have been proposed to promote synaptic plasticity processes underlying memory formation during REMS (B. Rasch & Born, 2013). In human studies, consistent evidence for involvement of REMS was found for tasks with a strong procedural memory component and emotional memories (B. Rasch & Born, 2013; Stickgold & Walker, 2005, 2007). Human perceptual skill learning (Karni, Tanne,

Rubenstein, Askenasy, & Sagi, 1994) and emotional memories (Nishida et al., 2009) was found to be REM-dependent. Although compelling evidence coming from majority of scientists point to a role of sleep in memory consolidation, some have opposing views especially with REMS involvement. REM deprivation studies and REM-related effects are confounded by effects of stress on changes in hormones and neurotransmitters (Siegel, 2001; Vertes, 2004; Vertes & Eastman, 2000).

However, there is evidence for NREMS involvement in simple motor tasks and REMS involvement in complex motor skill learning (Susanne Diekelmann et al., 2009; B. Rasch & Born, 2013; Stickgold, 2013; Stickgold & Walker, 2005). Human and rodent experiments on memory recall after sleep or sleep deprivation pointed to REMS involvement in efficient processing of cognitive procedural learning, while SWS and proportion of REMS-NREMS is important for declarative memories. According to active systems consolidation model, SWS rather than REMS after learning could preferentially cause selective memory reactivation and consolidation, eventually causing qualitative changes in long-term neocortical memory storage sites (Born & Wilhelm, 2012).

The general consensus of the dual process hypothesis proposes differential roles of sleep stages to different memory systems. One of the earliest studies that attempted to parse out the differential roles of REMS and SWS sleep in memory showed circadian effects with early SWS-rich sleep benefitting declarative memories and late REM-rich sleep in procedural memories (Gais & Born, 2004; Plihal & Born, 1997). A NREM-rich daytime nap selectively enhanced declarative, but not procedural memory (Tucker et al., 2006). Early nocturnal sleep rich in SWS facilitates hippocampus-dependent declarative memories and late nocturnal sleep rich in REMS facilitates non-declarative procedural, implicit and emotional memories.

1.10.4 Other contributing factors

1.10.4.1 Sleep cycles

Sleep cycles, rather than amounts of sleep stages, could be important according to the sequential hypothesis, which predicts distinct but complementary involvement of NREMS and REMS contribution in animals and human studies; adaptive responses strengthen during SWS and then integrate during REMS (Ambrosini & Giuditta, 2001; Giuditta et al., 1995). Effectively, systems consolidation during SWS and synaptic consolidation during REMS seems to be a possibility (Ambrosini & Giuditta, 2001; Susanne Diekelmann et al., 2009; B. Rasch & Born, 2013). Some electrophysiological and molecular data that have been tested to this effect, suggest that neuronal reverberation happens during SWS episodes post-acquisition, while plasticity-related transcriptional expression in the form of immediate early genes is triggered during REMS. In addition, the interplay between SWS and REMS propagates and transfers recent synaptic changes from the hippocampus to the cortex, in line with systems consolidation theories. Firstly, post-experience firing rate of neurons increased strongly during SWS for a short duration lasting for minutes in hippocampus and for a long duration lasting for hours in the cortex. Temporal dependence of firing rates was observed during REMS with cortical activity during experience predicting hippocampal activity in the first hour window after experience and this trend reversing in the third hour window. Upregulation of IEG expression was specific to REMS in the cortex, but not in hippocampus, again in line with systems consolidation theories predicting hippocampal disengagement over time. In addition, IEG expression was proportional to spindle power, but not to firing rates, indicating processing through dendritic input than somatic output. Neuronal activity during SWS and plasticity-related synaptic IEG expression proportional to SWS spindle power

during REMS, predicts a systems consolidation during SWS and synaptic consolidation during REMS (Ribeiro & Nicolelis, 2004; Ribeiro et al., 2007).

1.10.4.2 Circadian, social rhythm and aging

Circadian desynchronization due to social clock and sleep deprivation can also affect synaptic plasticity mechanisms in memory consolidation (Kelley, Evans, & Kelley, 2018). In addition, sleep quantity and quality has been shown to be correlated to learning and memory in both human aging (Mander, Winer, & Walker, 2017) and social rhythm-related sleep disruption in adolescents (Tarokh, Saletin, & Carskadon, 2016). Circadian rhythm disruption specifically results in memory consolidation deficits, without affecting normal learning/acquisition, as demonstrated in rats trained in the standard version of the Morris water task (Devan et al., 2001).

1.10.4.3 Future goal relevance

Consolidation processes during sleep could be selective in terms of motivational factors relevant for future goals and behaviour, by preferential recruitment of emotional and reward associated neocortical-hippocampal circuitry, mediated by hippocampal theta rhythm during awake encoding (Susanne Diekelmann et al., 2009; B. Rasch & Born, 2013).

1.10.4.4 Neurochemical influence

In addition to electrophysiological characteristics, neurochemical and hormonal changes also underlie the different sleep stages. It may not be the sleep stages per se, but changes in neurotransmitters and neuromodulators in the brain during sleep stages could mediate sleep-

dependent memory processing. Differential expression and regulation of neurochemicals during sleep and wakefulness could imply differential state-dependent functions.

Glutamatergic LTP plays a major role in synaptic consolidation with AMPA and NMDA receptor involvement (Feld & Born, 2020). Active systems consolidation of declarative memory may not recruit NMDA-mediated LTP mechanisms in hippocampal-cortical networks during sleep, contrary to encoding memory traces during awake learning; this could be the result of neuro-modulatory and neuro-oscillatory features during NREMS which could not just encode but transform memory traces.

GABAergic neurons are important for sleep induction and maintenance (Feld & Born, 2020). GABAergic drugs influence the generation and the interplay of sleep brain oscillations including SO, SWRs and spindles. GABA reuptake inhibitor, tiagabine, enhances SWA and SO observed during NREMS, but reduces SO-spindles coupling and hence does not improve memory consolidation. Zolpidem, a GABA A-positive modulator, strongly enhances the amount of sleep spindles resulting in enhanced declarative memory consolidation (Feld & Born, 2020).

According to synaptic tagging and capture hypothesis, dopaminergic activity during learning facilitates tagging of high-reward memories for subsequent preferential reactivation during active systems consolidation periods of sleep (Feld & Born, 2020; Redondo & Morris, 2011).

Levels of stress hormone cortisol and cholinergic activity is reduced during SWS than during REMS or waking and seems to be promising in neuromodulation-related sleep-dependent memory consolidation.

Gradient aminergic activity, including noradrenaline and serotonin, higher during waking, intermediate during SWS and minimum during REMS, could also play a neuromodulatory role during sleep (S. Diekelmann & Born, 2010; Feld & Born, 2020; B. Rasch & Born, 2013).

1.10.4.5 Cortisol, sleep and memory

The circadian modulation of cortisol is specifically relevant in the context of sleep-dependent memory consolidation. Cortisol levels are the lowest during early SWS-rich stage, start to rise during the middle of sleep and peaks during late REM-rich sleep leading to waking. REMS episodes coincide with cortisol peaks (Born & Fehm, 1998; U. Wagner & Born, 2008). A clear demonstration of the effects of cortisol on SWS-dependent memory consolidation was shown by the impairment of declarative but not procedural memories, by elevating plasma GC concentration during early sleep by administration of cortisol (Plihal & Born, 1999), and with the administration of a synthetic GC, dexamethasone (Plihal, Pietrowsky, & Born, 1999), leading to the conclusion that consolidation during early sleep depends on low cortisol levels.

Cortisol levels are under circadian influence, with low cortisol during SWS-rich early sleep and elevated cortisol levels during REM-rich late sleep (Born & Wagner, 2004). Increasing cortisol during early SWS-rich periods of nocturnal sleep impairs hippocampus-dependent declarative memory formation, without affecting procedural memory formation. Thus, the naturally-occurring inhibition of cortisol secretion (with the CNS glucocorticoid receptors being inactive) during early SWS-rich sleep in humans seems to be critical for hippocampus-mediated consolidation of declarative memories. On the other hand, preventing the natural increase in cortisol during REMS-rich sleep appears to enhance amygdala-dependent emotional memory, suggesting that the natural increase in cortisol during late sleep may diminish emotionality of memories. Thus, physiological cortisol feedback processes during early SWS-rich and late REM-rich sleep contributes to the differential effects of these sleep phases on memory formation. These findings are consistent with the view that cortisol, via activation of limbic glucocorticoid receptors generally diminishes

memory consolidation in humans (Born & Wagner, 2004). In general, increasing cortisol during early SWS-rich periods of nocturnal sleep impairs hippocampus-dependent declarative memory formation, while cortisol blockade during REMS-rich sleep appears to enhance amygdala-dependent emotional memory formation (Born & Fehm, 1998; Born & Wagner, 2004, 2007, 2009; U. Wagner & Born, 2008; U. Wagner, Degirmenci, Drosopoulos, Perras, & Born, 2005). In other words, the natural suppression of endogenous cortisol during early sleep phases enhances declarative memory consolidation, whereas the natural rise in cortisol during late sleep phases may prevent excessive emotionality of memories (Born & Wagner, 2004).

Very few studies have investigated interaction of cortisol and sleep for memory consolidation. Learning of temporal sequences distinctly improved by cortisol infusion during wake phase, but impaired by cortisol infusion during sleep (Wilhelm, Wagner, & Born, 2011), indicating fundamental differences in the mechanisms of brain state-dependent hippocampal memory consolidation (Kelemen, Bahrendt, Born, & Inostroza, 2014). Pre-encoding cortisol has been shown to interact with sleep to influence memory performance. Specifically, resting cortisol levels are strongly and specifically related to sleep-dependent emotional memory consolidation, suggesting that memory consolidation during sleep maybe cortisol dependent. In addition, higher pre-encoding cortisol increases sleep-dependent consolidation of emotional information that receives the most attention during encoding. Elevated cortisol during learning was found to promote a stronger relation between encoding- and successful retrieval-related activity in the amygdala and vmPFC, specifically in those who slept between encoding and retrieval (Bennion et al., 2015), at the same time probably reducing amygdala reactivity related to the retrieval of the consolidated, negative items (Van Marle, Hermans, Qin, Overeem, & Fernández, 2013).

1.10.5 Mechanisms of sleep-dependent memory consolidation

Overall, evidence from rodents and humans establish a powerful role for sleep in offline memory consolidation by bringing together molecular and cellular dynamics, network-level reorganization, inter-regional brain communication and behavioural levels of organization in different ways. Molecular signaling pathways involving gene regulation and protein synthesis affect long-lasting synaptic plasticity. Cellular excitability changes during NREMS and REMS affect synaptic plasticity in a Hebbian network model. Network level reorganization in memory is a result of coordinated replay of the firing pattern cell assemblies during post-learning sleep with the help of spatio-temporal coherence of regional brain oscillations (Abel, Havekes, Saletin, & Walker, 2013). Systems consolidation of hippocampus-dependent and non-hippocampus-dependent memory during sleep could be achieved by neuronal ensemble reactivation, synaptic rescaling and coordination of oscillations spanning stages of sleep and brain regions affecting local synaptic plasticity and distantly connected brain networks (Klinzing, Niethard, & Born, 2019). The active system consolidation hypothesis offers an explanation to this process through reactivations of neuronal ensembles with the involvement of inherent brain oscillations that bring them together in coherence.

1.10.5.1 Reactivation

According to active system consolidation hypothesis, aspects of both, dual process and sequential hypothesis, play a role through reactivation of newly encoded memory representations during sleep. Reactivation forms an important part of standard systems consolidation theory and active systems consolidation view of the memory function of sleep. Memories encoded parallelly into hippocampal and neocortical areas, are subsequently reactivated during offline sleep periods

by replay of the encoded memory trace, gradually strengthening cortico-cortical connections, eventually making the memory hippocampal-independent, thus, making the hippocampus a temporary store and neocortex a long-term storage site (S. Diekelmann & Born, 2010; P. W. Frankland & Bontempi, 2005). Thus, reactivations of memory representations during offline periods of sleep are thought to transform and distribute temporary fast-learning newly encoded hippocampal memories into long-lasting slow-learning neocortical networks for long-term storage (S. Diekelmann & Born, 2010; B. Rasch & Born, 2013).

Reactivation during SWS driven by SO, reactivate hippocampal memory representations during SWRs, and bring about neocortical plasticity, integration and consolidation during thalamocortical spindles, causing systems consolidation during SWS, followed by subsequent synaptic consolidation during REMS. This could explain the benefits of sleep on both declarative and non-declarative memory consolidation (S. Diekelmann & Born, 2010; B. Rasch & Born, 2013). Signs of neuronal reactivation have also been found during REMS with their activity correlated to theta phase, but its correlation with pre-task sleep makes this finding ambiguous for memory reactivation post-task encoding (Louie & Wilson, 2001).

Several lines of evidence, mostly from rodent studies, have been presented to support the phenomenon of reactivation during sleep. Neuronal activity of hippocampal place cells during the awake states, was found to influence the firing characteristics of the cells in immediate subsequent sleep episodes (Pavlides & Winson, 1989). Following up on this, sleep-dependent memory consolidation in the form of reactivation in a classic study, reignited the field of sleep and memory research forming the basis of neural mechanisms of consolidation processing that could preferentially occur during sleep. Correlation in activation patterns of place cell assemblies was found to be strikingly similar during subsequent SWS as was during task in hippocampal CA1 and dentate gyrus cells (Shen, Kudrimoti, McNaughton, & Barnes, 1998; M. A. Wilson &

McNaughton, 1994). The reactivation magnitude could be used as a predictor of memory performance during subsequent tests (Dupret, O'neill, Pleydell-Bouverie, & Csicsvari, 2010). This memory reactivation occurred within the first hour, typically within 20-40 mins of rest or sleep after learning (Battaglia, Sutherland, Cowen, Mc Naughton, & Harris, 2005). The reverberation could persist for upto 24 hours (Ribeiro et al., 2004); but this could be questionable (Tatsuno, Lipa, & McNaughton, 2006). Not only was there reactivation of neuronal assemblies, but the temporal order of reactivated cell assemblies was also preserved as time-compressed replay of about 10-20 times (A. K. Lee & Wilson, 2002; Nádasdy, Hirase, Czurkó, Csicsvari, & Buzsáki, 1999; Skaggs & McNaughton, 1996). Reactivation in hippocampal cell assemblies was correlated with SWRs (Kudrimoti, Barnes, & McNaughton, 1999). Reactivation was also found to occur in exploratory behaviour and not just trained tasks (O'Neill, Senior, & Csicsvari, 2006). To corroborate these findings, impaired hippocampal reactivation was found to be correlated with poor memory performance in spatial learning with NMDA blockers (Dupret et al., 2010) and in aged rats (Gerrard, Burke, McNaughton, & Barnes, 2008).

Reactivations of spatio-temporal patterns was subsequently also found in the mPFC with a 6-7 time compression factor (Euston et al., 2007; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009). The reactivation in mPFC correlated with cortical down-to-up state transition during SWS indicating a functional connectivity and association between reactivation, SWS and sleep spindles (Johnson et al., 2010). Temporal coordination between hippocampal and neocortical reactivation signals indicates the reciprocal hippocampal-neocortical functional connectivity where reactivation pattern in each region can precede and predict each other (Rothschild, Eban, & Frank, 2017). Reactivation of cell assemblies found in ventral striatum, parietal cortex and visual cortex, indicates its significance for memory consolidation beyond spatial learning, for place-reward and

decision-making, generalizing this as a mechanism for consolidation across several cortical regions (B. Rasch & Born, 2013; Schreiner & Staudigl, 2020).

The findings of reactivation of neuronal assemblies in hippocampus and other brain regions underscore the significance of time-compressed reactivation of cell assemblies during immediate SWS correlated with awake activity, in several brain regions and different behaviours, for memory processing and consolidation. Coordinated reactivation of memory-related traces has also been found in monkeys (Hoffman & Mcnaughton, 2002).

In addition to sleep stages, memory reactivation also emerges in awake animals during immobility, consummatory behaviour, grooming and task engagement (Karlsson & Frank, 2009; Ólafsdóttir, Bush, & Barry, 2018). Awake memory reactivation during SWRs could support task-learning and memory-guided decision-making behaviours (Jadhav, Kemere, German, & Frank, 2012).

Awake-state and sleep SWR-mediated memory replay possibly have differential roles, evident from the differences during these states. Local SWR-mediated awake replay seems to support memory-guided behaviour through retrieval and planning, with help from dopaminergic neuromodulator mechanisms, while subsequent non-local SWR-mediated sleep replay seems to support integration of memories through gradual consolidation into the framework of existing memories, with help from cholinergic neuromodulator mechanisms (Atherton, Dupret, & Mellor, 2015; Roumis & Frank, 2015; Tang, Shin, Frank, & Jadhav, 2017). An important difference between awake replay and sleep replay is the presence of sleep brain oscillations including slow waves and spindles. Although neuronal reactivation and replay during learning is stronger in the awake state compared with sleep states, mediation of sleep replay by these brain rhythms might offer a stronger temporal window to integrate experiences across larger spatial neocortical and hippocampal networks (Findlay, Tononi, & Cirelli, 2020; Tang et al., 2017).

The aforementioned findings in rodents have parallels in the human imaging and recording studies. Methodological constraints limit memory reactivation studies during sleep in humans, to examining similarity in the activity of brain regions during initial experience and subsequent sleep, using PET and fMRI. These and EEG studies in humans have provided initial evidence of reactivation during sleep, though not at a cellular level resolution like in rat reports (Maquet et al., 2000; Peigneux, 2014; Peigneux et al., 2004; Schreiner & Staudigl, 2020). Awake-state reactivation in humans indicated replay within 500 ms after cue onset prompting recollection and correlates with memory accuracy (Jafarpour, Fuentemilla, Horner, Penny, & Duzel, 2014). Depth electrode recordings from neurosurgical patients from hippocampus and lateral temporal cortex, showed pattern-completion related temporal coordination between the regions with item-specific reinstatement in the lateral temporal cortex, preceding the reactivation of item-context associations in the hippocampus, with increased levels of cortico-hippocampal communication during the reinstatement periods, and hippocampal-triggered cortical reinstatement during memory reactivation (Estefan et al., 2019). Multiple studies from different brain regions have shown that stimulus-related single-unit firing patterns are preserved between initial presentation of stimuli and during their successful retrieval (Gelbard-Sagiv, Mukamel, Harel, Malach, & Fried, 2008; Jang, Wittig Jr, Inati, & Zaghloul, 2017; Miller et al., 2013; Schreiner & Staudigl, 2020). Time-compression has also been observed in human studies in both awake-state and during rest, similar to rodent research (Y. Liu, Dolan, Kurth-Nelson, & Behrens, 2019; Schreiner & Staudigl, 2020; Yaffe, Shaikhouni, Arai, Inati, & Zaghloul, 2017).

With initial studies in rodent models, followed by non-human primates and human studies, hippocampal-triggered memory reactivation in episodic memory and goal-directed behaviour could be an important mechanism for rehearsal for awake-state and sleep-dependent memory consolidation (Carr, Jadhav, & Frank, 2011; Schreiner & Staudigl, 2020).

Computational modeling studies have also added to our understanding of replay in neuronal networks. Both N2 spindles and N3 SO promote sleep replay of neuronal spiking sequences learned during awake, with the replay being localized at trained network locations and subsequent improvements in memory performance. With training multiple memories in the network, local spiking sequences replayed distinct memory traces independently during spindles. SO was functionally relevant in promoting competition to allow replay of stronger memory traces and prevent replay of weaker memories in the network (Wei, Krishnan, Komarov, & Bazhenov, 2018). This hypothesizes extinction of weaker memories unless when N2 spindles preceded N3 SO as in natural sleep cycle and offers a testable mechanistic explanation for the role natural sleep stages for creating optimal conditions for memory consolidation. The strengthening of stronger memory traces and elimination of weaker synaptic connections is already predicted by synaptic homeostasis hypothesis (Tononi & Cirelli, 2003, 2006, 2014).

The cellular mechanism underlying reactivation of cell ensembles to consolidate memories could come from synaptic plasticity or remodeling changes at a cellular and molecular scale. Dendritic spine formation during initial activation and its strengthening and maintenance during subsequent sleep, facilitated by reactivation of initial network, could be a robust mechanism bridging network-level and cellular dynamics, possibly as evidence of synaptic tagging and capture hypothesis (Euston & Steenland, 2014; Redondo & Morris, 2011; G. Yang et al., 2014).

1.10.5.2 Synaptic rescaling and homeostatic mechanism

According to synaptic homeostasis hypothesis, the role of sleep in systems consolidation is embedded in a global homeostatic regulation of synaptic connectivity, through synaptic rescaling, reorganization and redistribution, especially during SWS. Selective parts of neuronal assemblies

involved in initial encoding of experience undergo homeostatic regulation and as a result, either downscale (depotentiate) or upscale (potentiate) the synapses, leading to weakening or strengthening of synaptic connections in the network respectively, thus, changing their contribution to memory representation within the network. Weaker connections are eliminated, and stronger ones strengthened resulting in memory enhancement by increasing signal to noise ratio (Klinzing et al., 2019; Tononi & Cirelli, 2014).

1.10.6 Learning during sleep

While the preceding section highlighted sleep-dependent memory consolidation of learning that happened during preceding awake stages, there is also some evidence of learning happening during sleep. Conditioning learning and implicit learning have been demonstrated to occur during different sleep stages. Studies in sleeping rats, infants and adults have shown that this learning can take place during sleep.

Paradoxical sleep seems to offer a window for processing of relevant information and formation of new associations which can be transferred to the awake state and be expressed in behaviour (Hennevin, Hars, Maho, & Bloch, 1995). This has been demonstrated in rats with multiunit activity recordings. CS presentation-induced changes in hippocampal multiunit activity were not observed before pairing; increased after pairing during paradoxical sleep and the cellular responses were elicited in post-learning awake state as well, suggesting that cellular conditioning established during sleep can be transferred to awake state (Maho & Bloch, 1992).

Delay eyeblink trace conditioning is a hippocampal-independent form of non-declarative memory paradigm subject to unconscious learning (Clark, Manns, & Squire, 2002). Classical conditioning, through external multimodal sensory stimulation and integration, is associated with

network-wide activity within the conditioned neural system in the developing cortex, and may influence its associated functional network architecture as shown by fMRI studies in neonates (Dall'Orso et al., 2021). Delayed eyelid/eyeblink conditioning paradigm (tone-air puff pairing) has been shown to be learnt by infants during sleep (Fifer et al., 2010; Tarullo et al., 2016). Enhanced frontal EEG SWA reflecting memory updating during later part of training sleep was also observed (Fifer et al., 2010). Given the fact that newborns spend most of their time in sleep, the ability to learn about external stimuli during non-awake states may be offer a crucial evolutionary survival advantage and rapid adaptation (Tarullo, Balsam, & Fifer, 2011). In addition, eyelid conditioning reflects a functional cerebellar circuitry, which could be used as a potential behavioural biomarker for early detection of several neurodevelopmental disorders including autism spectrum disorders, fetal alcohol syndrome, Down syndrome, fragile X syndrome, ADHD, dyslexia, language impairments, and schizophrenia (Reeb-Sutherland & Fox, 2015). Conditioning effect in adults during SWS was transferred to wakefulness (Ikeda & Morotomi, 1996).

Behavioral and EEG data in adults indicated the formation and presence of implicit memory trace for words presented during sleep (Andrillon & Kouider, 2016). Building on the idea of differential processing of sensory information during different stages of sleep, disassociated from behavioural responsiveness (Andrillon, Poulsen, Hansen, Léger, & Kouider, 2016), people could learn new sounds during spindle-mediated NREM2 and REMS and this learning was suppressed in SO-dependent SWS (Andrillon, Pressnitzer, Léger, & Kouider, 2017). Humans can also learn new information during sleep without later awareness of the learning process. Partial-reinforcement trace conditioning (odor-tone association) can be learnt during sleep and the acquired behavior persisted during later sleep and into subsequent awake state (Arzi et al., 2012). Humans can not only learn olfactory association in sleep, but this association can also alter behaviour (Arzi et al., 2014; Cellini & Parma, 2015); olfactory aversive conditioning during a

single night of sleep significantly reduced cigarette-smoking behavior in a sleep stage-dependent manner (NREM2 and REM), and this behavioural effect persisted for several days.

1.11 Sleep brain oscillations in memory: cortical-hippocampal communication

While the temporal involvement of hippocampus in memory recall is debated (reviewed in the section 1.3), major existing theories agree on hippocampal-cortical interactions (reviewed in section 1.5) involving cortical plasticity for memory consolidation. Neocortical-hippocampal communication has been found to be critical for most high-order cognitive processing including information processing, memory encoding, memory consolidation, memory retrieval and behavioural flexibility through decision making and based on past experiences. Several mutually non-exclusive functions have been attributed to brain oscillations including input selection, plasticity, connecting and binding cell assemblies, consolidation and integration of learned information and representation by phase information. Oscillatory coupling between different frequencies can enhance the spatial and temporal dimensionality of brain processing and integration for encoding complex temporal patterns and optimizing synaptic weights through combinatorial possibilities (G. Buzsaki & Draguhn, 2004). The active system consolidation hypothesis proposes a feed-forward system of interaction between temporary fast-encoding hippocampal networks and long-term slow-encoding neocortical networks through temporal oscillatory synchronization mechanisms resulting in reactivation and consolidation of memory traces. The synchronization between the oscillations enables an effective hippocampal-neocortical communication channel for transfer of reactivated information (S. Diekelmann & Born, 2010; Klinzing et al., 2019; B. Rasch & Born, 2013). Within the framework of memory consolidation models, hippocampal-neocortical functional connectivity has fundamental relevance through

interactions between their intrinsic oscillations underlying the temporal characteristics of memory consolidation facilitating inter-regional communication. Neocortical SO, hippocampal SWRs and thalamocortical spindles with k-complex, have been extensively demonstrated for their functional coupling and significance in sleep-dependent memory consolidation, as depicted in Fig 5.

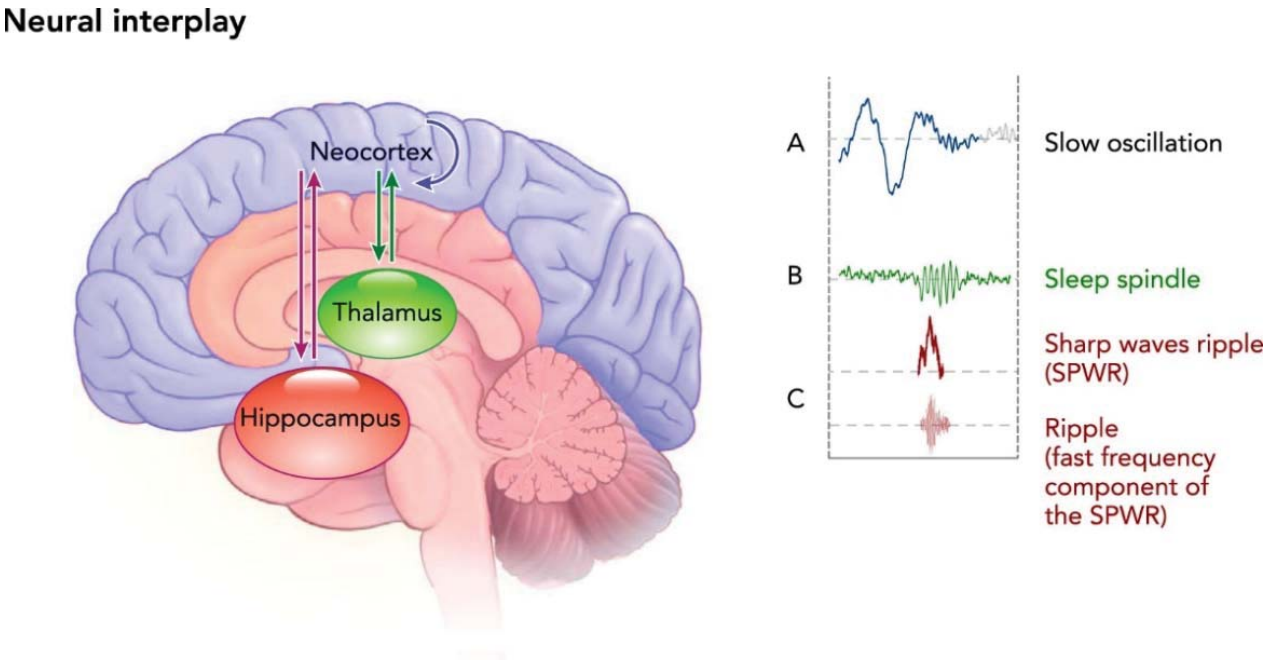


Figure 5: Model of interplay between brain oscillations of sleep-dependent memory consolidation

Note: adapted from: (Marshall, Cross, Binder, & Dang-Vu, 2020): Neocortical slow oscillations (A) temporally group activity in other brain structures including thalamocortical spindles (B) and hippocampal sharp-wave ripples (C). The timing of spindles is relative to the SO phase and the further coordinated reactivation of hippocampal SWRs are important in memory consolidation processes. This temporal coupling facilitates the transfer of encoded information from hippocampal traces by consolidation of neocortical memory traces. The timing and amplitude of the oscillations are not scaled.

Each of these oscillations have been implicated to have a causal role in learning and memory, and interaction between these oscillations has been found to be critical for sleep-dependent memory consolidation. This section will review the current understanding of the

individual role of each of these oscillations in memory processes and the functional coupling between them in all combinations.

1.11.1 k-complex and memory

KC was speculated to be related to stimulus relevance, reflective of a conditioning process during sleep, indicative of an arousal or a sleep protective reaction to a potentially arousing stimulus, reflect a fine balance between arousal and sleep protection correlated with autonomic activation, related to both spindles and delta waves being larger in SWS where arousal thresholds are highest in humans and involved in information processing (Colrain, 2005; Roth et al., 1956). KC could be a key element linking sensory system and slow waves in NREMS regulation in arousal-sleep decision at a network level highlighting a dual role for KC in region- specific sleep-promoting and arousal promoting responses (Halász, 2016; Latreille et al., 2020). It was found early on that conditioned discrimination acquired during awake state continued in sleep and was found to be associated to KC and reflected in the KC response, indicating a role for KC in stimulus preprocessing (D. G. McDonald et al., 1975).

Cortical multicell firing sequences organized into frames (periods of incremental neuronal population activity) were found to be correlated with KC and concurred with SO frequency range implying a relationship between SO and cortical frames brought together by KC (Ji & Wilson, 2007). Considering the observation that KC could represent the cortical DOWN state, its involvement in memory consolidation through processing of learned stimuli seems promising from a temporal perspective of oscillatory interactions (Cash et al., 2009). KC is also considered to be a DOWN-UP transition and it could provide the stimulus for neocortical-hippocampal-neocortical communication setting the time for hippocampal inputs to neocortical networks at the time of

optimal processing with no confounding activity indicating neocortical replay initiated by KC (Johnson et al., 2010; Todorova & Zugaro, 2020).

Expanding on the findings that disrupted slow waves linked to prefrontal atrophy and hippocampal-dependent memory impairment in aging (Mander et al., 2013) and this disruption of slow waves being linked to β-amyloid deposition (Mander et al., 2015), no significant changes of frontal ≤1 Hz SWA was observed in Alzheimer's patients, but frontal KC density decreases relative to the measured cognitive decline (De Gennaro et al., 2017); they hypothesize that partial overlap between 0.6-1 Hz slow wave EEG activity and KC can explain the contradiction. Overall decrease in amplitude and frequency of KC is observed with aging (Wauquier, 1993).

1.11.2 SO and memory

Soon after the discovery of SO during SWS, the memory consolidation effects of SWS have been indicated to depend on the neocortical SO (Born, Rasch, & Gais, 2006; S. Diekelmann & Born, 2010; Marshall & Born, 2007; Mölle & Born, 2011). Increased amplitude of SO was found in cortical areas associated with the task after learning compared to non-learning conditions in both humans and rats (Mölle, Eschenko, Gais, Sara, & Born, 2009). In one study, both amplitude of SO and length of UP-state corelated with memory performance (Heib et al., 2013). Increases in SWA EEG power as a reflection of SO, during post-learning sleep were reported to be correlated with memory retention in humans in a visuo-motor spatial task (Huber, Ghilardi, Massimini, & Tononi, 2004), declarative word list and procedural motor skill learning (Holz et al., 2012). Interestingly, SO power increase during sleep was restricted to the specific cortical regions that were associated with task acquisition during awake state (Huber et al., 2004). Learning of declarative memory also increased SO coherence across cortical regions during post-learning sleep (Mölle, Marshall, Gais,

& Born, 2004). In addition, cortical neuronal firing rates, firing synchrony and SWA have been correlated with homeostatic sleep-wake regulation, marker of sleep homeostasis after sustained wakefulness (Vyazovskiy, Olcese, et al., 2009).

Type I slow (SO) and type II slow wave (δ waves) have been shown to have distinct and competing roles in determining the extent of memory consolidation during NREMS indicating that these two phenomena drive differential activity-dependent processing as an outcome of reactivation of neural ensembles during NREMS (J. Kim et al., 2019). SO and delta waves could have competing roles, with reactivations during SO enhancing memory and delta wave mediating weakening of memory reactivations to facilitate forgetting (J. Kim et al., 2019).

Another recently proposed classification of slow waves considered transition frequency of slow waves as a distinguishing feature and found slow switchers (slow waves with a slow transition) and fast switchers (slow waves with a fast transition). Fast switchers were predominant in early sleep while slow switchers predominated later stages of sleep. The proportion of slow and fast switchers in normal sleep and recovery sleep, their pattern of homeostatic decline and underlying brain functional connectivity indicates a possibility of their differential contribution to sleep-dependent memory consolidation processes and sleep-dependent cognitive processes (Bouchard et al., 2021).

The possibility to experimentally manipulate the SO and examine the effects on SO dynamics, behavior and memory recall have helped in understanding their functions. Declarative memory improvements in humans and rats have been found to be correlated with enhancement of SO power through stimulation during post-learning sleep. Transcranial direct current stimulation increased SO activity and positively influenced memory recall in human studies (Marshall, Mölle, Hallschmid, & Born, 2004). tDCs at the endogenous SO frequency during sleep was reported to enhance memory consolidation of declarative memory in humans (Marshall, Helgadóttir, Mölle, &

Born, 2006) and rats (Binder, Berg, et al., 2014; Sonja Binder, Julia Rawohl, Jan Born, & Lisa Marshall, 2014). This has been interpreted as a causal role of SO in memory consolidation as the stimulation boosted SO power related to SWA and correlated with better memory recall. However, other studies have shown no effect of SO-tDCs (Eggert et al., 2013) and square wave tDCs (Sahlem et al., 2015). In addition, no entrainment of SO was found as a result of low frequency transcranial electrical stimulation (Lafon et al., 2017). Evoked SO through electrical stimulation have similarity with endogenous slow waves in terms of slope of waves and propagation across the cortex (Vyazovskiy, Faraguna, Cirelli, & Tononi, 2009).

Other stimulation techniques like auditory stimulation and transcranial magnetic stimulation have also been shown to enhance SO power correlated with memory improvements. A closed loop phase-locked auditory stimulation boosts SO and has shown to enhance declarative memory consolidation (H.-V. V. Ngo, Martinetz, Born, & Mölle, 2013). TMS-evoked localized potentiation of cortical EEG response was observed when TMS was applied to the motor cortex during wakefulness and this correlated to SWA enhancement in the same regions during subsequent sleep and the magnitude of potentiation predicted the localized increase in sleep SWA activity (Huber et al., 2007). The speculation is that increase in sleep intensity through the potentiation of SWA activity is related to cortical plasticity mechanisms (Huber et al., 2008).

Several mechanisms have been proposed for the memory effects of SO enhancing neocortical-hippocampal communication: coordinated inter-regional neuronal spiking activity (Ji & Wilson, 2007), thalamocortical plasticity processes of neuronal potentiation (Steriade, 2006; Steriade & Timofeev, 2003), SO-delta-spindle interactions (Steriade, 2006), temporal coupling between SO and ripples through interaction with hippocampus (Todorova & Zugaro, 2020), SO-spindle coupling through interaction with thalamic nuclei (Lisa Marshall et al., 2006; H.-V. V. Ngo et al., 2013; Steriade, 2006), triple coupling between SO with spindles and ripples through

interaction with thalamic nuclei and hippocampus (Zsófia Clemens et al., 2007; Mölle & Born, 2011; Sirota & Buzsáki, 2005; Sirota, Csicsvari, Buhl, & Buzsáki, 2003) and a slow wave hyperpolarization-triggered calcium-dependent post-synaptic LTP mechanism requiring AMPA and NMDA receptor coactivation strengthening neuronal connections (Sylvain Chauvette, Seigneur, & Timofeev, 2012). Another possibility is the electrophysiological and computational similarity between wakefulness and SO up-states in terms of neuronal activation and reactivation respectively, which could facilitate synaptic and cellular plasticity in thalamocortical networks during the UP-state related T-type Ca^{2+} channel-dependent bursts of neuronal activity (Destexhe, Hughes, Rudolph, & Crunelli, 2007).

1.11.3 Spindles and memory

1.11.3.1 Spindles correlated with learning

Since the indication of the involvement of SWS in memory consolidation, several studies have tried to parse out the role of specific regional or global brain oscillations. In addition to SO, sleep spindles and their interactions in the process of memory encoding and consolidation are of particular interest (Astori, Wimmer, & Lüthi, 2013; De Gennaro & Ferrara, 2003; Fernandez & Lüthi, 2020; S. M. Fogel & Smith, 2011; Klinzing et al., 2019; Marshall et al., 2020; McDevitt, Krishnan, Bazhenov, & Mednick, 2017; Peyrache & Seibt, 2020; Ulrich, 2016).

Learning of novel information or task has been shown to enhance subsequent spindles during sleep. Several human studies have shown post-learning increases in spindles or spindle density in several verbal and word association declarative tasks (Zofia Clemens, Fabó, & Halász, 2005; Gais, Mölle, Helms, & Born, 2002; Hoedlmoser et al., 2014; Mölle et al., 2009; Manuel Schabus et al., 2004) and in visuospatial memory tasks (R Bódizs, Lázár, & Rigó, 2008; Zsófia

Clemens, Fabó, & Halász, 2006). Spindle density increase post-learning correlated with learning difficult abstract words as a measure of encoding difficulty (Schmidt et al., 2006). Similarly, learning-dependent increases in spindle activity have also been observed for motor memories in procedural task learning tasks (SM Fogel, Jacob, & Smith, 2002; S. M. Fogel & Smith, 2006), visuomotor learning (S. M. Fogel, Smith, & Cote, 2007; Tamaki, Matsuoka, Nittono, & Hori, 2008) and in motor sequence learning (Morin et al., 2008; Tamaki et al., 2013). Motor task learning was found to be impaired after NREM2 sleep deprivation (C. Smith & MacNeill, 1994). In addition, odor-cued memory reactivation during NREM2 enhanced spindle activity during cueing, and this spindle-related reactivation was found to be correlated to gains in motor sequence learning performance the following day (Laventure et al., 2016). Studies have indicated that the degree of correlation between spindles and motor learning may be modulated by task complexity (S. M. Fogel et al., 2007; C. T. Smith, Aubrey, & Peters, 2004) and performance skill level (Peters, Ray, Smith, & Smith, 2008). Enhancement of spindles has also been reported following spatial episodic memory task (Meier-Koll, Bussmann, Schmidt, & Neuschwander, 1999) and in spindle-mediated reactivation of emotional memories (Cairney, Durrant, Hulleman, & Lewis, 2014). Post-learning sleep spindles predicted memory improvement for contextual aspects of hippocampal-dependent episodic memories (Van Der Helm, Gujar, Nishida, & Walker, 2011).

The findings in human studies have modest parallels in animals as well. Learning-associated changes in cortical sleep spindle activity during SWS has since then been shown in a few animal models, mostly rodents. One of the first studies to show a positive correlation between learning and subsequent sleep spindle density in an animal model was an increase in spindle density in the first hour of sleep following associative learning in an odour-reward pairing digging task (Eschenko, Mölle, Born, & Sara, 2006). This indicates its involvement in memory processing during post-learning immediate sleep. Sleep spindle density increased during post-learning SWS

in a shock avoidance task on the first training day, although the increase in spindle density was observed 21-24 hours after learning and not in immediate SWS period (S. M. Fogel et al., 2009). This could be evidence of sequential hypothesis of sleep-dependent memory consolidation with electrophysiological processes coordinating during both REMS and NREMS for memory consolidation (Ambrosini & Giuditta, 2001; Giuditta et al., 1995; C. T. Smith et al., 2004). Learning-dependent increase in spindles was also observed in dogs (Iotchev, Kis, Bódizs, van Luijtelaar, & Kubinyi, 2017).

In an attempt to replicate and compare results from humans (Gais et al., 2002; Mölle et al., 2002) and rats (Eschenko et al., 2006), Molle et al, 2009 found increase in spindle density in word-pair learning in humans and odor-reward task in rats, although the effect was more pronounced in humans (Mölle et al., 2009).

Studies have also discovered the role of spindles in integrating information into existing prior networks in the brain. Spindle density was predictive of specifically schema-related information retention with reduced decay of schema-related memories and correlated with disengagement of the hippocampus over a 24 hour period (Hennies, Ralph, Kempkes, Cousins, & Lewis, 2016). Correlation was also found between spindles and integration of new learned lexical information into existing neocortical networks indicating a role for spindles in novel information processing (Tamminen, Payne, Stickgold, Wamsley, & Gaskell, 2010). A TMR study also corroborated this with a similar finding that spindle-related consolidation of cued newly acquired information can be enhanced only if the cued memories are related to existing prior knowledge in cortical networks (Groch, Schreiner, Rasch, Huber, & Wilhelm, 2017).

Spindles can be involved in sleep-dependent motor memory consolidation in two ways: local reactivation of task-specific cortical networks and functionally connecting these cortical

regions with relevant sub-cortical networks of the hippocampus, putamen and thalamus (Boutin et al., 2018).

1.11.3.2 Spindles correlated with recall

In addition to increase in post-learning spindle activity (as reviewed in the preceding section), studies in humans (S. M. Fogel & Smith, 2011; Gais et al., 2002; Mölle et al., 2009) as well as in rodents (Eschenko et al., 2006) have also shown positive correlation between spindle activity and later memory retrieval as recorded during subsequent memory tests. Sleep spindles was found to be correlated with post-sleep retention of declarative memories (Zofia Clemens et al., 2005; Holz et al., 2012; Manuel Schabus et al., 2004). Individuals with increased spindle amplitude and duration showed better declarative memory recall and individuals that did not show an increase in spindle measures during post-learning sleep did not show improvement on the task (Manuel Schabus et al., 2004). Spindle activity during post-learning sleep is also correlated with memory recall in procedural learning (SM Fogel et al., 2002; S. M. Fogel & Smith, 2006; S. M. Fogel et al., 2007) and motor skill learning (Walker et al., 2002). These studies clearly indicate that the role of sleep spindles during post-learning encoding help consolidate memories for retention and retrieval.

1.11.3.3 Disrupting Spindles

Experimental manipulation of spindles by disrupting them, have provided additional evidence for the functional involvement of spindles in memory processing. Theta-frequency transcranial stimulation disrupted spindle power and reduced performance in a declarative memory task but not a procedural task (Marshall, Kirov, Brade, Mölle, & Born, 2011). Selective T-type calcium channel antagonist, TTA-P2 locally inhibit Cav3.3 T-type calcium channels and

selectively reduced frontal cortical spindle activity (Thankachan et al., 2019). Optogenetic inhibition of monosynaptic pathway from ventral hippocampus to mPFC during SWS reduced phase coupling of spindle-ripple and spindle-SO oscillations and adversely affected memory performance (Binder et al., 2019). Optogenetic stimulation of GABA/PV inhibitory input to TRN from basal forebrain parvalbumin neurons (BF-PV), at a frequency of 40Hz, similar to their discharge rate during wakefulness and REMS, immediately suppressed sleep spindles and promoted transitions to wakefulness (Thankachan et al., 2019). Optogenetic inhibition of parvalbumin-positive GABAergic neurons in mouse thalamic reticular nucleus suppressed spindle activity and was associated with decreased declarative and non-declarative memories in mice (Katsuki et al., 2017). These studies show that deficits in learning and memory can be induced by interfering with spindle activity and/or coupling of spindles with associated oscillations.

1.11.3.4 Other factors influencing the role of spindles in learning and memory

Several other points of consideration are important in the context of sleep spindles. Spindles show variability in terms of location, frequency, association with cortical SO, regional neuronal firing rates, time course across sleep cycles and circadian effects. Spindles can occur across multiple neocortical regions and can be spatially restricted as well. Slow and fast spindles show a topographically differential organization; slow frontal spindles and fast centroparietal spindles with a transition around supplementary motor area. These variations could be reflective of underlying thalamocortical projections to different cortical regions (Andrillon et al., 2011; De Gennaro & Ferrara, 2003).

1.11.3.4.1 Localized regional spindle enhancement

Several studies provide another line of evidence, that the effect of spindles was found to be preferentially localized to the cortical area involved in task performance and encoding, implying a role for regional sleep spindles in cortical plasticity in task-associated local brain regions (S. Diekelmann & Born, 2010; Johnson et al., 2012; Ulrich, 2016): PFC after encoding verbal word association learning (Zofia Clemens et al., 2005; Schmidt et al., 2006), parietal cortex after visuospatial learning (Zsófia Clemens et al., 2006), contralateral motor cortex in unilateral finger tapping motor skill learning task (Nishida & Walker, 2007), central and parietal regions in motor procedural learning (Milner, Fogel, & Cote, 2006), premotor and parietal cortices after visuomotor learning (Tamaki, Matsuoka, Nittono, & Hori, 2009), motor or premotor area in a motor-based brain-computer interface task (Johnson et al., 2012), posterior brain regions contralateral to the visual field being cued and involved in visuospatial processing in a word-location pair with odor task (Cox, Hofman, de Boer, & Talamini, 2014).

1.11.3.4.2 Baseline spindle activity

Although it was observed that baseline spindle activity is generally higher in highly gifted individuals, spindle increase post-learning correlates with memory improvement, independent of individual learning traits (Manuel Schabus et al., 2008). This indicates that while baseline spindle density could be a predictor of general cognitive abilities, learning ability is predicted by spindle increases during post-learning sleep. Individuals who showed increased spindle activity also had recorded high spindle activity prior to learning compared to those who did not show the increased effect, and the change correlated with memory performance (Manuel Schabus et al., 2004). Baseline sleep spindle activity correlated well with increased post-learning spindle activity and

memory recall in humans (Manuel Schabus et al., 2004), but this effect was found to be reversed in a rat study of avoidance learning (SM Fogel, Smith, & Beninger, 2010). Based on a retest in the avoidance task, rats were categorized into learners and non-learners. Non-learners had higher baseline sleep spindles which was unaffected by training, while learning rats had lower baseline sleep spindles which increased and correlated with learning. Thus, baseline sleep spindle density can be a predictor of learning and in some cases, higher baseline spindle activity may represent consolidation interfere with consolidation of adaptive information.

1.11.3.4.3 Spindles during daytime nap

Although melatonin (Dijk & Cajochen, 1997) and circadian rhythm (Dijk & Czeisler, 1995) affect sleep spindles, spindle activity increased after daytime napping in both declarative learning (Schmidt et al., 2006) and performance in motor learning task (Nishida & Walker, 2007). Some studies found learning-related spindle activity increased only in habitual daytime nappers predictive of learning (Kurdziel, Duclos, & Spencer, 2013; Milner et al., 2006). In addition, daytime naps (Dhand & Sohal, 2006) were found to be as good as night sleep in terms of memory processing during SWS (S. Mednick, Nakayama, & Stickgold, 2003) or REMS (Allen, 2003). These studies question circadian effects on at least some skill-specific spindle changes.

1.11.3.4.4 Stage of sleep effect

SWS in humans includes NREM 3 and 4 from earlier sleep classification (N3 according to current). Light sleep (N2) and SWS (N3) could have differential contributions to memory consolidation with light NREM in active potentiation and SWS in homeostatic regulation (Genzel et al., 2014). Several studies have shown spindle-learning association specific for NREM 2 or light

sleep in procedural and declarative tasks (S. M. Fogel & Smith, 2006; Milner et al., 2006; Ruch et al., 2012). SWS-specific spindle-memory potentiation has been shown in a declarative visual learning task and was predictive of memory performance (Cox, Hofman, & Talamini, 2012). A study in rats showed novel object learning-dependent increase in higher amplitude spindle-related pre-REM or intermediate sleep periods (during the transition between NREMS and REMS) increased within the first 2 hours after learning (Schiffelholz & Aldenhoff, 2002).

1.11.3.4.5 Spindle properties

Sigma power, spindle numbers, duration, amplitude, density and frequency have been reported as measures of spindle increase in different studies: *sigma power* (power overlapping in the spindle frequency band) (SM Fogel et al., 2002; S. M. Fogel & Smith, 2006; S. M. Fogel et al., 2007; Milner et al., 2006), *spindle numbers* (Zofia Clemens et al., 2005; Zsófia Clemens et al., 2006; Milner et al., 2006), *spindle amplitude and duration* was correlated with learning and predicted learning performance (Manuel Schabus et al., 2004; Manuel Schabus et al., 2008), *spindle density* **(**spindles per minute of SWS**)** (Eschenko et al., 2006; SM Fogel et al., 2002; S. M. Fogel & Smith, 2006; S. M. Fogel et al., 2007; Gais et al., 2002).

1.11.3.4.6 Slow and fast spindles

Slow and fast spindles, that have been characterized in humans, have been indicated to have differential roles in memory processing. Some studies investigating the role of spindles in learning and memory have not distinguished between the two (Zofia Clemens et al., 2005; Zsófia Clemens et al., 2006; Gais et al., 2002). Most studies have reported that learning-dependent spindle effects are more consistent for fast spindles. This has been shown in several motor learning tasks:

visuomotor (Tamaki et al., 2008), finger tapping (Barakat et al., 2011; Walker et al., 2002), motor learning (S. M. Fogel et al., 2007; Tamaki et al., 2009) and motor sequence learning (Morin et al., 2008). Only fast spindles were predictive of learning (Barakat et al., 2011; Tamaki et al., 2009). However, there are some exceptions: involvement of both fast and slow spindles (Hennies et al., 2016) and no positive correlation of either slow or fast spindles in declarative episodic memory task (Ackermann, Hartmann, Papassotiropoulos, de Quervain, & Rasch, 2015). Verbal memory has been shown to be encoded by slow spindles as demonstrated in high functioning individuals (Manuel Schabus et al., 2008) and in complex abstract encoding (Schmidt et al., 2006).

Differential activation of cortical regions during slow and fast spindles indicates distinct functional involvements as recorded from simultaneous EEG and fMRI. While fast spindles recruited sensorimotor cortical regions and hippocampus, slow spindles showed activation in superior frontal gyrus (Manuel Schabus et al., 2007). This indicates the selective involvement of fast spindles in hippocampal-dependent motor memory processing and slow spindles in declarative memory processing, as seen from studies that have shown correlations between them respectively. But recently, a TMR study showed that information processing is mediated by fast spindles. Associative memory cues (adjective-object and adjective-scene paired) during NREMS evoked transient fast spindles coupled to SO UP-states. During the narrow temporal of cue-evoked spindle activity, the categorical features of the cued representations (object or scene) are subject to reliable decoding which correlated with consolidation and retention (Cairney, El Marj, & Staresina, 2018).

Thus, the differential involvement of slow and fast spindles in memory processing could have more complex underlying mechanisms, which needs to be investigated deeper.

1.11.3.5 Other spindles functions

In addition to its role in sleep-dependent memory consolidation, sleep spindles have been reported to be involved in several other functions: cortical development, cognitive and intellectual measure, fluid intelligence, reasoning abilities and sleep maintenance (Fernandez & Lüthi, 2020).

Spindle bursts could have a mechanistic role in network reorganization during cortical development (Khazipov et al., 2004).

Spindle parameters have been found to be a physiological measure of general cognitive and intellectual abilities (Róbert Bódizs et al., 2005; Fernandez & Lüthi, 2020; S. M. Fogel & Smith, 2011; R. Nader & Smith, 2001; Ujma, 2021), learning potential (SM Fogel, Nader, Cote, & Smith, 2007; M Schabus et al., 2006) and reasoning abilities (Fang, Ray, Owen, & Fogel, 2019). Spindle amplitudes and correlations with specific brain region activations during sleep were found to be specifically related to reasoning abilities or fluid intelligence compared to STM or verbal abilities. Reasoning abilities were correlated with spindle time-locked activations of specific brain regions including thalamus, PFC, bilateral putamen, middle cingulate cortex, medial frontal gyrus, and precuneus indication inter-regional communication between thalamus, cortical regions, and basal ganglia during spindles for the use of logic, identification of complex patterns and problem solving (Fang et al., 2020; Fang et al., 2019).

Spindles could be involved in sleep maintenance (Steriade, 2003b). Spindles originate in the thalamus, which a sensory relay system for cortical inputs. Hence spindles could be involved in processing of sensory inputs during sleep through a sensory gating mechanism. Cortical activation as a result of sensory stimulation is strongly attenuated during spindle periods of NREMS. Spindles can thus modulate response to sensory stimuli during SWS in association with cortical SO DOWN states of cortical silence (Dang-Vu et al., 2011; M. D. Schabus et al., 2012). Sleep spindle density correlates with sleep quality and sleep stability indicated by higher resilience

to disruptive external stimuli processing in thalamocortical networks related to sleep maintenance and sensory gating (Dang-Vu, McKinney, Buxton, Solet, & Ellenbogen, 2010). Consistent with this function of sleep spindles, potentiating reticular thalamic nucleus burst activity enhanced spindles, improved sleep quality and increased arousal threshold (Wimmer et al., 2012).

1.11.3.6 Spindles and age-related changes

Age-related changes in SWS and associated with sleep spindles has been correlated for several spindle characteristics including density, duration, amplitude and topography (Campos-Beltrán & Marshall, 2021; Clawson, Durkin, & Aton, 2016; De Gennaro & Ferrara, 2003; Fernandez & Lüthi, 2020; M. Hahn et al., 2019; Landolt, Dijk, Achermann, & Borbély, 1996; Martin et al., 2013). Spindles change across the lifespan from early development to adolescence and aging. During early development spindles are initially seen in the central regions and then gradually develop over frontal and parietal regions. Spindle amplitude, density and duration are higher in the early years and steadily decline with age (Clawson et al., 2016). Age related changes in spindles are probably responsible for impaired motor skill memory consolidation (Stuart Fogel et al., 2017).

Disruption in slow-wave spindle coupling correlated with cognitive decline and medial frontal atrophy (Helfrich, Mander, Jagust, Knight, & Walker, 2018). Aging related reduction of prefrontal fast sleep spindles correlates with poor episodic learning and impaired hippocampal activation during recall (Mander et al., 2014). Given the significance of PFC and its interactions with the hippocampus in memory processing (Euston et al., 2012), these findings indicate mechanisms of age-related prefrontal atrophy-mediated impaired hippocampal-dependent memory processing, in addition to slow wave disruption (Mander et al., 2013), tying well into the role of

SO-spindle coupling in memory processing in neocortical networks (discussed in a following section 1.11.5).

1.11.3.7 Modulation of spindles in disorders

Properties of sleep spindles have been shown to be modulated and can be a promising biomarker in several neurodevelopmental disorders (autism-spectrum disorders or attention-deficit hyperactivity disorders), neurocognitive disorders (mental retardation, cognitive impairments), neuropsychiatric disorders (schizophrenia, depression, mood and anxiety disorders), age-related neurodegenerative disorders (Alzheimer's, Parkinson's, Huntingtin's, dementia), sleep disorders (insomnia, sleep-related movement disorders) and epilepsy (Fernandez & Lüthi, 2020). Impaired spindle-SO coupling is predictive of cognitive decline and pathological features of Alzheimer's disease (Winer et al., 2019).

1.11.3.8 Mechanisms of spindle-mediated memory effects

Behavioral states can modify neuronal activity as a function of network activity. Thalamo-cortical sleep spindles can mediate memory consolidation at both the network level and the cellular level of organization through mechanisms that are conducive to learning. At a network level, spindles can facilitate memory processing by activating corresponding neuronal networks in hippocampus and neocortical regions for memory information transfer. Coupling of spindles with neocortical SO and hippocampal ripples can be a mechanistic process of the information exchange and encoding (This is reviewed in following sections 1.11.5-1.11.8). At a cellular and synaptic level of organization, sleep spindles can facilitate plasticity through LTP mechanisms.

1.11.3.8.1 Functional connectivity and reactivation linked to spindles

Sleep spindle activity has been found to correlate with increases in neuronal activation in hippocampal and neocortical networks and increases in hippocampal-neocortical functional connectivity through the reactivation of memories. The observation that spindles occur during the temporal windows of coordinated activation of specialized cortical and sub cortical networks leads to the speculation that spindles can support sleep-dependent memory consolidation by the transient temporal synchronization and coordination of these activated networks. This has been demonstrated in both humans and rats. Combined EEG-fMRI recordings after learning of face-scene associations showed task-specific reactivation temporally coupled to spindle events restricted to face- and scene-selective visual cortical processing regions and hippocampus. The reactivation strength correlated with spindle amplitude, further highlighting the involvement of spindles in hippocampal-neocortical interactions in declarative memory processing (Til O Bergmann, Mölle, Diedrichs, Born, & Siebner, 2012). Enhanced connectivity between hippocampus and neocortical regions was found to be time-locked to sleep spindle occurrence from simultaneous EEG-fMRI measurements (Andrade et al., 2011).

Spindles can be involved in sleep-dependent motor memory consolidation in two ways: local reactivation of task-specific cortical networks in motor cortex and functional connection of these cortical regions with task-relevant sub cortical networks of the hippocampus, putamen and thalamus (Boutin et al., 2018). Spindles can modulate hippocampal neuronal activation pattern (Andrade et al., 2011; Sirota et al., 2003; Sullivan, Mizuseki, Sorgi, & Buzsáki, 2014) via cortical-entorhinal pathway (Isomura et al., 2006) or through nucleus reunions connecting to CA1 (Dolleman-Van der Weel, Da Silva, & Witter, 1997).

Fast sleep spindles were found to promote restructuring and network redistribution of neural representation of memory traces in PFC networks through direct modulation of distinct task-

dependent functional connectivity between hippocampus and PFC. Anterior hippocampus and ventromedial PFC were functionally connected for object-word pairs, while posterior hippocampus and posterior mPFC for scene-word pairs. This connectivity was found to be enhanced during recapitulation the next day and correlated with fast sleep density (E. Cowan et al., 2020).

Similar to human studies, unit firing and LFP recordings in rats have added to our understanding of this phenomenon at a better spatial and temporal resolution. Unit recordings for motor cortex in rats demonstrated more tightly bound synchronous replay of motor task-specific ensembles right after first skill learning with coincidence of higher slow wave and spindle activity. This further correlated with performance improvement in the post sleep periods. the observation that this replay of synchronous neuronal ensemble activity was observed only after the first skill learning and not during continued practice could indicate a role in neural plasticity and stabilization of early motor learning related cortical networks (Ramanathan, Gulati, & Ganguly, 2015).

Assessment of neuronal firing rates, LFP and IEG in a novel spatio-tactile experience experiment in rats showed several interesting observations (Ribeiro et al., 2007). Neuronal firing rates in the post experience showed strong increases during SWS, which lasted longer in cortical networks (for hours) compared to the hippocampal networks (for minutes). Expression of Arc in the somatosensory cortex was proportional to spindle amplitude, indicating dendritic input rather than somatic output representing tagging of new cortical representations for synaptic strengthening during spindles. Spindle activity during NREMS was predictive of Arc IEG expression in somatosensory cortex during later REMS. Activation of hippocampal- cortical networks during waking experience is followed by cortical plasticity during sleep in accordance with the active systems consolidation theory. In addition, Arc expression specifically in the cortex, and not in the hippocampus during later stages of REMS could be an indication of the disengagement of the hippocampus from memory processing over time, in line with the standard model of memory

consolidation. The study also indicated the involvement of both REMS and SWS in memory consolidation in line with the sequential hypothesis of sleep-dependent memory consolidation (Ambrosini & Giuditta, 2001; Giuditta et al., 1995).

TMR studies have further corroborated and provided additional support to this mechanism. TMR has been shown in several studies to enhance cue-induced spindles during post-learning sleep periods in the task-specific cortical processing regions. Odor-cued memory reactivation during NREM2 enhanced spindle activity during cueing, and this spindle-related reactivation was found to be correlated to gains in motor sequence learning performance the following day (Laventure et al., 2016). Increase amplitude and density of fast spindles in the posterior brain regions involved in visual spatial processing was recorded during olfactory cue-induced evoked spindles during NREMS after word-location association learning (Cox et al., 2014). Auditory-cued TMR helps better consolidation of emotional memories through spindle-mediated reactivation mechanisms (Cairney et al., 2014). The timing of cue presentation was found to be critical for memory reactivation related to sleep spindles, indicating a refractory period between optimal states of reactivation (Antony et al., 2018).

1.11.3.8.2 Spindles in network plasticity/LTP mechanisms

Plasticity is an alteration of neuronal properties including transient neuronal responsiveness, conductance of ionic currents, pre- and post-synaptic modifications, neurotransmitter release and postsynaptic sensitivity (Steriade & Timofeev, 2003). Several experimental approaches and speculations have led to the understanding that spindles can prime neocortical networks for memory-related long-term storage of representations through synaptic plasticity mechanisms.

Intra-thalamic and thalamo-cortical network generates highly synchronized spindle oscillations which can induce a combination of EPSP responses in neocortical pyramidal cells and IPSP through GABAergic pathways. Accordingly, pyramidal cells receive these highly synchronized powerful excitatory inputs from the thalamus and exhibit low rate of discharge during spindle oscillations, indicating dendritic inputs for processing. Thus, during spindles, synchronized high frequency activation from the thalamic neurons generate optimal conditions for cellular processing in the cortex by strong depolarization of dendritic membranes with calcium influx and simultaneously keeping the cell from firing action potentials. Spindles can thereby activate several calcium-dependent mechanisms of synaptic plasticity in cortical pyramidal dendrites: local protein synthesis, activation of CaMKII and activation of PKA as a molecular gate to enhance long-term plasticity related synaptic activation and gene expression (Sejnowski & Destexhe, 2000). Mechanistically, this brings together, calcium-mediated (Ghosh & Greenberg, 1995) dendritic integration (Yuste & Tank, 1996), post-synaptic protein modifications (Soderling & Derkach, 2000) and LTP mechanisms in memory (Lynch, 2004).

Several experimental observations corroborated these speculations. Rhythmic stimulation at 10 Hz in isolated cortical slabs and spontaneous spindles in intact cortex could increase post-synaptic responsiveness to stimuli for several minutes, indicating short- and medium-term neuronal plasticity (Timofeev et al., 2002). Reduced firing frequency of cortical pyramidal neurons during spindles in cats through peri-somatic inhibition (Diego Contreras, Destexhe, & Steriade, 1997a) and prevalence of inhibitory cell activity during sleep spindles in rats (Peyrache et al., 2011) explains the low level of discharge of cortical pyramidal neurons during spindles. The combined process of somatic inhibition through GABA-ergic interneurons and thalamic-mediated dendritic depolarization of cortical pyramidal neurons followed by a massive calcium influx can functionally isolate the dendrites from soma by triggering molecular plasticity mechanisms in the dendrites

maintaining neuronal silence. These neural plasticity-promoting mechanisms mediated by spindles, along with cortical reactivation mediated by SWR could bring together most factors for systems and synaptic consolidation (Peyrache & Seibt, 2020).

Rhythmic depolarizing spike-bursts or spike-trains in cortical and thalamic neurons can cause persistent excitability changes (Steriade, 1999). A direct *in vitro* demonstration of this phenomenon was shown in patch clamp recordings from layer 5 pyramidal cells of rat somatosensory cortex with evoked EPSP's in dendrites and action potentials in soma. A natural sleep spindle firing pattern *in vivo* could induce associative STP-LTP with NMDA receptor-dependent short-term potentiation (STP) and an L-type Ca2+ channel-dependent LTP, which was found to be a direct correlate of the number of spindle sequences in the pattern. Asynchronous spindle trains of EPSP's without simultaneous action potentials led to LTD, while repetitive spike bursts at 10 Hz mimicking a spindle stimulation pattern reliably induced STP-LTP in cortical synapses, indicating that repeated spindle-associated spiking activity can modify excitatory synapses in a Hebbian synaptic strength-dependent LTP mechanism (Rosanova & Ulrich, 2005). An *in vitro* demonstration of this phenomenon was the development of synaptic potentiation through LTP induction and increased reliability of evoked spindles through low intensity corpus callosum stimulation following a similar time course. This correlation between the strength of cortical layer 5 pyramidal cell activation and the efficacy of evoked spindles can bring together spindle oscillations at a network level and LTP mechanisms at a cellular level (Werk, Harbour, & Chapman, 2005). Increased calcium activity specific to neocortical layer 5 pyramidal dendrites was found to be synchronized to spindle oscillations in naturally sleeping rodents. This synchronization was neither found in the cell body nor in other cortical neurons within the column, suggesting a decoupling of calcium activity in the dendrites from somatic activity end the importance of these dendritic computations in memory consolidation process. Thus, spindle mediated plasticity

probably recruits a local dendritic non-Hebbian mechanism (Seibt et al., 2017). Indeed, nested spindles (spindles nested in the SO UP-state) induced high calcium activity in cortical pyramidal cell dendrites and this was associated with peri-somatic inhibition through parvalbumin-positive interneurons and reduced dendritic inhibition through somatostatin-positive interneurons (Niethard, Ngo, Ehrlich, & Born, 2018). Pre-thalamic stimulation during waking stage enhanced somatosensory evoked potentials during SWS, and was found to be associated with a post-synaptic calcium-dependent process involving both AMPA and NMDA receptors mediating LTP in cortical networks (Sylvain Chauvette et al., 2012). Excitatory synapses between thalamocortical and thalamic reticular nucleus neurons also show associative burst-dependent LTP mediated by NMDA receptors (Astori & Lüthi, 2013) and calcium-dependent mechanisms (Coulon et al., 2009). These effects during SWS could be mediated by spindles (Peyrache & Seibt, 2020). In addition, dendritic spine growth during NREMS associated with spindle-related memory consolidation could be a structural mechanism of synaptic plasticity (Ulrich, 2016).

Cortical spindles have been found to spatially and temporally organize global wave-like rotating patterns, organizing the neuronal activity through spike-time dependent plasticity in memory-related large-scale distributed networks (Muller et al., 2016). Spike-timing dependent plasticity mechanisms through coordination of ripple-spindle activity in hippocampal-cortical network (Wierzynski, Lubenov, Gu, & Siapas, 2009) and in firing patterns around spindles in thalamo-cortical network (Gardner, Hughes, & Jones, 2013) can mediate memory-related synaptic plasticity in neocortical networks. Using a technique called paired associative stimulation, in which electrical nerve stimulation is repeatedly paired with transcranial magnetic stimulation, LTP- or LTD- like effects on cortical excitability can be induced based on interstimulus interval and these changes were found to be correlated with local changes in slow spindles and the efficacy of the

stimulation the following day (Til Ole Bergmann et al., 2008). This study along with (Werk et al., 2005) indicate preferential triggering of spindles in recently activated networks.

1.11.4 Ripples and memory consolidation

Hippocampal SWRs have been studied and hypothesized to facilitate memory formation and consolidation through several observations indicating their role in learning, neuronal activation, reactivation and replay, synaptic plasticity, and systems consolidation through coordination with cortical network activities. The two-stage memory consolidation model postulated the active involvement of SWRs in neocortical-driven hippocampal neuronal activation during encoding, hippocampal-neocortical reactivation during replay facilitating synaptic potentiation/plasticity and strengthening of memory-related traces in both circuits and subsequent consolidation of memory traces in neocortical networks. During initial encoding, neocortical activation of SWR-triggered hippocampal activation leads to synaptic reorganization in CA3 as a temporary store of novel information. During subsequent offline periods of quiet wakefulness or sleep, CA3 recurrent network initiates spontaneous population activity triggering SWR-mediated CA1 neuronal activation, which in turn trigger neocortical reactivation leading to consolidation of memory traces in both circuits (Buzsáki, 1989). This is compatible with standard consolidation theory, transformation theory and indexing theory of memory consolidation.

Large excitatory output during SWR recruits downstream regions facilitating systems consolidation through cross-regional communication (Buzsáki, 1989). CA3-driven activation of CA1 generates ripples in CA1 (Hirase, Czurkó, Mamiya, & Buzsáki, 1999). The CA3 driven CA1 activation of ripples was demonstrated in mice experiments where post-training disruption of CA3 output impaired contextual fear memory and it correlated with reduced CA1 ripples in *in vivo*

hippocampal recordings and a significant reduction in the learning-dependent, ripple-associated reactivation of CA1 neurons, revealing that ripple-associated reactivation of hippocampal memory engram is critical for memory consolidation (Nakashiba, Buhl, McHugh, & Tonegawa, 2009). SWRs are also conducive for LTP at synaptic level to facilitate synaptic consolidation (discussed in section 1.11.4.5).

Disruption of SWRs has been reported in several animal models of disorders including epilepsy, Alzheimer disease, dementia, schizophrenia and in normal ageing, underscoring its significance in normal brain functions (Joo & Frank, 2018).

1.11.4.1 Ripples and learning

Learning can influence the physiological characteristics of hippocampal SWRs. Post-learning increases in SWR density, power and duration during SWS was found to correlate with memory performance in rats (Eschenko, Ramadan, Mölle, Born, & Sara, 2008). Increase in SWR density associated with learning and subsequent memory performance was also reported in other rodent studies (Girardeau, Cei, & Zugaro, 2014), in place-reward association task (Mölle et al., 2009; Ramadan, Eschenko, & Sara, 2009), non-human primates (Leonard & Hoffman, 2017) and in human studies (Axmacher, Elger, & Fell, 2008; Zsófia Clemens et al., 2007). Density of post-learning SWRs could also predict subsequent memory performance (Axmacher et al., 2008; Leonard & Hoffman, 2017; Mölle et al., 2009; Ramadan et al., 2009; Singer, Carr, Karlsson, & Frank, 2013). Blocking of learning-induced increase in the rate of hippocampal SWR by using soluble amyloid beta oligomers, impaired the formation of spatial memory, as evident from poor memory performance (Nicole et al., 2016). In addition, optogenetic activation of hippocampal pyramidal neurons artificially prolonged SWRs in rats performing learning tasks, and this increase

in duration was correlated with enhanced spatial memory, implicating ripples in both learning and memory recall processes (Fernández-Ruiz et al., 2019).

1.11.4.2 Disrupting ripples

In addition to studies showing evidence of correlation between learning, ripples and memory recall, the functional role of ripples in memory consolidation was demonstrated by studies that disrupted ripples and examined the effects on subsequent memory retrieval. Ripple-triggered single-pulse stimulation of ventral hippocampal commissure disrupted ripples, and transiently silenced spiking activity of hippocampal neurons, while firing of neocortical neurons was unaffected. Disruption of ripple activity during the transient silent period following stimulation could have prevented potential replay of place-cell sequences that were activated during prior learning. Disruption of ripples during the first hour post learning led to spatial memory deficits in subsequent testing (Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009). Another set of experiments in rodents that disrupted sleep ripples by electrical stimulation of CA1 afferents also found impaired memory recall in a spatial memory task (Ego-Stengel & Wilson, 2010). These studies demonstrate a causal relationship between ripples and memory consolidation.

Disruption of awake ripples in a spatial working memory task impaired performance, indicating a role for ripples in navigational decision making through integration of past experience with a known rule, based on the finding that fidelity of memory replay during awake ripples was found to be higher than during sleep (Jadhav et al., 2012). Optogenetic silencing of CA1 pyramidal cells to disrupt sleep ripples disrupted the reactivation of cell assemblies that gradually strengthened during first exploration (van de Ven, Trouche, McNamara, Allen, & Dupret, 2016). Awake ripple activity was found to be related to stabilization of place fields in a spatial memory

task, as optogenetic disruption of ripples during exploration, destabilized place fields during subsequent exploration (Roux, Hu, Eichler, Stark, & Buzsáki, 2017), while optogenetic inhibition of sleep ripples following exploration does not destabilize place fields (Kovacs et al., 2016). Disruption of spatial memory formation in a reward-based navigation task in mice was found to be related to reduced SWRs at goal location and during subsequent sleep, and impaired the memory performance when septal cholinergic neurons were optogenetically stimulated at goal location (Jarzebowski, Tang, Paulsen, & Hay, 2021).

1.11.4.3 Ripples and neuronal activation

Hippocampal neuronal depolarization/activation by either direct intracellular current injection or extracellular microstimulation near the soma during spontaneously occurring SWRs *in vivo*, can bias cellular responsiveness to be potentiated during subsequent SWRs, indicating that SWR is involved in activity of hippocampal cells, and that SWR-mediated Hebbian cell modification enhances its post synaptic sensitivity through spike-timing mediated LTP mechanisms (King, Henze, Leinekugel, & Buzsáki, 1999). The implication of this effect on learning was demonstrated in a hippocampus-dependent associative learning task, a classical trace eyeblink conditioning paradigm in awake rabbit behaviour. An auditory tone conditioned stimulus (CS) paired with air puff to eyes as unconditioned stimulus (US) were delivered in association with online detection of SWR activity. Ripple-associated training enhanced acquisition of the conditioned response compared to CS-US pairing not time-locked to SWRs. In addition, stronger theta phase-locking to CS and slower extinction slope in trained animals was observed. This indicated stimulus-associated SWR can phase reset theta oscillation resulting in enhances learning rate (Miriam S Nokia, Penttonen, & Wikgren, 2010). In a follow-up study, presentation of a bright

light during SWR activity in the awake inter-trial interval periods, did not disrupt the ripple, but induced a theta oscillation and impaired learning in the conditioning task (Miriam Shirin Nokia, Mikkonen, Penttonen, & Wikgren, 2012). Together these studies show that consolidation could depend on neuronal activity around SWR periods.

In addition to the role of SWRs during sleep, awake memory retrieval in humans was found to be correlated with increase in ripple density. Ripple coupling between medial temporal lobe and temporal association cortex, as a neural mechanism for memory retrieval, correlated with cortical neural activity that was recorded during encoding (Vaz, Inati, Brunel, & Zaghloul, 2019). A similar study also established that efficient encoding of visual information was associated with more ripples, and that successful memory recall was associated with transient reactivation of activation patterns present during encoding in higher visual cortical areas and was preceded by increased ripples (Norman et al., 2019). These studies suggest an important role for awake ripple coupling and neural activation in retrieval of memory representations through hippocampal-neocortical communication.

SWR-associated simultaneous coactivation of neuronal patterns have been recorded in the hippocampus and neocortical areas, implicating the role of ripple-triggered events in systems memory consolidation (Ji & Wilson, 2007; Mölle & Born, 2009; Peyrache et al., 2009; G. R. Sutherland & McNaughton, 2000; Wierzynski et al., 2009). Hippocampal ripples have been temporally found to be correlated with several cortical and subcortical areas including deep entorhinal cortex, mPFC, parietal cortex, somatosensory cortices, visual cortex and auditory cortex, providing evidence of ripple-mediated cortico-hippocampal communication either directly or relayed through entorhinal cortex. It has also been found in the reward processing regions of ventral tegmental area, ventral striatum, and locus coeruleus, and fear and aversive processing regions of

basolateral amygdala. These functional coactivations provide spatial, appetitive, and aversive context to hippocampal processing through ripple-mediated coupling (Todorova & Zugaro, 2020).

1.11.4.4 Ripples and replay

Reactivation forms an important part of standard systems consolidation theory and active systems consolidation view of the memory function of sleep. Reactivations of memory representations during offline periods of sleep are thought to transform and distribute temporary fast-learning newly encoded hippocampal memories into long-lasting slow-learning neocortical networks for long-term storage (S. Diekelmann & Born, 2010; B. Rasch & Born, 2013).

In rats, reactivation of awake spatial memory patterns has been observed at the cellular resolution as temporally ordered sequences of hippocampal neuronal activity linked to SWRs during subsequent post-task SWS (Nádasdy et al., 1999; Peyrache et al., 2009; G. R. Sutherland & McNaughton, 2000; M. A. Wilson & McNaughton, 1994). Similar reactivation patterns at a lower spatial resolution were observed in humans as well (Peigneux et al., 2004). Replay events associated with SWR activity have been observed during awake (Buzsáki, 2015; Diba & Buzsáki, 2007; Foster & Wilson, 2006; Roumis & Frank, 2015) and post-task SWS (Buzsáki, 2015; Ji & Wilson, 2007; A. K. Lee & Wilson, 2002; Nádasdy et al., 1999; Roumis & Frank, 2015) in both well-learned and novel tasks.

The speculation is that the population of CA1 pyramidal cells which are activated during ripple activity are ones participating in a particular information processing network (Gyorgy Buzsaki, 1998; N. Maier, Nimmrich, & Draguhn, 2003). In accordance with that, CA1 cells that were previously activated in mice exploring a novel environment, were found to be preferentially reactivated during spontaneous SWRs in hippocampal slices *in vitro* (Mizunuma et al., 2014).

Increased post-learning sleep reactivation was found to be strongest during SWRs (Kudrimoti et al., 1999; O'Neill, Senior, Allen, Huxter, & Csicsvari, 2008). Selective participation of neurons during SWR is dependent on recent activity in the network during experience. This could have strong implications for learning and memory in cell assembly replay, facilitated by SWR oscillations supporting consolidation within the network through synaptic strengthening (Buzsáki, 1989).

A moving threshold model predicted replay in forward and reverse direction. As threshold for spiking is lowered during SWRs, a cell with place field closest to the animal's current location receives the strongest subthreshold excitation, fires first with progressively weaker excitation cells generating a neuronal sequence of firing pattern as the threshold is progressively lowered (Buhry, Azizi, & Cheng, 2011; Buzsáki, 1989). In accordance with that, forward replay associated with SWRs has been observed during awake behaviour (Diba & Buzsáki, 2007) and NREMS (Ji & Wilson, 2007; A. K. Lee & Wilson, 2002; Nádasdy et al., 1999). Reverse sequence replay associated with SWRs has also been reported (Diba & Buzsáki, 2007; Foster & Wilson, 2006).

1.11.4.5 Ripples and synaptic plasticity

Population bursts of hippocampal activity during SWRs, capable of robust downstream activation in functional neuronal networks, could represent discrete quanta of transfer of stored representations to neocortical networks. Experience-induced changes in the CA3 recurrent presynaptic activity can trigger cooperative CA1 localized fast spiking dynamics and induce synaptic plasticity mediated by calcium influx in apical dendrites (Gyorgy Buzsaki, 1998). Since SWRs can recruit large neuronal population, the downstream activation of neocortical networks by

spatio-temporally coordinated CA1 SWRs can subsequently induce synaptic plasticity in neocortical networks and enhance connectivity for memory consolidation (Buzsáki, 1996).

The fact that strong LTP can be induced in post-synaptic cells by high frequency (100-300Hz) stimulation of presynaptic axons (Bliss & Lømo, 1973; Buzsáki, 1984; Douglas & Goddard, 1975; B. L. McNaughton, 2003; B. L. McNaughton, Douglas, & Goddard, 1978) and blocking NMDA receptor activity impairs LTP and learning (R. Morris, Anderson, Lynch, & Baudry, 1986), makes the high frequency bursts ideal to induce NMDA receptor mediated LTP for synaptic plasticity (E. R. Kandel, 2001a). Mimicking high frequency stimulation, 100-400Hz high frequency bursts of SWRs makes it conducive for endogenous LTP-mediated synaptic plasticity (King et al., 1999; Sadowski, Jones, & Mellor, 2011, 2016). Studies have also shown induction of LTP in CA3 to be important for ripple generation (Behrens, van den Boom, de Hoz, Friedman, & Heinemann, 2005).

Pharmacological induction has been shown to facilitate both SWR activity and LTP in rodent hippocampus with activation of cAMP and PKA as molecular components involved in synaptic plasticity (Rizwan ul Haq et al., 2016; R Ul Haq et al., 2012). Synchrony of spiking rates of CA1 cells is higher during ripples, conducive for Hebbian synaptic plasticity (Mizuseki & Buzsáki, 2013) and suppression of ripples attenuates neuronal firing rates (Talakoub, Schjetnan, Valiante, Popovic, & Hoffman, 2016). A recent report of involvement of SWRs in NMDA receptor mediated synaptic downscaling and learning suggests a selective role of SWRs in strengthening recent activation patterns and at the same time downscaling memory-irrelevant neuronal activity, tying well with synaptic homeostasis hypothesis of SWS (Norimoto et al., 2018; Tononi & Cirelli, 2003, 2014).

1.11.4.6 Awake and sleep ripples

In addition to sleep ripples, studies have also shown a positive correlation between awake hippocampal ripples and their functional role in spatial task learning and memory-guided decision-making behaviours (Jadhav et al., 2012; Jadhav, Rothschild, Roumis, & Frank, 2016; Papale, Zielinski, Frank, Jadhav, & Redish, 2016; Wikenheiser & Redish, 2012).

It is possible that awake ripples and sleep ripples have differential functions. Awake ripples may help memory-guided decision making by rapid retrieval of past experience. Sleep ripples may support consolidation of memories into episodic memory networks through reactivated associations of neuronal assemblies (Jadhav et al., 2016; Roumis & Frank, 2015; Tang & Jadhav, 2019). Awake-state and sleep SWR-mediated memory replay possibly have differential roles evident from the differences during these states. Local SWR-mediated awake replay seems to support memory-guided behaviour, through retrieval and planning with help from dopaminergic neuromodulator mechanisms, while subsequent non-local SWR-mediated sleep replay seems to support integration of memories through gradual consolidation into the framework of existing memories with help from cholinergic neuromodulator mechanisms (Atherton et al., 2015; Roumis & Frank, 2015; Tang et al., 2017).

1.11.5 Spindle-SO coupling

Although spindles can occur during both light sleep (N2) and deep sleep (N3), higher spindle density during SO-rich deep sleep positively correlated with declarative memory retention, suggesting an interaction between the oscillations specific to SWS (Cox et al., 2012). Spindles-cortical SO coupling has been demonstrated to be occurring in the UP state closely following delta waves (Florin Amzica & Mircea Steriade, 1997; Peyrache et al., 2011). Inherent hierarchical cross-

frequency phase-amplitude coupling between SO and spindles has been shown to facilitate memory consolidation (Mikutta et al., 2019). Human studies have shown SO-spindle coupling to be important for memory consolidation. Memory reactivation patterns of previously learned material was locked to SO-spindle complexes during post-learning sleep, with the precision of SO-spindle coupling predicting memory reactivating fidelity and reactivation strength predicted amount of consolidation (Schreiner, Petzka, Staudigl, & Staresina, 2021). Spindle-SO coupling related to cortical up and down states of depolarization and hyperpolarization respectively, and spindle activity temporally correlated with the transition to the upstate has been demonstrated in humans (Mölle et al., 2002) and in rodents (Sirota et al., 2003).

Fast and slow spindles may differ in their temporal phase-locking to SO. Fast spindles were found to be phase-locked to the depolarizing UP-state of SO, while slow spindles to up-to-down state transition of SO (Klinzing et al., 2016). The differential occurrence of slow and fast spindles in temporal relation to SO phases, gives a mechanistic advantage for the involvement of fast spindles through phase locking to SO UP-state, in sleep-dependent hippocampal memory processing through feedback mechanisms between hippocampus and neocortical networks (Mölle et al., 2011). In fact, fast spindles are precisely coupled to SO UP-states in younger adults, while the coupling shifts to the end of SO UP-state in older adults, and this shift correlates with poorer memory consolidation with aging (Muehlroth et al., 2019). Temporal grouping of localized hemispheric fast spindles in the SO UP-state seems to be a dynamic property of SWS modulated by pre-sleep learning-related activation patterns. SO UP-state was specifically associated with increased fast spindle activity coupling over the pre-activated hemisphere. (Yordanova, Kirov, Verleger, & Kolev, 2017).

In addition, the precise timing of spindles during the later stage of slow wave UP-state demonstrating a phase relationship between the two oscillations, correlated with and was predictive

of overnight memory improvement in a finger tapping motor sequence task in schizophrenia patients (Demanuele et al., 2017). SO-spindle coupling precision could also be an indicator of brain maturation from childhood to adolescence. The increase in coupling was found to be correlated with improvements in memory recall performance and sleep dependent memory consolidation in a declarative memory task, strongly indicating the development of hippocampal-neocortical memory systems as a factor of brain development. Cross-frequency coupling could be used as an indicator of network organization during development correlating with enhancements in memory formation (M. A. Hahn, Heib, Schabus, Hoedlmoser, & Helfrich, 2020).

Combined electroencephalography (EEG), structural MRI, and sleep-dependent memory assessment technique revealed that SO is responsible for temporal coupling of spindles and the quality of SO-spindle coupling predicts post-sleep memory recall. Selective atrophy in medial frontal cortex disrupted this cross-frequency coupling resulting in impaired post-sleep memory recall in older adults explaining age-related memory decline (Helfrich et al., 2018), in line with a study correlating hippocampal-dependent memory decline in aging to prefrontal atrophy and disrupted slow waves during NREMS (Mander et al., 2013). SO-spindle enhancement and coupling was shown as a predictor to facilitate consolidation of those memories which are relevant for future planning and behavior (Wilhelm, Diekelmann, et al., 2011). Only SO-coupled spindles and not isolated spindles are involved in hippocampal-neocortical information transfer, suggesting that is SO-spindle coupling is an indicator of hippocampal-neocortical network communication (Helfrich et al., 2019). Impaired spindle-SO coupling is predictive of cognitive decline and pathological features of Alzheimer's disease (Winer et al., 2019).

SO and spindles also interact to modulate the correlation of cortical neuronal firing. Greater synchronized firing in motor cortex neurons during spindle peaks occurred during spindles that occurred in close temporal proximity to SO (nested spindles) and showed a linear relationship, than

during spindles that were temporally remote from a SO (Silversmith, Lemke, Egert, Berke, & Ganguly, 2020). A similar observation was recorded using two photon imaging which showed unique high calcium activity in pyramidal cells and lower inhibitory interneuron activity during nested spindles to UP-state of SO compared with free spindles. This suggests a Ca2+-mediated facilitation of dendritic plasticity mechanisms during nested spindles within local cortical circuits and the SO-spindles coupling generate optimal conditions for that to occur (Niethard et al., 2018).

Neuronal replay in the motor cortex coincident with SO-spindle coupling was recorded during post-learning sleep after motor task, indicating that the coupling mechanism is specific to the brain region associated with the task and that the neural activity could mediate neuroplasticity mechanisms in neural networks during early motor learning (Ramanathan et al., 2015). Optogenetic inhibition of cortical neuronal spiking activity during the UP-states disrupted spindles, reduced the nesting of spindles to SO, reduced ensemble reactivation strength and was correlated to subsequent worsening of performance in a neuroprosthetic skill learning task (J. Kim et al., 2019).

1.11.6 Ripple coupling with SO (ripple-delta coupling)

In terms of directionality, ripples follow somatosensory neuronal activity and precede somatosensory delta waves (Sirota et al., 2003). The surge of activity during ripples could destabilize cortical UP state and allow transition to DOWN state (Jercog et al., 2017). Hippocampal ripples and the reactivation of prefrontal neuronal ensembles peaked before delta waves in post-task sleep, with prefrontal reactivation closely following hippocampal ripples (Peyrache et al., 2009). This suggests a possible role of ripple-delta coupling in memory consolidation in which delta DOWN state of neuronal silence could avoid interference of ripples with unrelated inputs during hippocampal-neocortical information processing. Ripple rate was reported to follow

DOWN-UP state transitions in multiple cortical areas (Battaglia, Sutherland, & McNaughton, 2004; Mölle et al., 2006) indicating a cortical UP state that might trigger hippocampal ripples. Increased cortical spindle, prefrontal neuronal activity and hippocampal ripples were observed in close temporal associations which could be decoded and analyzed to study the temporal dynamics of these oscillations during SO UP-state. SO UP-state-associated prefrontal neuronal activation precedes hippocampal SWRs indicating that prefrontal excitation can promotes hippocampal SWRs via efferent connection pathways. The preceding disfacilitation of hippocampal activity associated with cortical SO DOWN-state has been speculated to synchronize neocortico-hippocampal interplay for effective information transfer during the subsequent period of network-synchronized activation in the respective networks (Mölle et al., 2006). The dynamics of SO-ripple temporal associations was corroborated in a human study of intracranial recordings (Sanda et al., 2021). Early hippocampal ripples influence cortical transitions to UP-state, while cortical UP-states influence occurrence of the later ripples, which in turn influence transition to Down-state. The speculation of differential functional significance of ripples occurring at different phases of SO is related to their roles particularly in the context of encoding new information and reactivation of the information during memory consolidation.

High ripple periods with reactivation in hippocampus immediately precedes cortical DOWN-UP transitions. This alternation between DOWN and UP states could influence hippocampal excitability which in turn could also influence cortical states (Peyrache et al., 2011; Sirota & Buzsáki, 2005; Sirota et al., 2003). Another possible explanation is a common subcortical source which influences both cortical slow waves and hippocampal SWRs or slow wave dependent activation of ripples through entorhinal cortex (Isomura et al., 2006; Sirota et al., 2003). Prefrontal DOWN-UP transitions were found to be followed by entorhinal DOWN-UP transitions and correlated with increased activity in dentate gyrus and CA1 and ripples right after entorhinal

transition. SOs were found to bias hippocampal CA1 and CA3 neuronal activity in such a way that ripples during the DOWN-state may facilitate information processing in hippocampal circuits and ripples in the UP-state could facilitate hippocampal-driven information processing in neocortical circuits (Isomura et al., 2006).

Ripples during post-task sleep recorded from several association cortical regions including mPFC, parietal, retrosplenial, anterior cingulate cortices synchronized with hippocampal ripples in rats and humans, and occurred at cortical DOWN-UP transition, preceding spindles. This coupling suggests a possible role in memory consolidation (Khodagholy, Gelinas, & Buzsáki, 2017). A possible physiological implication of this bidirectional interaction in cortical-hippocampal circuits is reorganization of networks by coordination of cellular consolidation in distributed neocortical networks facilitating systems consolidation (Runyan, Moore, & Dash, 2019; L. R. Squire et al., 2015).

1.11.7 Spindle-ripple coupling

The co-occurrence of spindles and SWRs in close temporal precision is called spindle-ripple coupling. A study by Siapas and Wilson in 1998 was the first to show evidence of close temporal coupling of mPFC neocortical spindles and CA1 hippocampal ripples as an example of a global coordination of neuronal states during SWS following spatial navigation task. In addition, they found the following: hippocampal neurons show a high probability of firing within ripples and near the onset of spindles while cortical neurons fire close to ripples and immediately after spindles; firing of cortical and hippocampal neurons shows strong correlation around ripple and spindle events with hippocampal spikes preceding prefrontal spikes (Siapas & Wilson, 1998). This could be of functional significance in terms of encoding and reactivation for systems consolidation, given

the functional connectivity and interactions between the mPFC and hippocampus (Euston et al., 2012).

In addition to hippocampal activation, neocortical reactivations are also related to spindle-ripple coupling. Prefrontal reactivation of task-related cell assembly patterns coincides with ripples before spindle onset. Meaning, memory related reactivation of prefrontal neurons happens before the high spiking probability of neurons associated with spindles (Peyrache et al., 2009). Study of spike-timing relationships during sleep showed consistent firing of PFC cells within 100ms of hippocampal cell firing. In addition, amplitude of SWRs augments PFC response in the following spindles and these interactions are specific to NREMS and reduced in REMS suggesting functional coupling during SWS (Wierzynski et al., 2009).

Memory-related spindle-ripple coupling have since then been identified in rats (Maingret, Girardeau, Todorova, Goutierre, & Zugaro, 2016; Phillips et al., 2012; Siapas & Wilson, 1998; Sirota et al., 2003), mice (Xia et al., 2017) and humans (Zsófia Clemens et al., 2007; Zsófia Clemens et al., 2011; Mölle et al., 2009; Staresina et al., 2015) indicating a possibility of bidirectional hippocampal-cortical communication in which spindles correspond to a period of reorganization and plasticity in cortico-cortical synapses, and these nested ripples might then integrate this newly reconfigured cortical information into the hippocampal network (Todorova & Zugaro, 2020).

The timescale of ripple-spindle coupling varies by different cortical regions with spindles following ripples within seconds, and ripples being phase-locked to spindles in a finer temporal resolution (Todorova & Zugaro, 2020). SWRs can either be phase-locked to spindles (Zsófia Clemens et al., 2011; Sirota et al., 2003; Staresina et al., 2015) or precede spindles (Mölle et al., 2006; Peyrache et al., 2009; Siapas & Wilson, 1998), possibly indicating differences in information flow through functional connectivity. Fast spindles have been found to be more associated with

increased hippocampal activation, which could indicate their interactive role in sleep-dependent memory consolidation through phase-locking with hippocampal ripples (Mölle et al., 2011; B. Rasch & Born, 2013).

The functional significance of this coupling is underscored by the effects of disorder-related and experimental disruption of the process. Electrical stimulation of locus coeruleus during ripples disrupted spindle-ripple coupling during post-task sleep and resulted in memory impairment (Novitskaya, Sara, Logothetis, & Eschenko, 2016) highlighting a potential role for ripple-spindle coupling in memory processing. Ripple-spindle coupling was found to be disrupted in a rat neurodevelopmental model of schizophrenia, highlighting the importance of this coupling in normal brain functioning (Colgin, 2011; Phillips et al., 2012).

The mechanism proposed for neocortical plasticity mediated by spindle-ripple coupling is the following: ripple activity facilitates reactivation of memory-related cortical cell assemblies, and the following spindle activity induces synaptic plasticity in neocortical networks probably through a retroactive calcium-influx related potentiation of the recent reactivated synapses. Thus, ripple-triggered reactivated cortical memory traces undergo synaptic consolidation during the following spindles, bridging together systems and synaptic consolidation views (Todorova & Zugaro, 2020). mPFC responses to SWR events was different depending on spindle occurrence. In deep mPFC layers, pyramidal cells show phasic response to ripples not coupled with spindles and the phasic response is blocked during ripple-spindle coupling. Superficial neurons, especially interneurons are phase-locked and show tonic response during spindles suggesting local inhibition (Peyrache et al., 2011).

An additional observation is that hippocampal SWRs during SWS are associated with cortical activations at a time window when cortical inputs from other regions (subcortical thalamic, associational and midbrain neuromodulatory inputs) are supressed to allow optimal information

processing from hippocampus. Together, this shows that thalamic inputs induce local cortical inhibition during spindles, and that mPFC is more responsive to SWRs when not involved in thalamo-cortical coupling, indicating cortical processing by local synaptic consolidation during spindle-ripple coupling. (Logothetis et al., 2012; Peyrache et al., 2011).

1.11.8 Spindle-ripple-SO triple coupling

The memory consolidation effects of SWS have been indicated to depend on the neocortical SO, in terms of its frequency and essentially global occurrence, capable of temporal coupling of local intrinsic oscillations from different brain regions as a form of communication, especially hippocampal-neocortical communication, by temporally coupling neocortical spindles and hippocampal ripples in terms of local activity dynamics (Born et al., 2006; S. Diekelmann & Born, 2010; Marshall & Born, 2007; Marshall et al., 2020; Mölle & Born, 2011; B. Rasch & Born, 2013). This triple coupling has direct implications on systems consolidation between brain regions and synaptic consolidation with local regional networks, despite attenuated neuromodulatory activity which could otherwise be less conducive to synaptic plasticity (Klinzing et al., 2019; Schreiner & Staudigl, 2020). Fast local oscillations can facilitate recruitment of smaller number cell assemblies and synaptic plasticity, while slower oscillations can recruit larger spatial neurons and hence can facilitate temporal organization and synchronization of fast local oscillations across brain regions, acting as a pacemaker to set the rhythm of fast oscillations within local brain regions (B. Rasch & Born, 2013; Sirota & Buzsáki, 2005; Sirota et al., 2003).

Ripple-delta-spindle triple coupling has also been demonstrated in humans and several animal models (Abel et al., 2013; Zsófia Clemens et al., 2007). The first study in both rats and mice demonstrated coactivation of neuronal networks in somatosensory cortex and hippocampus

correlated with temporally fine-tuned interaction between neocortical SO, spindles and hippocampal SWR, indicated that this triple coupling is important in the underlying information transfer process between the regions (Sirota et al., 2003). To that effect, several studies in humans and rodents have demonstrated the correlation of this temporal triple coupling to memory formation during learning and subsequent performance indicating memory consolidation (Zsófia Clemens et al., 2007; Charles-Francois V Latchoumane, Hong-Viet V Ngo, Jan Born, & Hee-Sup Shin, 2017; Marshall et al., 2020; Mölle et al., 2009; B. Rasch & Born, 2013; Sirota et al., 2003; Skelin, Kilianski, & McNaughton, 2019).

Increased cortical spindle, prefrontal neuronal activity and hippocampal ripples were observed in close temporal associations which could be decoded and analyzed to study the temporal dynamics of these oscillations during SO UP-state. SO UP-state-associated prefrontal neuronal activation precedes hippocampal SWRs indicating that prefrontal excitation can promotes hippocampal sharp waves via efferent connection pathways. The preceding disfacilitation of hippocampal activity associated with cortical SO DOWN-state has been speculated to synchronize neocortico-hippocampal interplay for effective information transfer during the subsequent period of network-synchronized activation in the respective networks (Mölle et al., 2006). Synchronous DOWN-UP transition during SO could be a common physiological trigger for both spindles and ripples synchronizing them within very short temporal windows facilitating information processing between their local brain regions (Buzsáki, 2015). Learning-induced enhancement of SO linked to spindle-ripple coupling with temporal precision to SO UP-states has been demonstrated in both humans and rats (Mölle et al., 2009).

Induced fine temporal coupling between ripple-delta-spindle by ripple-triggered stimulation of delta-spindle sequences showed memory recall enhancement in object-place spatial memory task in rats. In addition, functional reorganization in prefrontal networks and increased

task-responsive prefrontal cells indicated that the underlying mechanism was the tight temporal ripple-delta-spindle coupling as the occurrence of delta-spindles did not increase compared to controls (Maingret et al., 2016). This could be a mechanism for SO-induced memory enhancement (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014; Lisa Marshall et al., 2006) that it was not just the boosting of SO, but the SO-triggered coupling between ripple and spindles (Todorova & Zugaro, 2020).

Learning-dependent patterns of mPFC reactivation of neuronal assemblies during UP-states of SO coincided with hippocampal SWRs with mPFC spindles lagging behind hippocampus by about 40ms. In addition, reactivation in both mPFC and hippocampus were higher just before spindle activity than after spindle activity, supporting the idea that SO-induced spindle-ripple coupling may facilitate previously activated synapses during reactivation (Peyrache et al., 2009). This could be of functional significance in terms of encoding and reactivation for systems consolidation, given the functional connectivity and interactions between the mPFC and hippocampus (Euston et al., 2012).

Additional support for the memory function of ripple-delta-spindle coupling comes from a detection of delta wave UP-state triggered optogenetic stimulation of thalamic spindles in a closed loop stimulation protocol. Cortical networks in UP-state were entrained resulting in hippocampal ripples phase locked to stimulation and enhanced memory performance in contextual fear condition task in mice. No memory enhancement was found in DOWN-state induced stimulation even though ripples were locked to stimulation (Charles-Francois V Latchoumane et al., 2017).

Neocortical SO has been speculated to be the driving force for hippocampal activity to subsequently activate neocortical reactivation (Ji & Wilson, 2007). Cellular processes initiated by learning in neocortical and hippocampal circuits are brought together by SO, through spindles and ripples to coordinate cellular consolidation in distributed neocortical networks which have already

been primed during initial learning (Runyan et al., 2019). SO characterized in the hippocampus has been found to follow cortical SO as an additional evidence of neocortical-hippocampal-neocortical reactivation network. Cortical SO has been found to lead hippocampal SO in EEG recordings (Wolansky et al., 2006). Intracranial human EEG experiment showed that SO-spindle coupling triggers hippocampal ripples which initiate subsequent information transfer to the neocortex and this timing ensures maximal information transfer during optimal neocortical processing capacity windows (Helfrich et al., 2019). The precise temporal coupling between SO, spindles and ripples have so been found to be linked, that they appear to be hierarchically nested within each other, with spindles being modulated by the UP-state of SO, and spindles modulating the ripples that they cluster in the spindle troughs (Staresina et al., 2015).

Activation of cortical neuronal patterns precedes and predicts hippocampal activation during SWRs with hippocampal activity patterns activating subsequent cortical activity patterns. The bidirectionality in communication through local oscillations is speculated to ensure optimal transfer of information between these regions. This has been shown in other studies as well that neocortex initiates ripple-triggered information transfer through oscillatory coupling; SO might orchestrate spindle-ripple coupling as ripples follow cortical delta waves, which are in turn followed by spindles (Maingret et al., 2016; Navarrete, Valderrama, & Lewis, 2020; H.-V. Ngo, Fell, & Staresina, 2020; Rothschild et al., 2017; Sirota & Buzsáki, 2005). The predicted sequence of cortical-hippocampal-cortical communication is as follows: Hippocampal SWR-associated replay during SWS would trigger neuronal reactivation in deep cortical layers during SO UP-state and the following delta DOWN wave would selectively isolate target synapses from other competing inputs to allow their network reorganization during the next UP-state transition and the following spindle possibly strengthens those synapses, which in turn effectively influence

hippocampal neuronal excitability and SWRs (Battaglia et al., 2004; Maingret et al., 2016; Mölle et al., 2006; Sirota et al., 2003).

1.12 Brain stimulation

Brain stimulation has taken several forms which broadly classify as non-invasive and invasive brain stimulation techniques. Non-invasive brain stimulation techniques include Transcranial electrical stimulation (tES), including Transcranial direct current stimulation (tDCs) & Transcranial alternating current stimulation (tACs) and Transcranial magnetic stimulation (TMS). Transcranial random noise stimulation (tRNs) is also a non-invasive electrical stimulation technique in which a weak alternating current oscillating at random frequencies is delivered from electrodes on the scalp. In addition to electrical stimulation induced by electrical or magnetic fields, other mechanisms of non-invasive sensory stimulation have also been studied for memory consolidation through the enhancement of SWA and SO. These include somatosensory, auditory, vestibular (slow rocking) and olfactory stimulation techniques either through targeted memory reactivation or subsequent effects on memory-related sleep oscillations. Deep brain stimulation and optogenetic stimulation are invasive techniques.

All of these stimulation techniques can be either applied during awake stage or sleep and have been found to modulate brain oscillations. The effects of this modulation can be tapped for studying the mechanistic functional role, as well as for applications to enhance or modulate the oscillations and their temporal relationships to each other, as a therapeutic strategy in several disorders, more specifically for memory enhancement (Brasil-Neto, 2012; Cellini & Mednick, 2019; Chase, Boudewyn, Carter, & Phillips, 2020; Ghorbani & Marshall, 2020; Grimaldi, Papalambros, Zee, & Malkani, 2020; Hanslmayr, Axmacher, & Inman, 2019; Malkani & Zee,

2020; Marshall & Binder, 2013; Marshall & Born, 2011; Riddle & Frohlich, 2021; Rosenblum & Dresler, 2021; Salfi, D'Atri, Tempesta, De Gennaro, & Ferrara, 2020; Sankar, Lipsman, & Lozano, 2014; Vosskuhl, Strüber, & Herrmann, 2018; Yavari, Jamil, Samani, Vidor, & Nitsche, 2018).

1.12.1 Electrical stimulation

The concept of electrical stimulation to modulate the nervous system dates back to ancient Romans who applied live electric ray *Torpedo nobiliana* to the head of a patient in order to alleviate headache, depression and epilepsy (Schwalb & Hamani, 2008). Effects of electrical stimulation on neuronal activity are measured by membrane potential changes, firing rate in single neurons, synchrony in neuronal populations, evoked population responses, entrainment of endogenous oscillations and modulation in pharmacologically induced active network. Effects of weak electrical stimulation modulating active neuronal networks depend on current dynamic state of the network determined by excitatory and inhibitory activity withing the network and influences from afferent pathways (Woods et al., 2016).

1.12.2 Transcranial electrical stimulation

Transcranial electrical stimulation is achieved by the application of mild electric current through electrodes placed on the cranium. tES does not generate action potentials in neurons. The passing of electricity through cortical regions can bidirectionally modulate neuronal activity by subthreshold decrease or increase of neuronal membrane potential and can thus influence the firing rate of neurons. Neuroimaging and neuromodulation studies with tDCs and tACs have shown activation in brain regions specific for motor skill learning, emotional processing and cognitive

processing and has demonstrated promising therapeutic effects in motor rehabilitation, depression, and memory functions (Yavari et al., 2018).

1.12.2.1 Transcranial direct current stimulation (tDCs)

tDCs involves application of a constant current over time through electrodes on the skull. Modulation of cortical excitability by positive or anodal and negative or cathodal current can result in depolarization or hyperpolarization of local cortical neurons respectively in the target area. The weak direct current of tDCs can influence the spontaneous firing rate of neurons by modulating the local membrane potentials. Modelling studies and recordings from intracranial electrodes suggest that the electrical field intensities caused by tDCs are well below the levels required to trigger action potential, while the weak currents can entrain oscillations (Chase et al., 2020). It merely changes the threshold for excitability without generating action potentials or LTP. Anodal tDCs over the frontal cortex in rat was shown to induce neuronal activation in cortical and subcortical regions (Takano et al., 2011).

Spontaneous activity of target neurons is modulated by the direction of current flow, in addition to other biological effects including changes in neurotransmitters, glial cell excitability changes, effects on vasculature and modulation of inflammatory responses. The modulation is dependent on current physiological state of neural network. It causes a tonic shift in neuronal membrane potential influencing neuronal excitability depending on polarity of stimulation. Surface anode produces inward flow from cortical surface causing neuronal depolarization and increasing excitability, while surface cathode produces hyperpolarization thereby increasing excitability threshold. Extent and direction of polarization of dendrites and axons will determine net changes in excitability (Woods et al., 2016). Bidirectional modulation of primary motor cortical excitability

changes with anodal tDCs causing excitation and cathodal tDCs causing reduction, measured by motor-evoked potentials, has been observed in humans (Furubayashi et al., 2008) and mice (Cambiaghi et al., 2010). This bidirectional effect of tDCs was also observed in primary somatosensory cortex in mice (Sánchez-León, Cordones, et al., 2021). Bidirectional effects of motor cortical excitability was observed with SO-tDCs in humans (Groppa et al., 2010).

Depending on the parameters of stimulation like shape, polarity, density, duration and electrode geometry, it can produce acute or long-lasting excitatory or inhibitory changes in cortical neuronal plasticity. These effects can be regional and across brain regional networks. Neuroplasticity mechanisms activated by stimulation could include changes in early gene activity, protein expression, NMDA receptor-mediated LTP-like effects of anodal stimulation, LTD-like effects of cathodal stimulation, intracellular concentrations of cAMP, calcium and BDNF, astrocyte-mediated changes, neurotransmitters and neuromodulators, which have all been studied in the pathway for plasticity at synaptic and systems level (Yavari et al., 2018). tDCs can cause BDNF-mediated synaptic plasticity and LTP when paired with thalamocortical stimulation (Fritsch et al., 2010). tDCs-induced LTP-mediated hippocampal synaptic plasticity has been reported (Rohan, Carhuatanta, McInturf, Miklasevich, & Jankord, 2015). Anodal tDCs has been shown to have direct and/or indirect effect on the dopaminergic system in the rat basal ganglia measured by increased extracellular dopamine levels for more than 400 min in the striatum (Tanaka et al., 2013). tDCs can directly influence glial cells and indirectly enhance cortical neuronal responses through astrocytic Ca2+/IP3 (inositol trisphosphate) signalling. tDCs was found to induce large-amplitude astrocytic Ca2+ surges across the entire mouse cortex without any changes in LFP. In addition, evoked cortical neuronal responses were absent in IP3R2 knockout mice, in which astrocytic Ca2+ waves are absent (Monai et al., 2016).

tDCs during wakefulness has been shown to be effective in a wide range of cognitive functions including perception, attention, working memory, motor learning, associative verbal learning, perceptual learning, decision making and emotion. Effects gave also been demonstrated in neurological/psychiatric disorders including depression, schizophrenia, mild cognitive impairment and Alzheimer's (Shin, Foerster, & Nitsche, 2015).

1.12.2.1.1 tDCs in memory

Short duration tDCs result in acute effects to membrane potential for the period of stimulation, while long duration stimulation can produce long-lasting neuroplastic effects in cortical activity including LTP and LTD indicating a possible physiological function in learning and memory in neuronal networks (Grimaldi et al., 2020).

Declarative memory enhancement was reported after day-time nap with application of tDCs with SO enhancement (Lisa Marshall et al., 2004). tDCs applied to temporal cortex had been found to be effective in Alzheimer's patients in visual recognition memory processing (Paulo Sergio Boggio et al., 2012; Paulo S Boggio et al., 2009; Ferrucci et al., 2008; Grimaldi et al., 2020).

The effects of tDCs have also been found to be specific to the region of the brain targeted for stimulation and its associated function. Anodal tDCs on PFC improved implicit task learning, while cathodal stimulation on the PFC and anodal stimulation on the primary visual cortex did not show learning effects (Kincses, Antal, Nitsche, Bártfai, & Paulus, 2004). tDCs targeted to the speech-specific Broca's area and not to other brain regions unrelated to speech processing, was found to enhance the acquisition of artificial grammar as a measure of implicit learning (De Vries et al., 2010). Anodal tDCs to primary motor cortex enhanced motor learning and increased the magnitude and duration of motor memories (Galea & Celnik, 2009). Anodal tDCs over the primary

motor cortex (M1) in humans has shown to enhance learning a novel and challenging motor skill task over an extended time course, demonstrating a promising therapeutic strategy motor rehabilitation after brain injury (Reis et al., 2009). tDCs has also been shown to be effective to enhance visuospatial working memory and skill learning in rats (Dockery, Liebetanz, Birbaumer, Malinowska, & Wesierska, 2011) and associative learning in rabbits (Márquez-Ruiz et al., 2012). In the latter study, eye blink conditioning was found to be either potentiated or depressed by the application of anodal or cathodal tDCs respectively.

There is significant variability in the findings of the effects of tDCs on memory consolidation. The effects of tDCs on memory-related spindles activity are variable from no effects to effects specific to slow or fast spindles or even a reduction of spindle activity. Other factors contributing to the effects include circadian effects of stimulation, amount of current to have effects beyond the superficial layers, timing and phase locking to intrinsic oscillations, individualized stimulation parameters to account for inter-individual differences, and the fact that changes to outlast the duration of stimulation for detection (Grimaldi et al., 2020).

1.12.2.1.2 SO-tDCs in memory

Most evidence of the beneficial effect of tDCs during sleep to enhance memory comes from oscillatory-tDCs (Grimaldi et al., 2020). The timing of neuronal activity within oscillatory neuronal networks is important for information encoding and transfer providing a mode of communication between distinct neuronal populations across different regions of the brain. Slow oscillatory stimulation at the resonance frequency of a network, time-locked to the local oscillatory patterns can facilitate communication between the hippocampus and the neocortex in memory processing. Given the importance of hippocampal-neocortical interactions in memory, slow oscillatory

stimulation can modulate local field potentials and neuronal firing rates between the regions (Marshall & Binder, 2013).

Application of SO-tDCs during night-time sleep, with enhancement of SO and spindles (Lisa Marshall et al., 2006) and daytime nap, with enhancements in SWA (Antonenko, Diekelmann, Olsen, Born, & Mölle, 2013) enhanced performance in declarative memories. Memory effects with concomitant enhancements in brain oscillations showed that the effects of the stimulation were mediated by changes to endogenous oscillations during SWS. However, in other studies using SO-tDCs (Bueno-Lopez, Eggert, Dorn, & Danker-Hopfe, 2019) and square wave tDCs (Sahlem et al., 2015) showed no effects on declarative memory consolidation.

Inter-individual differences like memory quotient can also modulate efficacy of SO-tDCs. SO-tDCs enhanced fast spindle parameters (counts, density, length and EEG power) in all subjects in acute and post-stimulation periods. Task-induced slow spindle density in the post-stimulation period increased in subjects with high MQ and decreased in those with low MQ. Higher learning and memory retention with SO-tDCs was shown in subjects with high memory quotient. (Koo, Mölle, & Marshall, 2018).

A recent study showed that it is not just the SO-tDCs, but the efficacy of stimulation on memory retention associated with SO-spindle coupling closer to SO trough (Dehnavi, Koo-Poeggel, Ghorbani, & Marshall, 2021). Stimulation time- and phase-locked to the frequency SO also seems to be an important determining factor for the efficacy on sleep-dependent memory consolidation, probably through SO-spindle coupling mechanisms (Grimaldi et al., 2020) as seen with tES (Cellini et al., 2019), SO-tDCs in older adults (Ladenbauer et al., 2016; Westerberg et al., 2015) and auditory stimulation (H.-V. V. Ngo et al., 2013; Santostasi et al., 2016).

Compared to the volume of SO-tDCs studies in humans, very few studies have investigated the effects of SO-tDCs application in sleep-dependent memory consolidation in animals (Campos-

Beltrán & Marshall, 2017). Pulsating wave of 12uA current for 45-sec with 15-sec ramp up and ramp down and 45 sec off applied to the medial cortex was one of the first studies in rats to study the effects of oscillatory currents (Albert, 1966). Anodal and cathodal currents between medial cortex and the back of rat showed differential effects on cortical excitability. Cathodal stimulation abolished memory retention and anodal stimulation improved memory consolidation. Better performance in terms of reduced error rate in radial arm maze task (Binder, Rawohl, et al., 2014) and better performance in object place recognition task (Binder, Berg, et al., 2014) were reported following SO-tDCs during post-learning sleep involving the hippocampal-mPFC network. This could tie in with the functional connectivity and interactions between the mPFC and hippocampus in encoding, decision-making and reactivation for systems consolidation (Euston et al., 2012).

Oscillatory stimulation of the frontal cortex from intracellular recordings in anesthetized rats and extracellular recordings from behaving rodents led to an intensity-dependent enhancement of neuronal firing during stimulation in cortical and hippocampal areas. Neuronal phase-locking to the electrical field stimulation increased with stimulus intensity and also depended on the behavioral state of the animal. In addition, 1.25 Hz stimulation entrained neurons during natural sleep highlighting that similarity between endogenous oscillations and stimulation frequencies is mechanistically and functionally important for stimulation efficacy in terms of modulation of brain oscillatory functions and neuroplastic changes (Ozen et al., 2010). Moderate intensity sinusoidal slow oscillatory electrical stimulation applied to the frontal cerebral cortex of anesthetized rats entrained hippocampal slow activity, increased SWR and cortical spindles, indicating the role of cortical hippocampal connections in memory consolidation (Greenberg, Whitten, & Dickson, 2016).

1.12.2.2 Transcranial alternating current stimulation (tACs)

tACs involves the application of a balanced sinusoidal alternating current through the electrodes. With alternating currents, the electrode alternates between being an anode and cathode in every cycle of the oscillation. It can cause non-linear modulation of cortical excitability during and post-stimulation depending on stimulation parameters like frequency, duration, amplitude and phase. It causes sub-threshold modulation of neuronal resting membrane potential depending on direction of current flow (Yavari et al., 2018).

tACs can have long lasting effects in terms of entrainment of endogenous brain oscillations (Helfrich et al., 2014). Entrainment is the process of a natural, intrinsic endogenous oscillation being phase- and frequency-synchronized to an external stimulation (Thut, Schyns, & Gross, 2011). tACs can entrain intrinsic oscillatory brain activity and modulate amplitude, frequency, phase-coherence, phase-synchronization of brain oscillations and these effects can be regional and across brain regional networks (Vosskuhl et al., 2018). Entrainment effect of tACs on neuronal oscillations is found to be strongest when the applied stimulation very close to the network's endogenously preferred frequency range or the systems resonance frequency (Fröhlich, 2015).

Given the high variability of resonance frequencies across individuals (T Zaehle, Lenz, Ohl, & Herrmann, 2010), a measure of individual frequency range can be taken into account to specifically modulate the individualistic endogenous oscillations, as applied and demonstrated for individual alpha frequency (IAF). Alpha activity peak frequencies can be clearly extracted in the individual frequency spectra and this information can be used to modulate the frequency of tACs to target the specific oscillation in a particular region of the brain. Personalized tACs over occipital cortex was found to entrain the neuronal oscillatory activity in their IAF range and elevated the endogenous alpha power in parieto-central (Tino Zaehle, Rach, & Herrmann, 2010). Enhanced IAF power lasted for at least 30 mins when tACs was applied during low endogenous IAF power, while

IAF power was not further enhanced by the application of tACs during high endogenous IAF power, indicating that the lasting modulation effects of tACs is dependent on the range of the stimulation frequency and endogenous oscillation frequency power as a measure of brain states (Neuling, Rach, & Herrmann, 2013).

The alternating current can modulate the neuronal spike timing in an oscillatory mode at the frequency of the local or global endogenous brain oscillations. Simulation studies in a network of spiking neurons indicated that selective modulation of synapses by STDP was dependent on the resonance frequencies of the neural circuits that the synapses belong to. Thus, the effects of tACs on neural activity as an after-effect, could be mediated by STDP (Tino Zaehle et al., 2010).

140Hz tACs increased M1 excitability as measured by TMS-generated motor evoked potentials (MEPs) during and for up to 1 h after stimulation (Moliadze, Antal, & Paulus, 2010), indicating cortical excitability changes lasting beyond the duration of stimulation. High frequency tACs can induce neuroplastic effects (Yavari et al., 2018).

tACs targeted to a large neuronal population can modulate frequency-dependent oscillatory network activity and membrane potential of individual neuronal membranes. Fluctuating membrane potentials between depolarization and hyperpolarization in neuronal compartments and networks depends on frequency of stimulation with higher frequencies being less effective. Frequency, phase electric field strength and current density of the oscillating stimulation determines polarization, entrainment effects and its duration. tACs is thought to be most effective at entraining oscillations when it is frequency-locked to endogenous neuronal oscillation (Arnold's tongue). Cross-frequency interactions, phase coupling interactions and harmonics of stimulation frequency can also be affected by tACs. Through oscillatory modulation, it can be effective in a range of cognitive functions including perception, attention, working memory, declarative memory, fluid intelligence, decision making, and self-awareness when dreaming (Woods et al.,

2016). Frequency-specific tACs with changes in intensity and phase, can be targeted towards several brain regions to modulate specific brain oscillations for desired physiological outcomes (Antal & Paulus, 2013). With the optimization of stimulation parameters to spatial and temporal dynamics of endogenous brain oscillations, targeting individual frequency localization and cross-frequency coupling, tACs can be used as a potential therapeutic intervention in range of neurocognitive and neuropsychiatric disorders including dementia, depression, schizophrenia, chronic pain, ADHD and OCD, most of them with altered brain oscillations (Elyamany, Leicht, Herrmann, & Mulert, 2021).

1.12.2.2.1 tACs in memory

tACs frequency- and phase-locked to endogenous SO delivered in a closed loop system during overnight sleep showed visual memory enhancements (A. P. Jones et al., 2018; Ketz, Jones, Bryant, Clark, & Pilly, 2018). The stimulation also increased slow wave power and enhanced coupling with spindles (Ketz et al., 2018).

1.12.2.3 Transcranial random noise stimulation (tRNs)

tRNs is the latest modified version of tACs. The tRNs device generates alternating current following a white noise structure, with all frequencies having equal power, with a Gaussian amplitude structure. Low-frequency tRNs (0.1–100 Hz) and high-frequency tRNs (100-640 Hz) are defined based on up to half of the sampling rate of 1280 Hz in typical human EEG recordings (Moret, Donato, Nucci, Cona, & Campana, 2019). It is based on the concept of aperiodic brain signal which meaningfully tracks the excitatory-inhibitory balance of neural activity as a mutable neural phenomenon. Accordingly, relatively greater power in the low-frequency range can be

inhibitory and relatively greater power in the high-frequency range can be excitatory (Riddle & Frohlich, 2021). The effects of tRNs were found to depend on the width of the selected frequency range (Paulus, 2011). Higher frequencies (100-640 Hz) effectively generated excitability increase (Terney, Chaieb, Moliadze, Antal, & Paulus, 2008). More specifically, only the full band (100-700 Hz) significantly modulated cortical excitability by enhancing MEPs after stimulation (Moret et al., 2019). The narrower frequency band within the full range was found to be ineffective.

The primary effect of tRNs seems to be increases in cortical excitability (Moliadze, Fritzsche, & Antal, 2014; Moret et al., 2019; Terney et al., 2008). tRNs was originally introduced and demonstrated in human study and the application of an alternating current with random amplitude and wideband frequency showed consistent excitability increases over the motor cortex lasting 60 min after stimulation, measured by increases in motor-evoked potentials, and positively correlated with behaviour (Terney et al., 2008).

The excitatory effects have been attributed to several factors. Stochastic resonance effect, specifically at the higher frequency ranges, mediated by repeated subthreshold stimulations was proposed in the original study (Terney et al., 2008) and speculated from significant increase in auditory steady state responses to 40 Hz frequency modulated tone and increased excitability in the auditory cortex (Van Doren, Langguth, & Schecklmann, 2014). Stochastic resonance is a phenomenon where a normally weak signal (for electrode detection) can be boosted by the addition of broad-band noise to the signal. It can non-selectively boost all frequencies such that the endogenous activity regardless of its frequency band is amplified (Antal & Herrmann, 2016; Riddle & Frohlich, 2021). This is possibly mediated by increased synchronization of neural firing through amplification of existing subthreshold oscillatory activity, which determines the frequency of suprathreshold signal. Thus, the induced neural synchrony can reduce the amount of endogenous noise, thus improving signal-to-noise ratio in the targeted brain regions with sensitization of

sensory processing functions leading to enhanced perception and cognitive performance (Antal & Herrmann, 2016; Pavan et al., 2019). At a neuronal level the effects are attributed to increased sensitivity of neuronal networks to modulation, kinetics of activation and inactivation of Na^+ channels (Antal & Herrmann, 2016; Paulus, 2011; Remedios et al., 2019) and NMDA-receptor independent but sodium-channel blocker dependent and benzodiazepines-sensitive plasticity (Chaieb, Antal, & Paulus, 2015). Chronic tRNs at low-density currents is capable of increasing excitability by decreasing focalized GABA levels in the PFC of juvenile mice (Sánchez-León, Sánchez-López, et al., 2021), leading to the speculation that behavioural enhancements seen with tRNs could be due to decreased GABA. tRNs of the DLPFC was correlated with neurovascular-coupling mediated hemodynamic changes in the DLPFC and corresponding improvement in calculation- and memory-recall based arithmetic learning, which lasted for 6 months after the stimulation (Snowball et al., 2013), indicating a robust long-term enhancement of cognitive brain functions.

Studies with high frequency tRNs have shown enhancements in several cognitive functions. Enhancement with tRNs have been demonstrated in sensory visual perception (van der Groen & Wenderoth, 2016), facial identity (Romanska, Rezlescu, Susilo, Duchaine, & Banissy, 2015) and perception of emotions (Penton, Dixon, Evans, & Banissy, 2017). It has also been found to enhance learning and memory as shown in studies enhancing perceptual learning (Fertonani, Pirulli, & Miniussi, 2011), arithmetic and calculation skills (Snowball et al., 2013), visual learning (Herpich et al., 2015) and working memory (O. Murphy et al., 2020). Therapeutically, it has the potential to desynchronize pathological rhythms (Paulus, 2011). It has found therapeutic applications in tinnitus, depression, schizophrenia, pain reduction in multiple sclerosis, decreasing motor excitability in Parkinson's disease tRNs (Moret et al., 2019).

1.12.2.4 Transcranial pink-noise stimulation (tPNs)

tPNs is the latest version of tRNs, in which the power distribution of the stimulation waveform is matched to the 1/f characteristics of the a priori measured endogenous activity of the brain. Similar to tRNs, the generation of electrical waveform of tPNs uses a random phase for each frequency but with a different amplitude for each frequency based on 1/f parameters (Riddle & Frohlich, 2021).

High-density tPNs targeted to the ACC had physiological effects of reduction in food craving in women with obesity (Leong et al., 2018). The effect was speculated to be due to reduction in low-frequency beta oscillations over ACC caused by suppression of higher frequency activity by the relatively higher power in low-frequencies from tPNs.

tPNs can be personalized to a certain extent to individual frequency localization, given that slope of the aperiodic signal is estimated for stimulation, to modulate a steeper or flatter slope of the aperiodic signal of the brain. Aperiodic tPNs signal with a superimposed, individualized in-phase periodic component and a random phase for the other frequencies could generate a unique waveform, approximating the endogenous neural signals to enhance the physiological validity of the stimulation waveform, with the potential of most effective modulation of endogenous neural activity, compared to traditional tACs. Further, this could help to causally dissociate the functional roles periodic and aperiodic signals in cognition (Riddle & Frohlich, 2021).

1.12.3 Transcranial magnetic stimulation

TMS involves the application of high intensity short lasting electromagnetic field to deliver electric current pulse to superficial layers of the cerebral cortex, which have the capacity to generate above threshold activation and action potential generation in neurons. It has higher temporal and spatial resolution compared to TES (Grimaldi et al., 2020; Yavari et al., 2018). The effects of TMS

are seen in local neurons unto about 2 cm depth superficial cortical neurons with potential to activate functionally connected brain regional network. The effects of TMS on neuronal modulation is frequency- dependent, with lower frequencies at around 1 Hz decreasing neuronal excitability and high frequency stimulation between 5-20 Hz increasing neuronal excitability. In terms of the mechanism of action, TMS can modulate LTP-like and LTD-like cortical plasticity through spike timing dependent synaptic changes in paired association stimulation paradigm. The effects of TMS can last well beyond the stimulation period for even up to 70 minutes (Grimaldi et al., 2020).

1.12.3.1.1 TMS in memory

Repetitive TMS (rTMS) is the application of TMS with frequency of 1 Hz or higher. rTMS stimulation targeted to the dorsolateral prefrontal cortex (DLPFC) has been shown to be effective in several cognitive functions including working memory (Bagherzadeh, Khorrami, Zarrindast, Shariat, & Pantazis, 2016; Brunoni & Vanderhasselt, 2014), episodic memory (Gagnon, Schneider, Grondin, & Blanchet, 2011), attention (Hwang, Kim, Park, Bang, & Kim, 2010) and procedural skills (Vanderhasselt, De Raedt, Baeken, Leyman, & D'haenen, 2006).

DLPFC is crucial for working and episodic memory processing indicating interaction between them. DLPFC uses information from episodic memories as episodic buffer (A. Baddeley, 2000, 2003) to operate on new information in working memory (Balconi, 2013). DLPFC has a top-down effect on posterior neocortical regions' sensory input processing. High frequency facilitative TMS of left DLPFC reduces memory performance in verbal episodic memory tasks indicating that activation of this region interferes with memory encoding. Slow rTMS can have inhibitory effects on neural activity. Application of slow rTMS on left DLPFC during episodic memory encoding

enhances memory performance via disinhibition of posterior parietal regions' stimulus processing functions (van der Plas, Braun, Stauch, & Hanslmayr, 2021).

Awake rTMS has been shown to be effective in enhancing cognitive functions in Alzheimer's patients and mild cognitive impairment patients, probably through an increase in functional connectivity between the stimulated cortical networks and the hippocampus (Grimaldi et al., 2020). Given the understanding that learning and synaptic potentiation during awake state can increase subsequent sleep SWA and synaptic strength, that neural connections within cortical networks are modulated by SWA, and that thalamo-cortical circuits are silenced during the down states, SO could be a potential mechanism of sleep homeostasis in line with predictions of synaptic homeostasis hypothesis.

Studies have also shown sleep-dependent memory consolidation effects of TMS through the enhancement of SO, SWA and spindle parameters during SWS. rTMS during sleep has been shown to trigger SO spreading over the cortex with higher spindle amplitudes during UP-states (Marcello Massimini et al., 2007). TMS induced enhancement of sleep SO could induce synaptic potentiation through a similar mechanism (M Massimini, Tononi, & Huber, 2009).

1.12.4 Sensory stimulation

Several non-invasive sensory stimulation techniques including olfactory, auditory and vestibular stimulation mechanisms have been studied in the context of brain stimulation during sleep and associational cue-dependent learning and reactivation during subsequent sleep (Cellini & Mednick, 2019).

1.12.4.1 Olfactory stimulation and aromatherapy

Olfaction can be an exceptional stimulus due to privileged cortical access during sleep without thalamic relay system and enhanced functional connectivity between olfactory, limbic, and neocortical areas during SWS (Cellini & Parma, 2015). Inhalation aromatherapy during sleep can facilitate olfactory sensory stimulation to promote sleep, SWS (Goel, Kim, & Lao, 2005) and associated oscillations like delta waves during SWS (Ko, Su, Yang, Liu, & Su, 2021), offering an effective therapy for insomnia (Cheong et al., 2021). Odor presentation during sleep was found to enhance power in NREM delta (0.5-4 Hz) and slow spindle (9-12 Hz) frequency range in proportion to odor duration (Perl et al., 2016). The effect of olfactory stimulation can be differentiated from auditory stimulation in that it does not evoke k-complexes (Perl et al., 2016), which can be evoked reliably by auditory stimulation during sleep and can possibly cause arousal depending on the salience of the stimulus (refer to section on KC 1.11.1). In addition, olfactory sniffing response can signal consciousness in unresponsive TBI patients (Arzi et al., 2020). The response can significantly discriminate between unresponsive and minimally conscious states with a high predictive ability for regaining of consciousness and long-term survival rates, which offer better end-of-life decisions and therapeutic pain management strategies.

1.12.4.2 Acoustic stimulation

Given the observations that k-complex can be induced by auditory stimulation during sleep and that k-complex and SO are closely related to each other, presentation of auditory stimuli during SWS has been found to cause phase synchronization of neuronal population firing leading to an increase in SO and SWA. Depending on the intensity, frequency and timing of the acoustic stimulation, it can have different effects and recruit differential mechanisms of slow wave

enhancement. Rhythmic auditory stimulation can also entrain intrinsic brain oscillations. Acoustic stimulation phase locked to UP-DOWN transitions during SO can enhance SO (Bellesi, Riedner, Garcia-Molina, Cirelli, & Tononi, 2014). Closed loop auditory stimulation time locked to UP-state transition of SO has shown promising results in sleep dependent memory consolidation in young (H.-V. V. Ngo et al., 2013; Ong et al., 2016), as well as older individuals (Papalambros et al., 2017).

1.12.4.3 Targeted memory reactivation

TMR is based on the mechanistic evidence for memory consolidation facilitated by neuronal reactivation and replay in neuronal networks recruited for encoding new information during learning. In TMR, the sleeping brain is exposed to a cue (typically an olfactory or auditory cue) that was part of the learning context. When the engram is cued, neuronal replay can be artificially triggered through pattern completion in contextual encoding networks of the memory, promoting memory consolidation by recruiting the brains natural mechanisms triggered by spontaneous reactivations (Schouten, Pereira, Tops, & Louzada, 2017).

1.12.4.3.1 Olfactory TMR

Re-exposure to odour during SWS was found to improve the retention of hippocampus-dependent declarative memories but not of hippocampus-independent procedural memories (Björn Rasch, Büchel, Gais, & Born, 2007). Odor omission during learning and odour re-exposure during REMS or wakefulness was ineffective in memory retention. In addition, significant hippocampal activation response time-locked to odor re-exposure during SWS was revealed with fMRI.

1.12.4.3.2 Auditory TMR

Replaying of sounds during sleep, that had been associated with objects at specific spatial locations during learning, showed memory recall for locations specific for the sounds that were played during sleep and not for those that were not played (Rudoy, Voss, Westerberg, & Paller, 2009). Auditory stimulation can thus facilitate systems consolidation during sleep.

1.12.5 Deep brain stimulation (DBS)

DBS electrodes are implanted into deep regions of the brain to modulate local neural functions with the use of electric current. Human stereotactic techniques allow the implantation of the electrodes targeted to specific brain regions to be stimulated. An implantable pulse generator placed under the skin below the clavicle contains a battery and electronic components which control stimulation parameters (frequency, pulse width, and voltage) to deliver electrical stimulation (Lozano et al., 2019; Pycroft, Stein, & Aziz, 2018). Several technological advancements in the field of DBS have taken place over the last several decades. We now have 'closed-loop' or 'adaptive' DBS, in which the stimulator can record the ongoing local neural activity and modulate the stimulating parameters accordingly (Pycroft et al., 2018).

DBS is mostly studied and applied in humans for treatment of neurological or psychiatric conditions. DBS has been well established and used extensively in the clinical management of treatment-resistant parkinsonian movement disorders (Hitti et al., 2019; Paff, Loh, Sarica, Lozano, & Fasano, 2020; Soh, Ten Brinke, Lozano, & Fasano, 2020). Current applications and research in DBS have shown potentially promising efficacy in the treatment of several neurological conditions including essential tremor, dystonia, Tourette's, Alzheimer's, Huntington's, pain, cluster headache, epilepsy, obesity and neuropsychiatric conditions including major depressive disorder, anxiety,

PTSD, addiction, anorexia and OCD (D. J. Lee, Lozano, Dallapiazza, & Lozano, 2019; Lozano et al., 2019; Pycroft et al., 2018).

1.12.5.1 Mechanisms of DBS

Several principles of electrical excitation play an important role in the mechanisms of DBS. Based on electrophysiological principles, the intensity of electric current decreases with the distance from the tip of the electrode, and hence the types of neural elements that will be affected will differ depending on the distance from the current source. Electric current will affect all the neural elements around the electrode tip including extracellular matrix, cellular membranes, axons, dendrites and soma. Frequency, amplitude, and pulse width of the electric current determine the effects (Dostrovsky & Lozano, 2002; Herrington, Cheng, & Eskandar, 2016). The amount of current injected end the conductivity of the tissue can determine the strength and extent of electrical potentials generated by the stimulation and this is modulated by current-distance relationship and electrode-tissue interface where the electrical charge of the stimulator is converted into ionic charge carriers in the local tissue. In neuronal networks, the excitability of axons is much higher than soma and large myelinated axons are more excitable than unmyelinated axons. Axonal excitation is a factor of strength-duration relationship and size of axonal fiber, with lower excitation threshold of large diameter axons (Dostrovsky & Lozano, 2002; Herrington et al., 2016). In terms of the medium and cell membranes, the resistivity of extracellular medium, membrane potential of cellular membranes, permeability and conductive properties of membranes are some of the determining factors for the effect of extracellular electric currents. Hence, the effect of DBS largely depends on what is being stimulated and the downstream connectivity of the stimulated element. The effect of stimulating a region largely depends on potential activation of projection neuronal

axons and/or axons of afferent inputs to the nuclei, axons passing in or near the nuclei. Stimulation beyond the action potential threshold in axonal membranes would generate orthodromic and antidromic action potentials. Whether this leads to an activation or inhibition of neural networks depends on the type of axon being activated, the effects of neuromodulators and neurotransmitters being released at the axonal terminals and the balance between excitation and inhibition. There is evidence that high frequency stimulation could either cause excitation of neurons in the region stimulated, or it can decrease the firing rate of neurons leading to depression of neural activity (Dostrovsky & Lozano, 2002; Herrington et al., 2016). Anodal stimulation and cathodal stimulation can have differential effects in terms of membrane depolarization (Brocker & Grill, 2013). DBS can have local and systemic effects due to afferent and efferent effects and regulation of neuronal networks by neurotransmitters and neuromodulators. Electrical stimulation can also modulate pathological network activity, normalize, disrupt or resonate with local physiological oscillatory patterns at a network level of organization.

Several possible DBS-mediated mechanisms have been proposed and investigated. There is substantial discussion and controversy in the field regarding the outcomes of electrical brain stimulation in terms of inhibition or activation (Vitek, 2002). High frequency (HF) stimulation can cause inhibition mediated by several mechanisms of membrane hyperpolarization mediated by either stimulation of inhibitory afferents or by reduction of neuronal excitability by activation of calcium-dependent potassium currents or by inactivation of voltage gated sodium and calcium channels or by depolarization block due to increases in extracellular potassium, which depolarizes the membrane to inactivate the voltage gated sodium channels or inhibition due to hyperpolarization. Frequency, amplitude, pulse width, electrode configuration and duration of stimulation can define its outcome. Low-intensity stimulation can produce short duration inhibition. HF stimulation can cause inhibition due to temporal summation or short duration

excitatory response. HF low-intensity stimulation can cause long duration inhibition due to temporal summation. Inhibition can be reduced after longer trains of stimulation probably due to desensitization of the GABA receptors. HF high-intensity stimulation produce a complex of inhibition and excitation caused by neurotransmitter release and channel dynamics resulting in early inhibition followed by rebound excitation after cessation of hyperpolarization or GABA release and a subsequent inhibition (Dostrovsky & Lozano, 2002).

At a cellular level, DBS can affect neurons directly or indirectly through glial cells, modulate gliotransmitters and neurotransmitters, confer neuroprotection through neurotrophic factors, confer neuroplasticity through enhanced neurogenesis and electrotaxis through activation of transcription factors and gene expression (Ashkan, Rogers, Bergman, & Ughratdar, 2017; Herrington et al., 2016). The cellular and molecular mechanisms of DBS have been reviewed and summarized (Jakobs, Fomenko, Lozano, & Kiening, 2019), as depicted in the following figures 6 and 7.

Given the complexity of nervous system anatomy including neurons and glial cells, multiple pathways, neuronal components, cellular structures with a range of electrophysiological properties in any microscopic region, the outcome of stimulation can be varied and complex due to differential effects at a spatial resolution on this multi-component dynamic system (Vitek, 2002).

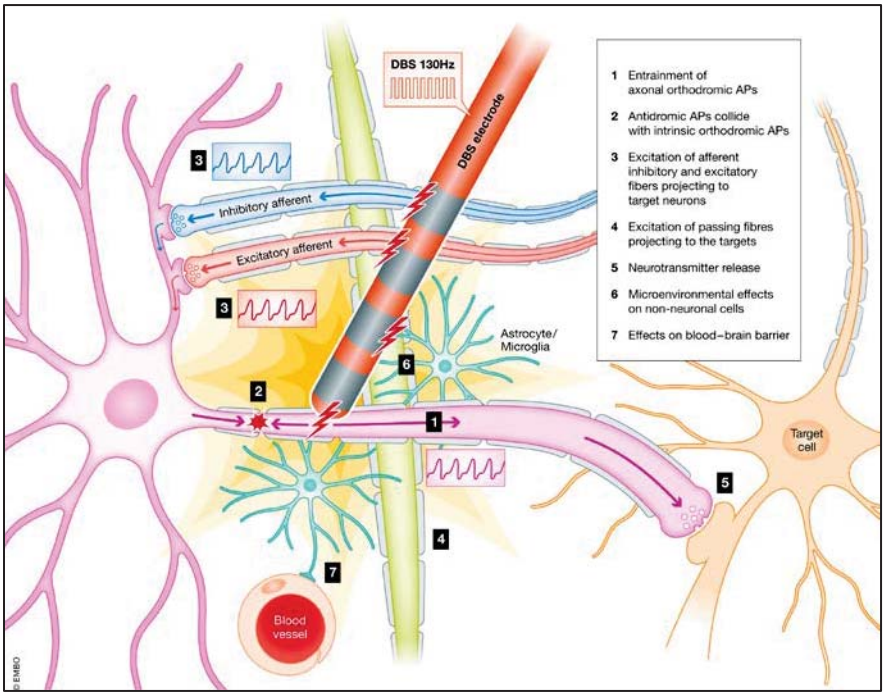


Figure 6: Effects of DBS in the brain microarchitecture

Adapted from (Jakobs et al., 2019): High-frequency stimulation from DBS electrode causes several changes in the microarchitecture of the brain volume as listed (1-7) in the figure.

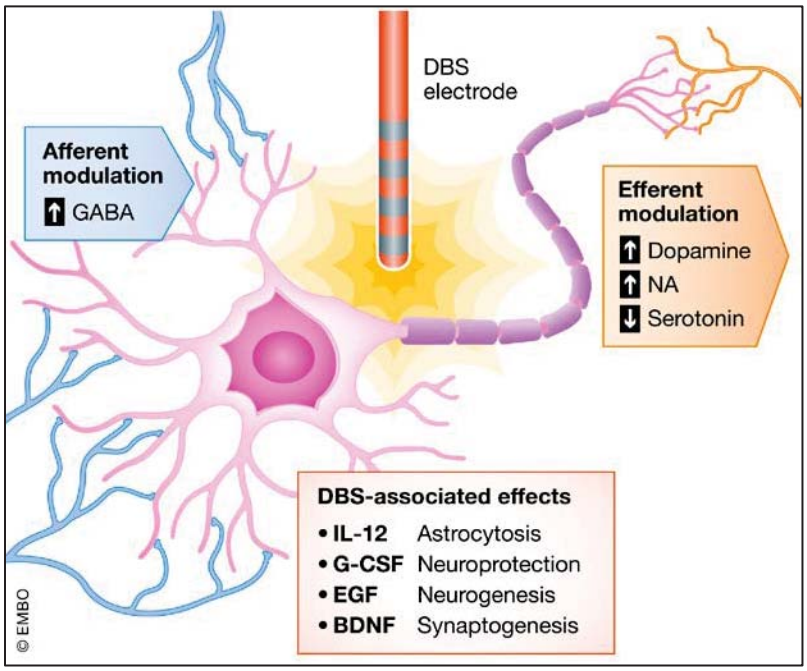


Figure 7: Effects of DBS on the molecular level

Adapted from (Jakobs et al., 2019). Molecular effects of DBS include modulation of both inhibitory and excitatory neurotransmitters, growth factors and interleukins.

1.12.5.2 DBS in memory and Alzheimer's

Given the importance of Papez circuitry in human memory processing, DBS has been targeted to the different regions involved in the circuitry and functionally connected regions including the hippocampus proper, entorhinal cortex, fornix, related limbic structures like the amygdala and septal nuclei, anterior nucleus of thalamus, nucleus basalis of Meynert in the basal forebrain, temporal and frontal cortices (Mankin & Fried, 2020). Fornix connects subiculum and hippocampus to mammillary bodies and septal nuclei via post-commissural fibers. The fornix also has cholinergic axons projecting from septal area to the hippocampus. Mammillothalamic tract connects mammillary bodies to anterior nucleus of thalamus which projects to cingulate gyrus. new cortical projections from the cingulate feedback into the hippocampus through entorhinal cortex (Lozano & Lipsman, 2013). The fornix has been a leading target for DBS in memory loss and cognitive decline associated with Alzheimer's and dementia. Fornix DBS has been suggested to be involved with increased functional connectivity, neurotransmitter levels and enhanced neuroplasticity. DBS close to the fornix can stimulate both orthodromic and antidromic directions and activate hippocampus and entorhinal cortex and exert its influence on memory and cognitive processing. Cholinergic DBS stimulation of Nucleus Basalis of Meynert of the basal forebrain also can improve memory function in dementia. Stimulation of the entorhinal cortex during memory encoding in humans has shown to enhance memory performance. DBS of the hippocampus and medial temporal lobe structures has shown improve memory performance in neurosurgical patients (Ezzyat et al., 2018). In rodent studies, high frequency stimulation of the perforant pathway which connects the easy to the hippocampal formation can improve memory encoding of novel information mediated by theta phase resetting, cholinergic stimulation and LTP mechanisms (Sankar et al., 2014).

Overall, DBS has the potential to target memory circuitry in Alzheimer's disease and has shown promising results in early clinical trials in terms of enhancement of cognitive function and reduced atrophy in humans and neurogenesis and improved memory performance in animal studies (Mirzadeh, Bari, & Lozano, 2016).

1.13 Manipulating spindles through brain stimulation and other techniques

Modulating sleep spindles may be important in several situations as a therapeutic strategy to treat underlying cognitive deficits. Sleep spindles have been found to be elevated in patients with insomnia (Spiegelhalder et al., 2012) and disrupted in schizophrenia (Göder et al., 2015). Spindle activity during NREM2 correlates well with behavioural recovery in stroke and TBI patients. In stroke patients, increase in power and coherence of sleep spindles was observed during chronic recovery (Gottselig, Bassetti, & Achermann, 2002). In TBI patients, there is a correlation between sleep spindles measured by EEG and higher levels of cognitive recovery measured by fMRI and PET neuroimaging techniques (Forgacs et al., 2014; Urakami, 2012).

Several techniques of non-invasive and invasive brain stimulation techniques have been investigated for their effects on enhancing sleep and sleep related brain oscillations as therapeutic strategies against several neurocognitive and neuropsychiatric disorders. Non-invasive brain stimulation techniques include transcranial direct current stimulation, transcranial alternating current stimulation, transcranial magnetic stimulation, acoustic, vestibular and somatosensory stimulation techniques have shown to enhance spindle parameters (Cellini & Mednick, 2019; Fernandez & Lüthi, 2020; Grimaldi et al., 2020).

The current section will review the brain stimulation techniques that have been employed to experimentally manipulate spindles.

1.13.1 SO-tDCs

SO-tDCs to the cortex increased spindle power and SO power with concomitant enhancement of memory performance in humans (Lisa Marshall et al., 2006). SO-tDCs enhanced fast spindle parameters (counts, density, length and EEG power) in all subjects in acute and post-stimulation periods. Task-induced slow spindle density in the post-stimulation period increased in subjects with high MQ and decreased in those with low MQ. Higher learning and memory retention with SO-tDCs was shown in subjects with high memory quotient (Koo et al., 2018). A recent study showed that it is not just the SO-tDCs, but the efficacy of stimulation on memory retention associated with SO-spindle coupling closer to SO trough (Dehnavi et al., 2021). Some contradictory results were also obtained in studies related to the effect of SO-tDCs in older adults in visual spatial learning and memory performance. In one study it showed a significant improvement in memory retention after sleep (Ladenbauer et al., 2016), while in the other study such an effect was not observed (Paßmann et al., 2016). Both the studies showed that SO-tDCs significantly increased frontal SWA and spindle activity.

The memory enhancing effects are limited to SO-tDCs. Theta-tDCs during NREMS caused global reduction in SWA and decrease in frontal slow-EEG spindles with a reduced performance in a declarative but not procedural memory task (Marshall et al., 2011).

1.13.2 tACs

A 12-Hz spindle-like waveform tACs in a closed-loop system in the frontal regions locked to real-time detection of endogenous spindle activity during SO-spindle coupling in SWS, increased fast spindle activity and enhanced performance in finger tapping motor memory task (Lustenberger et al., 2016).

1.13.3 TMS

Using TMS, slow waves and spindles were found to be reliably triggered during sleep (Marcello Massimini et al., 2007). It was also found that spindles are preferentially triggered in networks that were recently active, indicating a temporal association between network activity and spindle oscillations. This was experimentally demonstrated in a human study combining paired associative stimulation of the median nerve with TMS of contralateral motor cortex, which increased local spindles (Til Ole Bergmann et al., 2008).

1.13.4 Sensory stimulation

1.13.4.1 Auditory stimulation

A very early study showed that acoustic tones during sleep can evoke SO/K-complex followed by spindles (Davis et al., 1939). Auditory closed loop stimulation in-phase, but not out-of-phase to SO, boosted sleep spindles and SO-spindle synchrony and enhanced memory in young (Lustenberger et al., 2018; H.-V. V. Ngo et al., 2013) and in older adults (Papalambros et al., 2017). The in-phase stimulation effect could be due to spike-order dependent synaptic plasticity (Ulrich, 2016).

Frequency-specific enhancement of spindles was observed with oscillating auditory stimulus during afternoon nap: increased slow spindle with 12-Hz stimulation and fast spindle with 15Hz. (Antony & Paller, 2017), while in another study frequency-specific effect was not observed, 14Hz and 40Hz stimulation increased spindles (Lustenberger et al., 2018). The differences could be due to stimulation from specific regions and differing on-off cycles of the tone.

Timing of rhythmic auditory stimulation seems to be important in the effects in sleep electrophysiology and declarative memory consolidation. While closed-loop auditory stimulation phase-locked to SO UP-sate enhances SO, spindles and declarative memory (H.-V. V. Ngo et al., 2013; H.-V. V. Ngo et al., 2015), open loop stimulation not phase-locked to SO, showed increase in SO, but reduction in phase-locked spindles, spindle power and no memory improvement compared to controls (Weigenand, Mölle, Werner, Martinetz, & Marshall, 2016). Slow wave frequency phase-targeted closed-loop auditory stimulation in rats specifically altered delta (slow waves) and sigma (sleep spindles) power persistently over chronic periods of stimulation (continuously over 16 days), without affecting the 24-hr sleep-wake cycle and positively correlated with motor performance learning (Moreira et al., 2021). This demonstrates efficacy and reliability of long-term stimulation to enhance learning measures.

1.13.4.2 Olfactory stimulation

Olfactory stimulation using odors during sleep has been found to modulate sleep physiological characteristics including increase in SWS (Goel et al., 2005) enhancing SWA (Perl et al., 2016) and slow spindles (Cox et al., 2014; Perl et al., 2016). Re-exposure to the paired odours in a visuospatial learning task during sleep was found to be associated with increases in fast spindles correlated with reactivation in contralateral posterior brain regions (involved in visuospatial processing) to the cued visual field (Cox et al., 2014) and was associated with memory improvements (Rihm, Diekelmann, Born, & Rasch, 2014), indicating a spindle-mediated memory replay event in the localized cortical regions.

1.13.4.3 Vestibular stimulation: Bed rocking

Vestibular sensory stimulation has also shown to enhance spindle activity in a bed-rocking experiment in humans and also enhanced sleep maintenance, with the rocking cycle influencing a temporal clustering of SO and spindles (Bayer et al., 2011; Perrault et al., 2019). Both the studies showed better post sleep memory performance and the spindle power during NREMS was positively correlated with enhancement of memory. A possible explanation for this phenomenon are the direct connections from the vestibular system to the thalamic system.

1.13.5 Auditory-cued Targeted memory reactivation (TMR)

Auditory-cued TMR helps consolidation of emotional memories through spindle-mediated reactivation mechanisms (Cairney et al., 2014). Following up on their, earlier study, Cairney et al demonstrated functional involvement of spindle-mediated processing of memory information in an association task (Cairney et al., 2018). In a declarative memory task, association between words and pictures of objects or scenes was established during learning. Better memory performance was observed with only those pictures, the words associated with which, was played during NREMS. Words associated with objects or scenes elicited high fast spindle activity. During the critical time window of evoked spindle activity, the activation pattern allowed to decode the category (object or scene) associated with the word, and the fidelity of decoding was predictive of consolidation. The activation pattern differed for those words that were not a part of the memory task. This indicated a functional involvement of sleep spindle-mediated processing of associated memory information (Cairney et al., 2018). Spindle-mediated TMR was found to be related to increases in spindle density and SO-spindle coupling during the cueing period in a procedural task and was shown to evolve over several weeks after the cueing (Rakowska, Abdellahi, Bagrowska, Navarrete,

& Lewis, 2021). Some studies showed successful declarative memory recall following TMR, where the cue presentation itself did not enhance spindles, but reactivation was correlated with spindle activity (Antony, Gobel, O'hare, Reber, & Paller, 2012). While verbal cueing improved vocabulary learning in younger subjects without increased spindle activity (Schreiner & Rasch, 2015), memory enhancement was not found in older adults (Cordi, Schreiner, & Rasch, 2018). This is probably due to a aging-related disruption of SO-spindle coupling in SO UP-state (Helfrich et al., 2018).

Timing of cue presentation with respect to spindles has been shown to be critical for effectiveness of reactivation. Reactivation may occur during sleep spindles (Cairney et al., 2018). Studies investigating the relationship of reactivation to spindles, show that a critical time window may exist with respect to spindle activity when cue presentation might be most effective with least interference. Auditory cues played at the right time just before an endogenous spindle reactivated memory content and enhanced memory performance in a 'sound associated with objects' learning paradigm (Antony et al., 2018). And TMR cues were less likely to elicit reactivation and memory performance if occurring right after a spindle (B. Wang et al., 2019). This timing effectiveness with respect to endogenous spindles suggests that TMR may recruit the brain's natural reactivation machinery during NREMS (Schönauer, 2018). Based on these studies, it was proposed that a refractory period following spindles may facilitate local processing of memories in neocortical networks in the form of reactivation, with the optimization of spindle-ripple interactions (Antony, Schönauer, Staresina, & Cairney, 2019).

1.13.6 Pharmacological manipulations

Several hypnotic agents, like benzodiazepines and non-benzodiazepine Z-drugs have been used to enhance sleep, with most of them with pharmacological augmentation of sleep spindles and spindle density (Fernandez & Lüthi, 2020).

Zolpidem increases sigma power, fast spindle activity and SO-spindle coupling, with the maintenance of sleep architecture in humans via potentiation of intrathalamic GABA-receptor-mediated synaptic inhibition, with correlative enhancement of verbal memories and possibly emotional memories. It has also been found to enhance ripples in rodents, opening up other possible mechanisms of actions through spindle-ripple coupling (Fernandez & Lüthi, 2020). Zolpidem produced spindle-mediated exceptional improvements in declarative memories more than what sleep can offer, but not perceptual or motor learning.

Midazolam and zopiclone have similar half-life dependent actions in increasing spindle activity in initial sleep stages and depressing SWA in later stages without affecting homeostatic sleep regulatory mechanisms, probably through activation of GABAergic processes in TRN (Aeschbach, Dijk, Trachsel, Brunner, & Borbély, 1994). Agents like sodium oxybate enhance SWA at the cost of reduced spindle density, with no effects in declarative or emotional memories (S. C. Mednick et al., 2013). Blocking of thalamic output by tetrodotoxin leads to reduction in SWA and suppression of spindles (David et al., 2013), while pharmacological stimulation of TRN with MT2 melatonin receptor agonist enhances spindle activity-mediated NREMS (Ochoa-Sanchez et al., 2011).

Eszopiclone in schizophrenic patients increased sleep spindle density with corresponding improvement in finger-tapping motor task (Wamsley et al., 2013). Antidepressant drugs, selective serotonin-(fluvoxamine) and norepinephrine-(reboxetine) reuptake inhibitors, have been found to increase fast sleep spindle activity and density, with correlative enhanced performance in a finger

sequence tapping motor learning task (Björn Rasch, Pommer, Diekelmann, & Born, 2009). On the other hand, Tiagabine, a GABA reuptake blocker, enhanced SWA, increased SWS, decreased REMS, decreased SO-spindle coupling of both slow and fast spindles, with no enhancements in performance in procedural sequence finger tapping (Feld et al., 2013). These studies also indicate the importance of spindle activity in the consolidation of sequence tapping task.

$GABA_A$ receptor agonistic drugs enhance spindles while concurrently decreasing SWA. Because of these opposing effects on SO and spindles, they cannot be reliably used to study effects of spindle enhancements alone. The effects may be confounded by consolidation mechanisms recruiting SO-spindle coupling. In addition, these drugs have been shown to impair LTP, LTP-dependent plasticity in mature and developing brains (B. Rasch & Born, 2013).

Genetic manipulation of low-voltage gated T-type Ca^{2+} channels (T-channels) and small-conductance Ca^{2+}-activated type-2 K^+ channel (SK2) channels have been shown to selectively enhance spindle activity due to their functional involvement in intrinsic rhythmic bursting of TRN neurons underlying spindle generation (Astori et al., 2013). Knock-out of Cav3.3 channels selectively impaired spindles, reduced sigma power, especially at NREMS-REMS transition periods (Astori et al., 2011). SK2 overexpression in mice enhances TRN bursting activity with corresponding sustained spindle activity, prolonged NREM episodes and higher arousal response by modulation of thalamic sensory gating mechanisms (Wimmer et al., 2012). This effect ties in with the involvement of sleep spindles in sleep maintenance and correlated higher arousal threshold, as discussed in section 1.11.3.5 related to other functions of spindles.

1.13.7 Invasive electrical stimulation in rats

Low intensity electrical stimulation applied to cortex evoke spindles, which propagate from the site of stimulation, while high intensity electrical stimulation can evoke spindles simultaneously in multiple cortical regions (Diego Contreras, Destexhe, Sejnowski, et al., 1997). Enhanced reliability of spindles can be effectively triggered by electrical stimulation in networks that were activated in the recent past (Werk et al., 2005). Pulse electrical stimulation in the neocortical networks has shown to trigger SO followed by spindles and enhance the coupling between SO, spindles and hippocampal ripples, highlighting their role in memory consolidation processes through communication between hippocampus and the neocortex for information transfer (M. J. Eckert, Iyer, Euston, & Tatsuno, 2021; Maingret et al., 2016; Vyazovskiy, Faraguna, et al., 2009).

1.13.8 Optogenetic stimulation

Optogenetic stimulation of parvalbumin-expressing inhibitory neurons in TRN enhanced spindle density. Induced spindles in-phase with SO UP-state but not out-of-phase spindles, improved consolidation of hippocampal-dependent memory. Stimulated spindles nested ripples within their troughs, enhanced spindle-ripple coupling and SO-spindle-ripple triple coupling. In phase optogenetic inhibition of thalamic spindles showed impairment of hippocampal dependent memory (Charles-Francois V Latchoumane et al., 2017), highlighting the importance of the ripple/delta/spindle trifecta coordination for memory consolidation. Optogenetic stimulation of TRN has been shown to reliably induce spindles during SWS (Halassa et al., 2011; A. Kim et al., 2012; Thankachan et al., 2019). Spindle-like optogenetic stimulation of TRN neurons during NREMS was found to increase spindle oscillations, and this modulation enhanced memory in mice

(A. Kim et al., 2012). Optogenetic induction of spindles also correlates with increased duration of NREMS and the NREM-REMS transitions.

1.13.9 DBS in humans

Daytime central thalamic deep brain stimulation (CT-DBS) in a minimally conscious state individual with TBI, for the promotion of arousal regulation, increased spindle frequency in stage 2 sleep, the amount of sustained SWS and SWS delta power (Adams et al., 2016). In a follow-up study, these enhancements in sleep electrophysiology, specifically sleep spindle activity and SWS delta power regress after CT-DBS discontinuation (Gottshall, Adams, Forgacs, & Schiff, 2019). These studies show that daytime CT-DBS promotes arousal regulation during the day and can enhance sleep spindles at night through homeostatic regulation. In addition, they underscore the role of sleep spindles in behavioural and clinical recovery.

Chapter 2 Exploration- and learning-dependent modulation of sleep spindles

2.1 Background

Sleep spindles have been shown to be critically important and correlate with learning and memory as reported in several human and animal studies (reviewed in detail in earlier section 1.11.3). Enhancement of sleep spindles has been correlated with memory encoding (reviewed in detail in Section 1.11.3.1) and memory retrieval (reviewed in detail in Section 1.11.3.2). In addition, enhanced spindles through experimental manipulation correlated with better learning and memory (reviewed in detail in Section 1.13), while conversely, disrupting spindles has been shown to interfere with memory performance (reviewed in detail in Section 1.11.3.3). Further, the observations that sleep spindles happen during neuronal reactivation after learning, and that they can facilitate plasticity through LTP mechanisms (Section 1.11.3.8) further strengthen the possibility of spindle-associated memory consolidation processes.

Compared to human literature, very few studies in animals have shown modifications in sleep spindles as a result of hippocampus-dependent learning tasks (Fernandez & Lüthi, 2020). Given that these have been extensively reviewed in the corresponding sections in Chapter 1, some of the relevant studies leading to the present work are recapitulated only briefly here.

Spindles have been shown to be correlated with active exploration and learning in a few animal models. In a mouse model, the largest and fastest discrete local spindles during NREMS have been documented in the barrel cortex, which is one of the rodents' primary modalities for active exploration and, presumably, experience-dependent learning (Fernandez & Lüthi, 2020; Fernandez et al., 2018). This clearly leads to the speculation of the involvement of SWS spindles in the consolidation learning occurring during active spatial exploration. Exposure to new information leads to an increase in sleep spindles in dogs and some other mammalian species

(Iotchev et al., 2017; Iotchev & Kubinyi, 2021). The earliest study in rats involved exposing the animals to novel objects, a task thought to require episodic memory. A sleep stage known as pre-REM, associated with high spindle activity, increased within the first 2 hours after learning (Schiffelholz & Aldenhoff, 2002). The first correlative study between SWS spindles and learning demonstrated that learning an odor-reward association lead to enhanced power in the sigma band and increased spindle density in two time windows: between 0-30 and between 30-60 min post-learning (Eschenko et al., 2006). This was further validated in a follow-up study where learning lead to increased spindle coupling to the up-state of the SO (Mölle et al., 2009). Learning measured after a period of consolidation in rodent object-place memory task was found to be correlated with increased power in the spindle frequency band (Binder et al., 2012). Both SWS-related SWA and spindles were found to be important for this consolidation process.

Increase in spindle frequency band and spindle density specifically in the 21-24-hour period, but not at any other time after the first training session, was positively correlated with avoidance task performance (SM Fogel et al., 2010; S. M. Fogel et al., 2009). Baseline sleep spindle activity correlated well with increased post-learning spindle activity and memory recall in humans (Manuel Schabus et al., 2004), but this effect was found to be reversed in a rat study of avoidance learning (SM Fogel et al., 2010). Based on a retest in the avoidance task, rats were categorized into learners and non-learners. Non-learners had higher baseline sleep spindles which was unaffected by training, while learning rats had lower baseline sleep spindles which increased and correlated with learning. Thus, baseline sleep spindle density can be a predictor of learning and in some cases; higher baseline spindle activity may represent consolidation of maladaptive information and suppression of adaptive processes through homeostatic regulation.

Coupling of spindles with the neocortical SO may be one of the mechanisms whereby spindles can enhance memory. One of the first studies to show a causal effect of SWS spindles in

hippocampus-dependent learning used optogenetic stimulation to promote thalamic synchronization during the active state of the SO, thereby inducing spindles. These spindles were shown to facilitate contextual fear memory in mice (Charles-Francois V Latchoumane et al., 2017). Another study investigated the interactions between SO and spindles that contribute to the modulation of correlation structure of primary motor cortex neuronal firing after rats practiced on a novel motor task (Silversmith et al., 2020). During spindles, primary motor cortex neurons fired at a preferred phase, with neural pairs demonstrating greater neural synchrony, or correlated firing, during spindle peaks. Temporal proximity between SO and spindles correlated with changes to the distribution of neural correlations. Interestingly, after animals practiced a novel motor task, pairwise correlations of primary motor cortex neuronal firing increased during nested spindles, consistent with targeted strengthening of functional interactions between neocortical SO and thalamo-cortical spindles. Real-time, closed-loop optogenetic inhibition of pyramidal cells and interneurons in the primary motor cortex of rats during cortical UP-states modulated coupling of spiking activity to the SO (J. Kim et al., 2019). This manipulated the amounts of SO-spindle or delta-spindle nesting events and lead to corresponding changes in task performance with a neuro-prosthetic limb, a measure of procedural memory. Coupling to spindles drove reactivations of novel awake experiences in the primary motor cortex and differentially drove selective memory consolidation versus memory weakening. This indicates the physiological relevance of SO-spindle coupling. Temporal coupling can show bidirectional effects either strengthening memory traces or promote forgetting. Thus, spindle nesting could be a key mechanism to support memory consolidation.

Coupling of spindles with hippocampal SWRs may be another mechanism whereby spindles can enhance memory. Ripple-spindle correlations enhanced after exploratory behavior along with preferential engagement of recently active CA1 neurons during SWRs and their phase-

locking to spindles (Varela & Wilson, 2020). Individual cycles of the spindle oscillation were speculated to provide a key temporal window for processing that could combine and organize the activation of recently active CA1 cell ensembles, and subsets of cells in thalamocortical networks. This study further suggests that individual spindle cycles provide optimal temporal windows in which the activation of groups of thalamic cells during spindles could facilitate the integration of recently acquired novel hippocampal memory traces into neocortical networks, given the predominantly thalamic origin of spindles with neocortical inputs (discussed in section 1.9.4.1 of the introduction chapter). In addition, high frequency 100 Hz ripple-triggered stimulation of the locus coeruleus during post-learning sleep transiently blocked generation of ripple-associated cortical spindles and caused a reference memory deficit in a radial maze task. This suppression of ripple-spindle synchrony was thought to interfere with hippocampal-cortical communication and reduce the efficiency of off-line memory consolidation (Novitskaya et al., 2016).

Phase locking of specific neuronal activity has been shown to be linked with spindle activity, indicating encoding of information in specific neuronal networks. Visual stimulus-dependent (orientation-specific response potentiation, OSRP) neuronal spiking coherence in V1 in mice was found to be phase locked to spindles consistent with spindle-mediated plasticity (Durkin et al., 2017). During post-stimulus NREMS, spike-field coherence (SFC) of lateral geniculate nucleus (LGN) neurons increased with primary visual cortex V1 delta (0.5–4 Hz) and spindle (7–15 Hz) oscillations, with neurons most responsive to the presented stimulus showing greater SFC. Rhythmic optogenetic activation of corticothalamic V1 neurons induced coherent firing in LGN neurons. In addition, they showed that optogenetic disruption of corticothalamic activity reduces spindles, impairs OSRP, and impairs potentiation of neuronal responses in the visual cortex during post-experience NREMS. Similarly, visual cortex neurons become more strongly phase-locked to thalamocortical spindle oscillations during sleep after perceptual learning in adult mice (Aton,

Suresh, Broussard, & Frank, 2014) and developing cats (Aton et al., 2013). Sleep-dependent V1 response potentiation (associated with a shift in orientation preference in favor of the presented stimulus, ORSP) was found to be proportional to phase-locking of principal neuronal spiking activity with spindles in the adult mouse visual cortex (V1) (Aton et al., 2014), indicating experience-dependent synaptic potentiation. Further, during post-monocular deprivation NREMS in cats, the visual cortex principal neuron firing increases and becomes more phase-locked to SWA and spindle oscillations (Aton et al., 2013). Ocular dominance shifts in favor of open-eye stimulation, which is evident only after post-monocular deprivation sleep. Spindle-mediated entrainment of mPFC neuronal firing has also been observed. Inhibitory neurons across all layers show increased firing rate phase-locked to spindles, while the majority of superficial, but not deep, excitatory neuronal firing rates are modulated by spindles (Peyrache et al., 2011). Thus, spindle-locked enhancement of neuronal firing could possibly induce plasticity mechanisms in neocortical circuits through thalamocortical inputs during spindles.

Spindles have also been speculated to recruit long-term plasticity mechanisms in neocortical circuits, presumably involved in memory consolidation processes. As a measure of IEG-mediated plasticity, Arc gene expression in the cortex was found to be proportional to LFP amplitude in the spindle-range (10-14 Hz) but not to firing rates, indicating that signals were more related to dendritic input than to somatic output (Ribeiro et al., 2007). It has been demonstrated in rats that induction of LTP in the sensorimotor cortex by electrical stimulation of corpus callosum or ventrolateral thalamus, which include cortical or thalamic inputs to the sensorimotor cortex, results in increased reliability of evoked sleep spindles (Werk et al., 2005) and that sleep spindle-like activity can produce LTP in *in vitro* preparations of rat somatosensory cortex (Rosanova & Ulrich, 2005). Thalamocortical activity during NREMS was shown to trigger cortical LTP-like

responses in the cat neocortex, as demonstrated by a combination of *in vivo* intracellular and *in vitro* recordings (Sylvain Chauvette et al., 2012).

Considered together, the above mentioned evidence provides some support for the idea that sleep spindle oscillations in post-task sleep are learning-dependent, especially in humans.

2.2 Study Objectives

The broader question asked was "Does learning enhance spindles?". The evidence for this is still weak especially from rodent studies. The task-dependence of enhancement of spindles also needs investigation. The current study was designed to follow-up on the observation by Eschenko et al., 2006, that task-learning in odor-reward association correlated with enhancement of spindle density within the first hour of post-learning sleep and predicted retrieval. In addition, the study showed that this correlation was specific to task learning and reported no enhancement of spindles associated with an exploration task.

The first question we asked was "Do we really need a task to drive consolidation-related brain activity?" In other words, is it possible that novel environment exploration might be sufficient to increase spindle activity? The rationale was that encoding of information and spatial learning happens during exploration and not necessarily only during a learning task. Hence, one would expect that memories are encoded and consolidated during the exploration of novel environments. Indeed, such a correlation was reported in a human study in which spatial exploration preferentially altered N2 sleep spindles (Meier-Koll et al., 1999). To test whether encoding of a novel environment might be sufficient to lead to enhanced spindles, we compared a standard novel environment to two environments with enhanced learning (novel objects and novel social experience).

In addition to exploration of novel environments and building upon the few rodent studies reporting correlations between spindle increases and task learning, we wanted to investigate the changes in spindle density following other types of hippocampal-dependent learning not yet reported in rodent literature. To test whether learning could enhance spindles, we included a modified version of Morris Water task and a version of fear conditioning task.

Further, to test whether a cognitive demand in a familiar task, one which clearly involves some form of learning, might also lead to enhanced spindles we used a task switching behavioural paradigm. The task switching experiment is distinct from our other tasks as the learning involves a switch in strategy on an otherwise familiar task. The details of the task and the rationale are described in the relevant methods section.

Based on the described speculations, our hypothesis was that spindle density would be increased during post-learning sleep in the first 30-min window after SWS onset in all tasks relative to a control condition in which learning was minimized.

2.3 Methods and study design

The methods described here apply for all behavioural experiments tested, except for the switch-task, which was the reported in (Insel & Barnes, 2015) and dataset generously shared by Dr. Nathan Insel for analyses for this thesis as it was in line with the current investigations.

Animals and handling

The data was collected from four male Fisher/Brown Norway hybrid rats, which were bred in-house within the University of Lethbridge animal facility. The rats were kept on a 12 h light / 12 h dark cycle with lights ON from 0730-1930 and lights off from 1930-0730. Water and food

were available *ad libitum* in their standard clear plastic cages. Rats were housed in pairs before surgery and handled daily after transfer to the experimental protocol. In addition, before starting the experiments, animals were handled daily for 10 min in the preceding week to accustom the animals to the experimenter and recording conditions. This presumably reduces stress during behavioral testing. Four Fisher 344/Brown Norway hybrid rats were between 4-8 months old and weighed between 350-450g at the time of surgery. The rats were housed individually after electrode implantation surgery, with *ad libitum* food and water access while being kept on the same 12 h light-dark cycle. They continued to be handled at regular intervals between experiments. They were also habituated regularly to the recording chamber, recording room, recording conditions and procedures for a week before the first experiment and then for a few days before each experiment, as described in more detail below, under the section Electrophysiological Sleep Recordings. Behavioural experiments and recording sessions took place during the light phase. All the animal protocols were approved by the Animal Welfare Committee of the University of Lethbridge. All experimental procedures were performed in accordance with the Canadian Council of Animal Care and the Animal Welfare Committee guidelines at the University of Lethbridge (national and institutional regulations respectively for care and use of laboratory animals).

Electrodes

LFP electrodes: Teflon-insulated annealed (AN) stainless steel wire 0.002 inch diameter (A-M systems, Sequim, WA, USA; 793600) was used for all cortical and hippocampal LFP recording electrodes, which were manufactured in-house. This was made into a twisted pair of electrodes with the help of a magnetic rotator and the exposed ends stripped of insulation. The ends were offset by 0.5 mm to act as a bipolar electrode pair.

Ground screw electrodes: The base of a torx 000-120 screw head was soldered to a 0.005 inch stainless steel insulated wire half-hardened (HH) (A-M systems, Sequim, WA, USA; 791500) as customized ground recording screw electrodes.

EMG electrodes: The methodology for implanting EMG electrodes was devised specifically for our surgical and recording purposes. A 20 cm multistrand stainless steel wire (Cooner wire Inc, Chatsworth, CA, USA; AS632) was knotted about 3-5 times in the center. At a distance of about 1 cm from the knot on one side, the insulation was carefully removed for around 1mm length of the wire, for muscle electrical activity recording. The electrode wire was then doubled over and twisted for 25-30 rotations using a magnetic rotator by holding the other end with a clamp. The wires were then briefly heated using a heat gun so that the insulation would stick together and prevent the electrode from unwinding. Insulation was also removed from the free ends. These ends were inserted into a 21G 2-inch, or 22G 1.5-inch needle tip cut off from the bases, soldered and secured. This needle tip was slightly bent to aid in insertion and threading of the wire from the neck to the skull (as described below).

Surgery and recovery:

All stereotaxic electrode implantations were performed under isoflurane anesthesia. Hair on the head (for stereotaxic electrode implantation) and about an inch bilaterally on the neck (for EMG electrode insertion) was shaved using standard clippers. Animals were anesthetized with isoflurane (1-1.5 % by volume at oxygen flow rate of 1.5 L/min). The animal was secured in the stereotaxic apparatus with ear bars. Anaesthesia was maintained with isofluorane delivered via a stereotaxic-mounted nosepiece. A nose cone in the front and ear bars on the sides restrained the head. Bregma and Lambda were levelled by adjusting the height of the stereotaxic nosepiece. A thermostat-controlled heating pad regulated the animals body temperature during surgery. A rectal

temperature probe, heart rate monitor and oximeter were connected to the animal. The eyes were covered with ophthalmic ointment. A thick piece of aluminum foil cut in the shape of eye shades was secured on the eyes to protect them from the lights used to illuminate the surgical field. Throughout the surgical procedure, fluid support was provided with subcutaneous (SC) pre-warmed sterile isotonic saline (0.9% NaCl) at a volume of 5 ml rats at regular intervals of 3 hours. After sterilizing the surgical area with chlorohexidine and alcohol, lidocaine (0.1 ml of 2 %: 20mg/mL) with Epinephrine was injected SC along the midline of the skull under the planned incision site as a local anesthetic. The epinephrine is a vasoconstrictor and so helps to reduce bleeding, while lidocaine is an analgesic. The incision was then made along the midline, extending from roughly 5 mm in front of bregma to the occipital ridge at the back of the skull. The exposed skull was cleared of fascia. Holes were drilled at the appropriate stereotaxic locations with respect to bregma for the insertion of ground screws, anchor screws, as well as stimulating and recording electrodes.

Recording electrodes were surgically implanted bilaterally in the motor cortex, hippocampus, and neck for continuous chronic recordings of LFP and EMG, respectively. More specifically, four bipolar LFP electrodes, two EMG electrodes and two ground electrodes were implanted at appropriate locations according to coordinates obtained from the rat stereotaxic atlas (Paxinos & Watson, 2006). Bipolar twisted-pair LFP recording electrodes were implanted bilaterally above the motor cortex (anterior 1.6 mm from bregma, lateral +/- 1.5 mm for bilateral implantation, at a depth of 1.8 mm to 2 mm from skull surface) and hippocampus (posterior 3.5 or 4 mm from bregma, lateral +/- 2.5 mm for bilateral implantation at a depth of 2.5 mm from surface of dura or 3.5mm from skull surface) under microscopic guidance.

Two EMG electrodes were implanted bilaterally in the neck muscle using a specialized method. An incision was made in the skin with a scalpel on either side of the back of the next about

an inch from the back of the skull incision. The curved needle tip attached to the customized EMG electrode was inserted through the skin incision and advanced until the tip protruded through the skull-top incision. The knot at the end of the EMG electrode helped secure the electrode under the skin and the skin incision was closed with tissue glue. This ensured that the exposed part of the electrode was secure in the neck muscle to record myocardial activity. Once the EMG electrode projected out of the head region, the needle tip was slipped off leaving the exposed end of the EMG electrode to be eventually connected to the vias of the custom-designed printed circuit board (PCB) connected to a Mill-max connector for recording purposes.

Four anchor screws were placed at the edges of the surgical field. The electrodes were carefully connected into the vias of the custom-designed printed circuit board by putting a drop of solder into each via with the inserted electrode tip. Vias have respective connections through wires to the pads which were soldered to the Mill-Max Mfg. Corp. connectors. The connector was secured to the skull by dental acrylic, with a visible bump mould around the diameter, to secure the recording headstage with a tissue tape to secure it during recordings.

Following surgery, the animals were housed individually with food and water available ad libitum. They were handled daily during the following recovery week for post-operative monitoring, injections, weighing, and familiarization with experimenter. The antibiotic, Baytril (5mg/ml 10.0 mg/kg SC), was administered every 24 hours for 5 days and the analgesic, Meloxicam (Metacam, 5mg/mL 5-10 mg/Kg SC) was administered every 24 hours for 3 days during post-operative care to control inflammation, pain, and infections. The rats were allowed to recover for 7 days from the surgical procedure before any experiments and procedures.

Electrophysiological sleep recordings/Data acquisition:

After recovering from surgery, the animals were placed in a recording chamber located in a quiet, dimly lit room daily for about 3 hours for 3 days. The purpose was to habituate them to the experimenter and recording conditions. They were also habituated to the process of attaching recording cables to the skull connector for an additional week before the first experiment. On experiment days, rats were taken to the recording room, connected to the recording system, and placed in the recording chamber where they rested. Typically, experiments used to investigate the role of sleep in memory consolidation record sleep before (pre-training) and after training (post-training) using electrophysiological LFP and EMG recordings to score arousal state (e.g., sleep/wake) and different sleep stages. Accordingly, each recording session consisted of two 2-hr sessions: a baseline pre-task recording and a post-task recording session for each behavioural experiment. The procedure was repeated for every training day for each task. The vivarium maintained a 12-h light cycle (7:30 a.m. on/7:30 p.m. off) and the animals were tested in the same order every day such that recordings on different days occurred at approximately the same time (±1 h).

Recording was done with a Neuralynx data acquisition system (Digital Lynx SX, Neuralynx, Boseman, Montana). The electrophysiological recordings were acquired in a dimly lit room as the rats rested in a glass box with metal rims (16-inch cube box). The movements of the rats were tracked by a standard video camera mounted to the ceiling directly above the recording chamber connected to the data acquisition system. The rat was connected to the amplifier by a cable allowing free movement within the box. LFP and EMG was recorded with a unity-gain headstage, HS-27 (Neuralynx, Bozeman, MT) and multi-wire tether cables (TETH-HS-27-3M, Neuralynx, Bozeman, MT) and a 128 channel commutator (PSR-36-4, Neuralynx, Bozeman, MT). A counter-weight system was customized to reduce the weight of the headstage and tethers for the

rats to relax and rest. The counter-weight was attached to the tether cable above the headstage using fishing line running through a plastic pulley attached to the bottom of the commutator. Data was recorded using Digital Lynx neurophysiology data acquisition system and Cheetah data acquisition software. The signals were sampled at 2 kHz. All signals were amplified, digitized and bandpass filtered between 0.01 and 1000 Hz via a Digital Lynx system (Neuralynx, Bozeman, MT) and referenced to a skull ground screw electrode. Two standard computers running Microsoft Windows 7 were used: one for running the Cheetah data acquisition software and one running the MATLAB (MathWorks, Natick, Massachusetts) software for real-time detection of sleep stages as described in Eckert et al, 2021.

Offline analyses of sleep, SWS and spindle detection was performed. High EMG power/low EMG power was used to select the threshold for separating awake and rest periods based on the EMG activity. Rest periods greater than 20 second was selected for getting the timestamps of rest periods. For SWS detection during the rest periods, LFP recording was used to calculate the Fourier transform of the hippocampal and EMG signals. For the hippocampal LFP, the ratio of delta (1-4 Hz) and alpha (10-15 Hz) to theta (6-10 Hz) power was calculated. The hippocampal delta*alpha/theta power ratio was used to obtain a numerical estimate of the sleep state, referred to as the "SWS score". For each animal, the threshold value of 2SD of the SWS score was set to detect SWS periods. For spindle detection, the cortical LFP was filtered between 10-20 Hz, then squared and smoothed (175 ms rectangular window, step size 1 sample) to obtain a power signal. Periods of significant spindle activity were determined by finding peaks greater than 2 SD in the power signal, and then the start/end timestamps were found by measuring the power before/after the peak until the power fell to 0.75 times the peak threshold. This method yielded a list of timestamps indicating the start and end timepoints of each spindle. Detected events shorter than 200 ms and longer than 2 sec were discarded.

Video from an overhead camera was also acquired via the Neuralynx Digital Lynx system. The video was synchronized and time-stamped with electrophysiological recordings and stored on the computer for off-line analysis.

Behavioral procedure and experimental schedules:

Rats were run through a series of experiments in the order shown in Table 1:

Order of Task	Task	# of days for task	# of weeks after the previous task
1	Novel exploration task 1	8	n/a
2	Novel exploration task 2	8	5 weeks
3	Novel exploration task 3 / Social expt	8	4 weeks
4	Morris water task	14	4 weeks
5	Fear task	13	4 weeks

Table 1: Series of behavioural tasks tested

The order of the behavioural tasks was designed to go from the least stressful to the most stressful experience in rats. The highest levels of stress may have a confounding influence affecting the interpretation of results in other behavioural tests, if done in different order.

Novel environment exploration experiment 1:

The rats were allowed to explore a novel context in a novel room for 20 mins. The exploration box had several objects and toys (combination of plastic and metal toys and objects of different shapes and sizes, typically used in environmental enrichment studies) for visual and somatosensory exploration of the spatial context, as shown in Figure 8. In addition, there were some novel gustatory stimuli (in the form of chocolate sprinkles, cheerios, fruit loops) and olfactory stimuli in the form of some scents (combination of pine scent, banana scent, eucalyptus scent) spread in the context as well. The exploration task was recorded by a standard video camera from the top giving

full view of the rat exploring the context. During baseline sleep recording, rats were taken into the recording room and electrophysiological brain state activity was recorded for 2 hours. On day 8, a 2-hr sleep session was recorded before and after exploration task, comprising the pre-exploration and post-exploration sleep respectively.

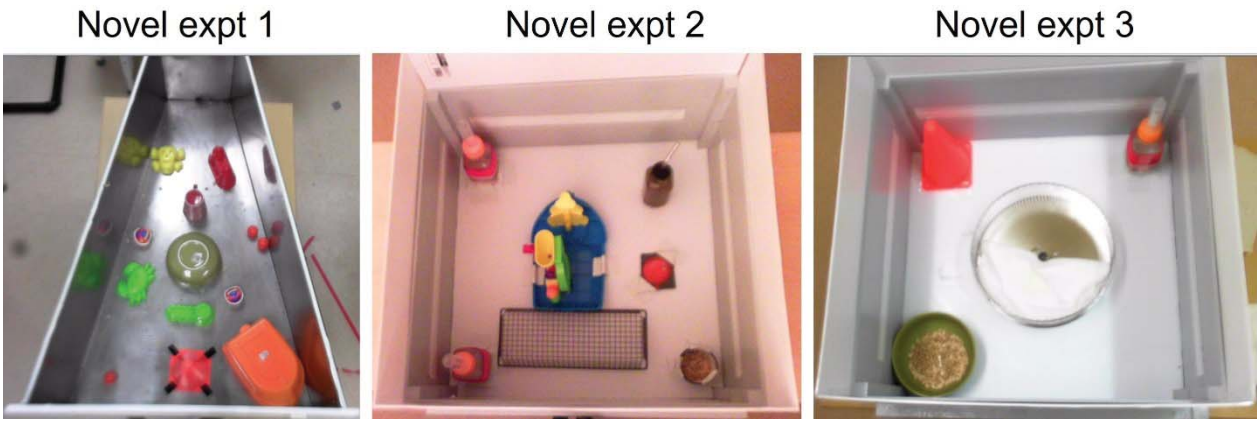


Figure 8: Novel environment exposure contexts for expt 1, 2 and 3

The experimental schedule was as described in the following table:

Experimental day	Behavior	Recording
1-4	Habituation days	2-hr sleep
5-7	Baseline sleep	2-hr sleep
8	Novel context exploration: 20 mins	2-hr pre-exploration and 2-hr post-exploration sleep

Table 2: Experimental schedule for Novel environment exploration experiment 1

Novel environment exploration experiment 2:

In addition, to another novel context and room different from experiment 1, the rats were allowed to explore the context with different set of objects and scents from experiment 1, as shown in Figure 8. The spatial location of the objects and scents within the context was kept the same in experiment 2 for the 3 days of exploration. During the home-cage baseline days, rats were taken to

their respective home cages, instead of the task room, for an equivalent amount of time of 20 mins in between two 2-hr sleep recordings. The experimental schedule was as described in the following table:

Experimental day	Behavior	Recording
1-2	Home-cage habituation days	2-hr pre-sleep and 2-hr post-sleep
3	Home-cage Baseline control	2-hr pre-sleep and 2-hr post-sleep
4-6	Novel context exploration: 20 mins	2-hr pre-exploration and 2-hr post-exploration sleep

Table 3: Experimental schedule for Novel environment exploration experiment 2

Novel environment exploration 3/ social expt:

In addition to the information from novel exploration task 2, rats were allowed to explore a context (different from the previous two experiments; different combination of objects and sensory stimuli, as shown in Figure 8) in the presence of another rat it never interacted with so far, in an effort to try to maximize the novelty in the context in terms of an additional component of social interaction. Rats were paired randomly (Rats 11-12 and rats 13-14), but the same pair was allowed to interact on all the exploration days. Rats were placed in the same order in quick succession and from the same direction each day. During the home-cage baseline days, rats were taken to their respective home cages, instead of the exploration task room, for an equivalent amount of time of 20 mins in between the two 2-hr sleep recordings of pre-task and post-task sleep. The experimental schedule was as described in the following table:

Experimental day	Behavior	Recording
1-2	Home-cage habituation days	2-hr pre-sleep and 2-hr post-sleep
3	Home-cage Baseline control	2-hr pre-sleep and 2-hr post-sleep
4-6	Novel context exploration: 20 mins	2-hr pre-exploration and 2-hr post-exploration sleep

Table 4: Experimental schedule for Novel environment exploration experiment 3/social experience

Morris water task:

We used a modified version of the standard Morris water task (R. G. Morris, 1981; Terry Jr, 2009). The modified version was an adaptation of experiment 2 from the following research article (Bye & McDonald, 2019). To briefly describe the task as given by Bye and McDonald: A large circular fibreglass pool (45 cm height and 125 cm diameter) in the center of the room was filled with water made non-transparent with non-toxic white paint. A white plastic circular platform (12 cm diameter; approximately 2 cm below the surface of the water) served as the escape platform. Rats could see cues on walls beyond the maze. The pool was cleaned and refilled with water every evening after the task so that the water comes to room temperature by the next day. Each room had several distinct visual cues in the form of pictures of colored shapes on the walls in addition to a computer, sink, black shelf, and entrance door. Rats were transported into the testing room in individual cages on a wheeled cart and placed into the SE corner of the room. Animals were run in groups of 2, one after the other. For pretraining in Room A, rats were trained to find a hidden platform located in the NW quadrant of the pool. Each rat had 12 trials per day over 4 days, for a total of 48 trials. The rat was placed in the pool at one of the random start positions facing the pool wall and allowed to swim until they reached the hidden platform or until 60 s had elapsed. After every trial the rat would be left on the platform for 10 s in order for them to rear and grasp the visual cues for spatial learning of the environment. Each training session took approximately 30 min with an average inter-trial interval of approximately 3-4 min. 24 hours after pretraining, the platform was moved to the SE quadrant, opposite to that of pretraining, for 12 trials on that day. This reversal training consisted of 12 trials on day 5. Similar to pretraining, rats were placed in the pool at one of the directional positions in random order, with 60 s to find the platform, and 10 s on the platform. A probe trial was performed on day 6. For the mass training session, animals were

mass-trained to a new platform location in a new room B (separate room at a different in the facility, with distinct extra maze cues than room A; different style of transport cage between pre-training and mass-training) for 12 trials. Latency to find the platform was the primary measure of learning and memory test. All trials were grouped in four-trial average blocks.

Experimental day	Behavior	Recording
1-2	Home-cage habituation days	2-hr pre-sleep and 2-hr post-sleep
3	Home-cage Baseline control	2-hr pre-sleep and 2-hr post-sleep
4-7	Pretraining Room A: 12 trials	2-hr pre-task and 2-hr post-task sleep
8	Reversal training Room A: 12 trials	2-hr pre-task and 2-hr post-task sleep
9	Mass-training Room B: 12 trials	2-hr pre-task and 2-hr post-task sleep

Table 5: Experimental schedule for modified Morris Water task experiment

During the home-cage baseline days, rats were taken to their respective home cages, instead of the task room, for an equivalent amount of time of 20 mins in between two 2-hr sleep recordings.

Context pre-exposure and immediate shock deficit fear conditioning:

This behavioural paradigm has been described in (Fanselow, 1986; Rudy & O'Reilly, 2001), given the finding that immediate shock deficit reflects a deficit in the formation of association between contextual stimuli and shock. The details have been given in experiment 3 of that reference. The behavioural task was conducted as described in the Figure 9. The modified version of the task from the reference, was based on the observation that the memory representation that is active at the time of shock is more important for the development of conditioned fear response than the physical cues present at the time of shock. The rats could be conditioned to the memory representation activated by the transport context and not to the physical cues present at the time of shock.

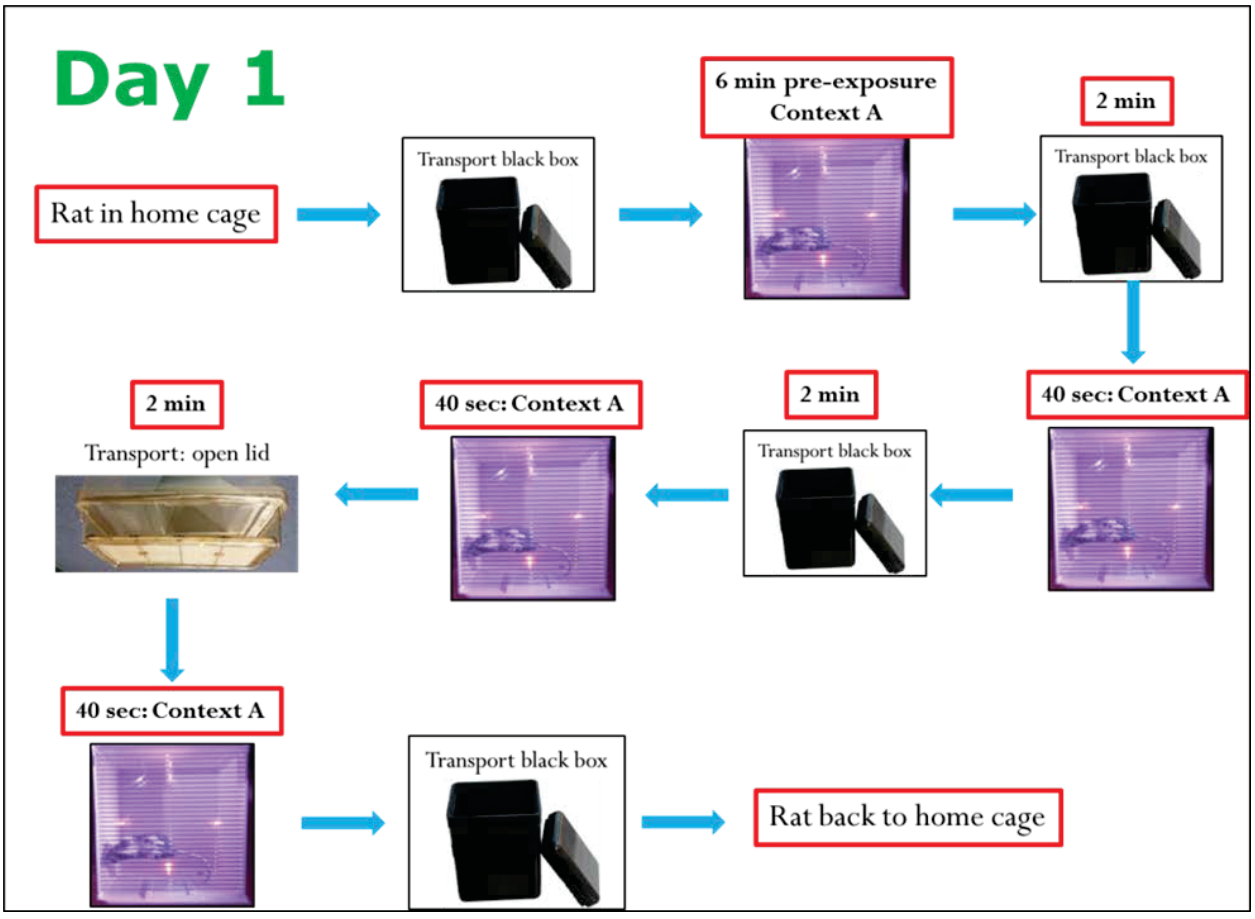
As described by Rudy and O'Reilly, we established a transport-context association through preexposure to context A and then this transport context is used to bring rats into a different context (context B) for immediate-shock conditioning. Context A was composed of a square large white

interior chamber that sat on a smooth Plexiglas floor, illuminated by 4 led lights on the lid. Context B was designed to be very dissimilar to Context A. Context B was a black triangular box with grid floor that permitted the delivery of foot shock, black lid, no lighting in the box and no illumination in the room. It was illuminated minimally with red light, just enough to permit the experimenter to conduct the experiment in the room, put the rat in the chamber, to deliver shock. An infrared camera was placed under the glass countertop to record the rat behaviour to a computer during preexposure and testing days.

The rats were preexposed to Context A on day 1 and shocked immediately in context B on day 2. The preexposure was designed to give the rats time and opportunity to explore the context A and successfully form a memory representation of the context. The immediate shock in context B prevented the rat from forming a spatial memory representation of the shock-context. We then tested the rats in the pre-exposed, original associated context and the actual conditioning context. On day 1, the rats were transported to the pre-exposure context A in the black bucket transport box. The rats were given multiple exposures to Context A on day 1. The first exposure was 6 min; thereafter, all the remaining 3 exposures were 40 sec. in between the first 3 exposures, the rats were placed in the black transport box (for 2 min) and after the third exposure the rats were placed in an open plastic pan (for 2 min). On Day 2, the rats were fear-conditioned. They were taken from their home cage in the black transport bucket, placed in Context B, and shocked immediately for 2-sec. The transport context was the same during both day 1 and 2. On day 3, the rats were transported in an entirely different environment (the one used between preexposure trials 3 and 4) and tested for freezing behaviour in both contexts A and B with the order of testing counter-balanced between the rats.

If the rats display freezing in the original associated context and not in the actual conditioning context, this would be strong support for conjunctive representation view of context, presented by Rudy and O'Reilly that the rats were conditioning to the memory representation active at the point of shock, and not to the physical cues during the time of immediate shock. This is the immediate-shock effect.

During the home-cage baseline days, rats were taken to their respective home cages, instead of the task room, for an equivalent amount of time of 15 mins in between two 2-hr sleep recordings.



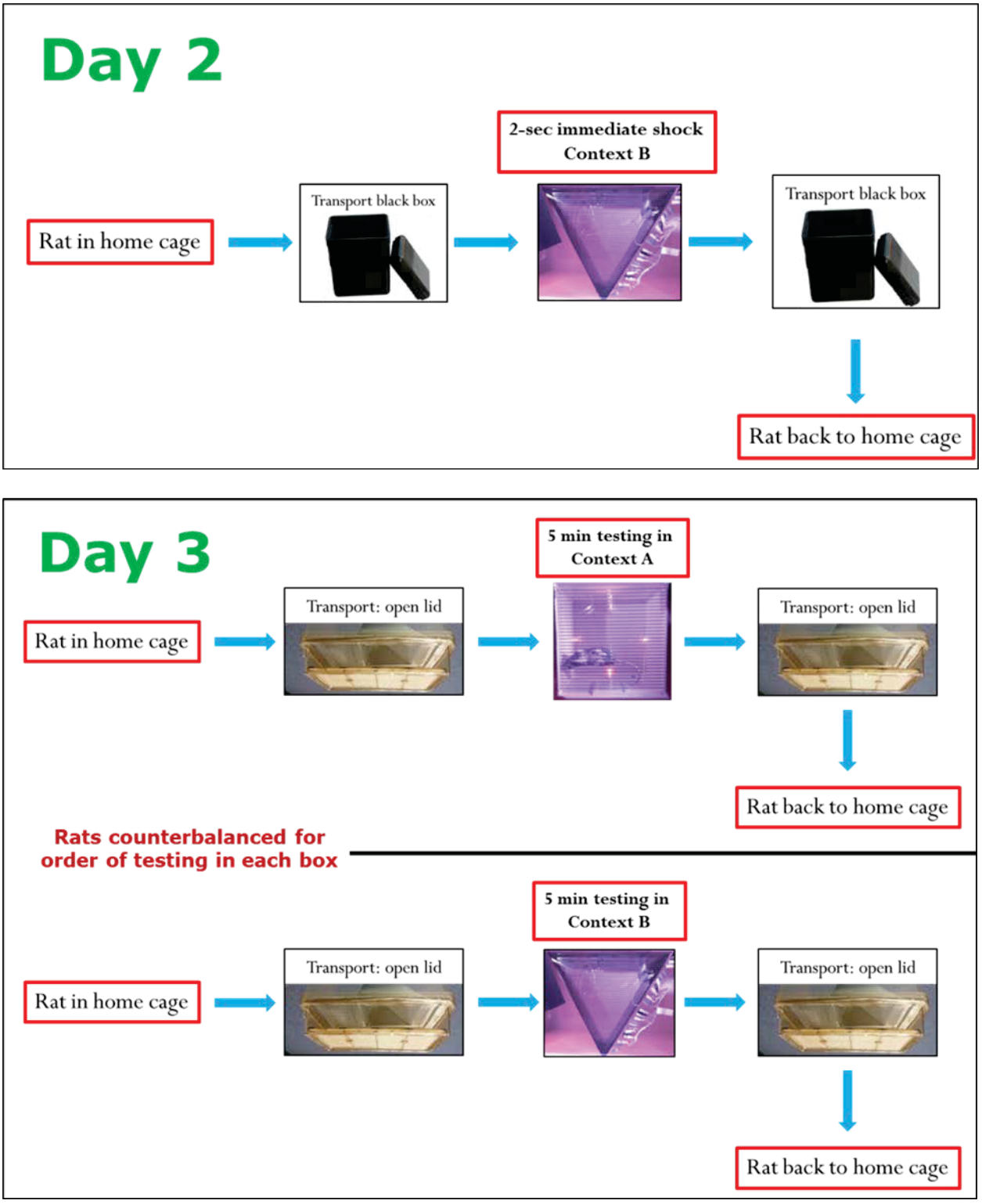


Figure 9: Context pre-exposure and immediate shock deficit fear conditioning experiment

Experimental day	Behavior	Recording
1-2	Home-cage habituation days	2-hr pre-sleep and 2-hr post-sleep
3	Home-cage Baseline control	2-hr pre-sleep and 2-hr post-sleep
4	Pre-exposure to context A	2-hr pre-task and 2-hr post-task sleep
5	Immediate shock in context B	2-hr pre-task and 2-hr post-task sleep
6	Day 1 testing: 5min exposure to contexts A and B counterbalanced between rats	2-hr pre-task and 2-hr post-task sleep

Table 6: Experimental schedule for Context pre-exposure and immediate shock deficit fear conditioning

Switch-task:

The details of the animals, behavioural experiment and recordings is as described in (Insel & Barnes, 2015): "The experimental setup was 3-arm platform radiating from a circular, central region. Rats were trained to shuttle to the ends of platform arms to follow an auditory cue (a 10-kHz tone broken each 50 ms by a 25-ms delay) and visual cue (a 4-Hz blinking white light). A 40-min session on the task took place once each day and consisted of up to several hundred trials. A rat initiated a trial by entering the circular, central zone (the cue zone), at which point an auditory and visual cue were presented from the ends of randomly selected arms. The trial was completed when the rat reached the end of an arm (the feeder zone), where it would receive a drop of liquid food reward if it had correctly followed the rewarded cue, or alternatively, encounters an error sound if it had not. During a given session, only one of the two cues was rewarded. The rewarded cue was switched following 8 sessions, referred to as a task-switch". The dataset for this experiment was shared by Dr. Nathan Insel, University of Montana. For the purposes of this study, changes in spindle density were measured in the post-task sleep following the task switch.

Data processing and statistical analysis:

Offline electrophysiological data processing was performed using MATLAB software similar to that described in Eckert et al, 2021. Briefly, the awake state was identified by the presence of low-amplitude fast activity associated with high EMG tone. SWS was identified by continuous high-amplitude slow activity, high sigma power (power overlapping in the spindle frequency band), and low EMG activity. REMS was characterized by strong theta activity, low-voltage fast activity, and an absence of EMG tonus. All automated scoring of behavioral states were additionally verified by video recording and by visual inspection of the signals using Neuraview software (Neuralynx, Bozeman, MT).

EMG power spectra was used for automated scoring of sleep-wake episodes. For the EMG signal, the ratio of power in the high (100-300) to low (10-20) frequencies was calculated. The EMG signal from each session was thresholded manually to segment the recording into epochs of rest and movement. Power spectra of delta (0-4 Hz), theta (5-10 Hz), and spindle (10-20 Hz) frequency bands from the LFP traces were used to further classify rest epochs as SWS or REM based on the method for the detection of SWS based on the ratio (delta power * alpha power / theta power). Periods with SWS power of 2SD were categorized as SWS periods. This was also visually verified with Neuraview (Neuralynx, Bozeman, MT) software to validate the automated detection. Periods of significant SWS power activity were determined by finding peaks greater than 2 SD from the mean in the power signal to take into account considerable differences in LFP signal amplitude using the 'findpeaks' function in MATLAB. The start/end timestamps were found by measuring the power before/after the peak until the power fell to 0.75 times the peak threshold: an upward-rising crossing defined the SWS onset, and the next falling crossing defined the end of the SWS episode. The start/end time stamps marked all the threshold crossings of the SWS power rms

signal. This method yielded a list of timestamps indicating the start and end timepoints of each SWS episode.

Sleep spindles are easily detected in the rat cortical LFP recordings and serve as indicators of SWS. Spindles were detected automatically based on thresholded power signals of filtered LFP recordings (spindle 10–20 Hz). Threshold values for automated detection of LFP features were selected using data from the pre-task recording from each day. Once set, the same thresholds were used for the post-task recording session on the same day. For spindle detection, the cortical LFP was bandpass filtered between 10-20 Hz, then squared and smoothed (175 ms rectangular window, step size 1 sample) to obtain a power signal. Periods of significant spindle activity were determined by finding peaks greater than 2 SD from the mean in the power signal to take into account considerable differences in LFP signal amplitude using the 'findpeaks' function in MATLAB. The start/end timestamps were found by measuring the power before/after the peak until the power fell to 0.75 times the peak threshold: an upward-rising crossing defined the spindle onset, and the next falling crossing defined the end of the spindle. The start/end time stamps marked all the threshold crossings of the sigma rms signal. This method yielded a list of timestamps indicating the start and end timepoints of each spindle. Detected events shorter than 200 ms and longer than 2 sec were discarded. Threshold values for automated detection of LFP features was done while inspecting the pre-task recording. Once set, the same thresholds were used for post-task recording sessions. The spindle analysis was restricted to SWS episodes with continuous spindling activity (≥1 spindle per 10 s), a sleep state closely analogous to spindling activity in human NREM2 (De Gennaro & Ferrara, 2003; Eschenko et al., 2006). Spindle density was calculated as the number of spindles per minute of SWS. For each SWS episode, the average was calculated to obtain a single value for that epoch. The average spindle density of all SWS epochs in the 2-hour recording session was used to compute a mean and SD which could be used for statistical comparisons with other sessions.

Since the temporal pattern of sleep and SWS episode onset and duration varied greatly between individual rats across experimental conditions, sleep indices were averaged over 30 min time intervals from the onset of SWS. Reliability of SWS and spindle-detection algorithms was verified by visual observation.

Studies have shown that most robust effects of learning on spindle density (Eschenko et al., 2006) occur during the first hour of post-learning SWS. Taking this observation into account, the results were limited to the first hour of post-experience SWS starting with the onset of the first episode of SWS, divided into 30-min periods for analyses purposes.

2.4 Results

Spindle density was calculated for each of the experiments for the first 30 minutes after SWS onset, when we expect to see experience-dependent changes in spindles (Eschenko et al., 2006). In experiments where pre-sleep and post-sleep sessions were recorded, the difference between these two spindle density measurements is reported. Difference in spindle density between post-experience and pre-experience sessions used for statistical comparison and each experimental day was compared corresponding with the home-cage control sessions.

Novel environment exposure experiment 1:

In the first experiment, rats were introduced to a novel environment to see if this would increase spindle density after this learning experience. During baseline sessions in this experiment, rats were taken from the home cage to the recording room and the electrophysiological activity was recorded over a 2-hr period. Baseline is the average of 3 sessions immediately preceding the exposure sessions. Spindle density was computed for the novel exposure session and compared to

the average spindle density over three baseline days during which rats stayed in the sleep chamber undisturbed for the two-hour recording sessions. A t-test between the baseline average and post-exposure session was used for statistical comparison.

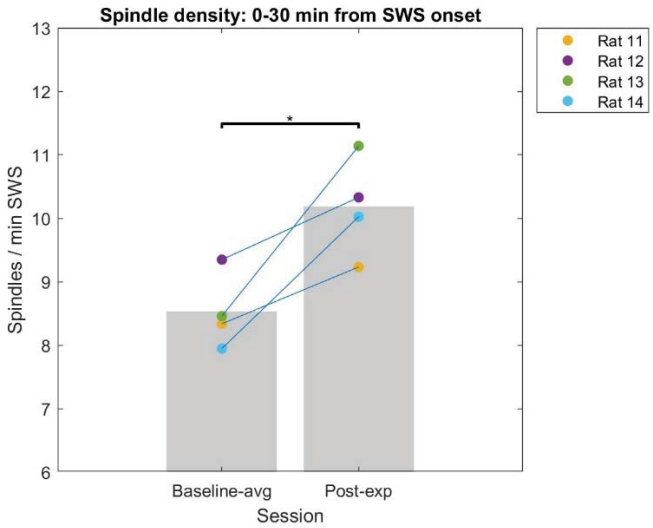


Figure 10: Spindle density in the first 30 minutes after SWS-onset in Novel experiment 1.

Spindle density was significantly higher ($t(6) = -3.3658$; $p = 0.0151$) after a 20 minute exposure to a novel environment exploration task compared to baseline sleep sessions. There was just one comparison in this experiment, so we did not have to correct for multiple comparisons using the Bonferroni method, to account for the criteria for significance. Additionally, it is interesting to note that the analyses of the 30-60 minute window after SWS-onset showed continued increases in spindle density post-exposure (as demonstrated in supplementary figure S13).

Novel environment exposure experiment 2:

In the second novel environment exposure experiment, rats were introduced to a novel environment for 3 days consecutively (all 3 days in the same environment; Exp1, Exp2 and Exp3 respectively) to see if this would increase spindle density after this learning experience. HC refers

to home-cage control days. During home-cage (HC) baseline sessions in this experiment, rats were taken from the home cage to the recording room and the electrophysiological activity was recorded over a 2-hr period, brought back to the home-cage for 20 min (equivalent to the exposure time on experimental days) and taken back to the recording room for the post-homecage 2-hr recording session. Only the last day of home-cage baseline recording was considered for comparisons, like was used in earlier work (Eschenko et al., 2006). Differences in spindle density between the pre-task and post-task sleep were computed for the novel exposure session and compared to the spindle density difference (post-task minus pre-task) on the home-cage (HC) baseline days. HC-baseline was compared with each of the exposure days using a t-test for statistical comparison.

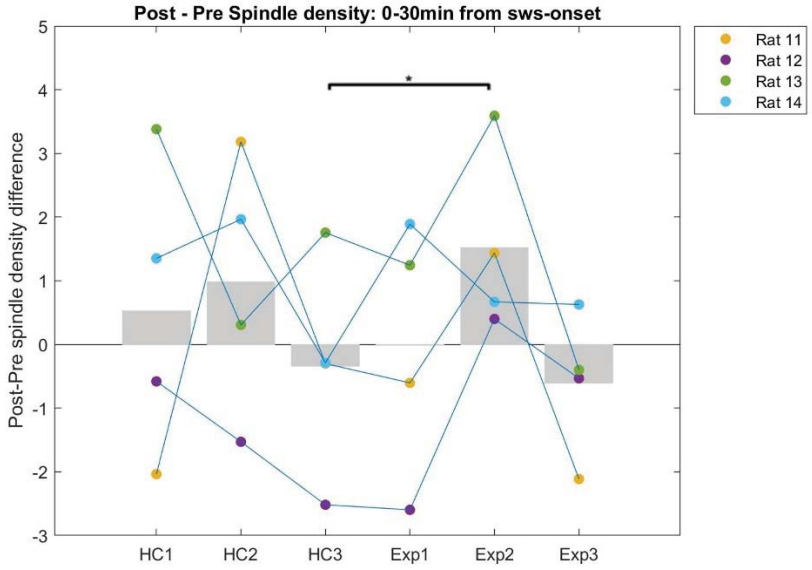


Figure 11: Difference in spindle density between post-experience and pre-experience sessions in the first 30 minutes after SWS-onset in Novel experiment 2

Spindle density difference was significantly higher on day 2 of exposure ($t(6) = -4.6181$; $p = 0.0153$) after a 20 minute exposure to a novel environment exploration task compared to baseline sleep session. We corrected for multiple comparisons using the Bonferroni method, the criteria for

significance would have been p>0.016 and novel exp day 2 would have been significant. The other tests (exp1 and exp3) were non-significant. Additionally, it is interesting to note that the analyses of the 30-60 minute window after SWS-onset partly was in line with our hypothesis, at least in terms of the trends we had expected (as demonstrated in supplementary figure S14), even though no comparison reached statistical significance. The highest increase was seen in Exp 1, with the increase slowly declining over the 3 days, as the rats habituate and become familiar with the environment.

Novel environment exposure experiment 3:

In this novel environment exposure experiment, rats were introduced to a novel environment for 3 days consecutively (all 3 days in the same environment; Exp1, Exp2 and Exp3 respectively) along with a social experience component in the form of a partner rat (the same pair of rats on all the 3 exposure days) to interact with, for all the 3 days of exposure to the novel context, to see if this social interaction would increase spindle density after this learning experience. HC refers to home-cage control days. During home-cage (HC) baseline sessions in this experiment, rats were taken from the home cage to the recording room and the electrophysiological activity was recorded over a 2-hr period, brought back to the home-cage for 20 min (equivalent to the exposure time on experimental days) and taken back to the recording room for the post-homecage 2-hr recording session. Only the last day of home-cage baseline recording was considered for comparisons, like was used in earlier work (Eschenko et al., 2006). Differences in spindle density between the pre-task and post-task sleep were computed for the novel exposure session and compared to the spindle density difference (post-task minus pre-task) on the home-cage (HC) baseline days. HC-baseline was compared with each of the exposure days using a t-test for statistical comparison.

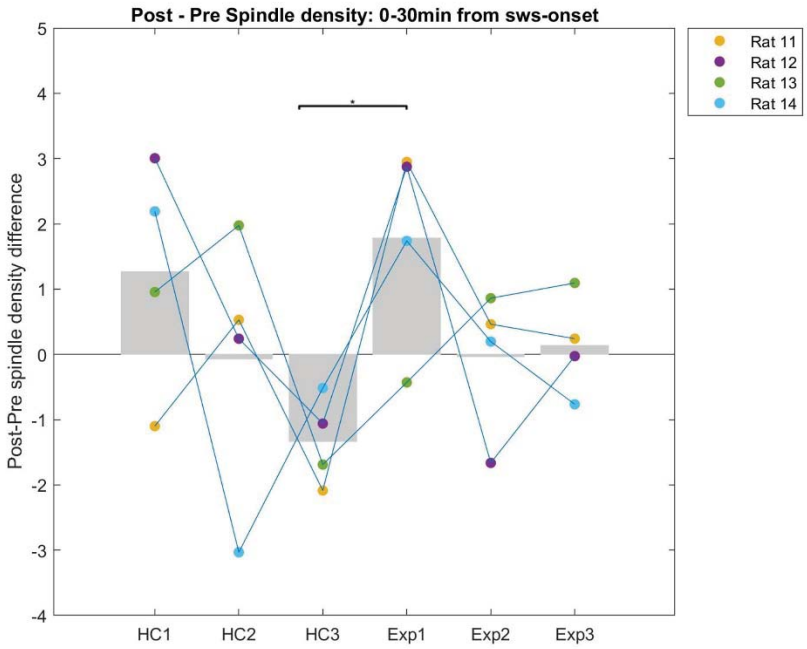


Figure 12: Difference in spindle density between post-experience and pre-experience sessions in the first 30 minutes after sws-onset in Novel experiment 3

This social experience seemed to drive experience-related spindle density the highest on day 1 of exposure. Spindle density difference was significantly higher on day 1 of exposure ($t(6) = -3.6264$; $p = 0.0110$) after a 20 minute exposure to a novel environment exploration task compared to baseline sleep session. We corrected for multiple comparisons using the Bonferroni method, the criteria for significance would have been $p>0.016$ and novel exp day 1 would have been significant. The other test comparisons (Exp2 and Exp3) were non-significant. Additionally, it is interesting to note that the analyses of the 30-60 minute window after SWS-onset showed continued increases in spindle density in Exp 1, and additionally in Exp 3 (as demonstrated in supplementary figure S15).

Water task experiment:

In this modified version of the Morris Water task experiment, rats underwent a series of behavioural trials in between two 2-hr recording sessions (pre-task sleep session and post-task sleep session respectively. HC refers to home-cage control days. During home-cage (HC) baseline sessions in this experiment, rats were taken from the home cage to the recording room and the electrophysiological activity was recorded over a 2-hr period, brought back to the home-cage for 20-30 min (equivalent to the behaviour time on experimental days) and taken back to the recording room for the post-home cage 2-hr recording session. Hab refers to habituation days, similar to HC. On experimental days, rats were given 12 trials in the water task, as described in the methods section. A1-A4 are the pretraining trials over 4 consecutive days in room A. Rev A is reversal trials in room A with platform position reversed to the diagonally opposite quadrant to that during pretraining days. B refers to mass training in room B. The results of latency to find platform location on all days of this experiment are reported in supplementary figure S16.

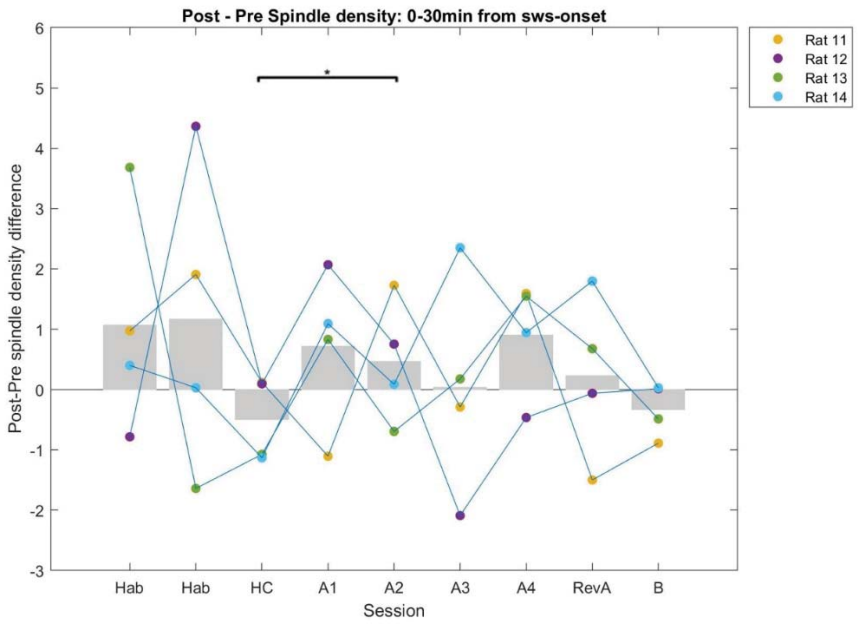


Figure 13: Difference in spindle density between post-experience and pre-experience sessions in the first 30 minutes after sws-onset in modified version of water task experiment

Our predictions were as follows: A1 would involve a lot of learning for the rats in terms of the task, swimming in the water pool, the concept of finding a platform to escape, the spatial organization of the room and the spatial location of the escape platform with respect to extra-maze cues in the room, to name a few. Hence, we expected significant increases in spindle density to account for all this learning. For A2-A4, rats were trained to the same platform location in room A as on A1 and we expected the increase in spindle density to be slightly lower than A1, but still higher than home-cage baseline to account for spatial learning. Rev A is reversal trials in room A with platform position reversed to the opposite quadrant to that during pretraining days. We anticipated the increase in spindle density to account specifically to learning the new platform location stripped off all the additional task learning from A1. B refers to mass training in room B. We expected significant increases in spindle density in B, lower than A1 and higher than RevA. Thus, specifically accounting for learning the new platform location in a new room stripped off all the additional task learning from A1. We anticipated this amount of spindle density increase to specifically account for contextual spatial learning in an already learnt task.

Only the last day of home-cage baseline recording was considered for comparisons, like was used in earlier work (Eschenko et al., 2006). Differences in spindle density between the pre-task and post-task sleep were computed for the novel exposure session and compared to the spindle density difference (post-task minus pre-task) on the home-cage (HC) baseline days. HC-baseline was compared with each of the experimental days using a t-test for statistical comparison. Spindle density difference was significantly higher on day 2 of the task ($t(6) = -3.4993$; $p = 0.0395$) after a Water task learning compared to baseline sleep session. The other test comparisons were non-significant. We corrected for multiple comparisons using the Bonferroni method, the criteria for significance would have been $p>0.0083$ and none of the comparisons would have been significant. It is interesting to note that the analyses of the 30-60 minute window after SWS-onset partly was

in line with our hypothesis, at least for A2, A3 and B, correlative with learning on days 2 and 3 of pre-training and mass training in room B respectively (as demonstrated in supplementary figure S11), where the spindle density increases were statistically significant.

Context pre-exposure and immediate shock deficit fear conditioning experiment:

In this modified version of the Context pre-exposure and immediate shock deficit fear conditioning experiment, rats underwent a series of behavioural tests in between two 2-hr recording sessions (pre-task sleep session and post-task sleep session respectively. During home-cage (HC) baseline sessions in this experiment, rats were taken from the home cage to the recording room and the electrophysiological activity was recorded over a 2-hr period, brought back to the home-cage for 15 min (equivalent to the behaviour time on experimental days) and taken back to the recording room for the post-homecage 2-hr recording session. HC refers to home-cage control days. Hab refers to habituation days, similar to HC. On experimental day 1, rats were pre-exposed to context A. On experimental day 2, rats were given an immediate 2-sec shock as soon as they were introduced into to context B. On experimental day 3, rats were tested in both contexts, preexposure context A and shock context B.

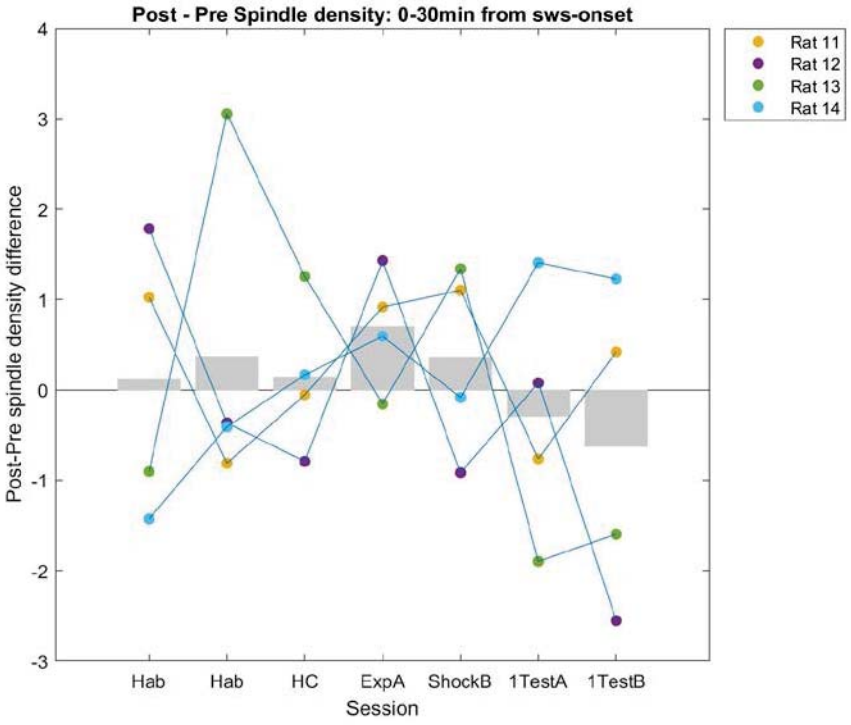


Figure 14: Difference in spindle density between post-experience and pre-experience sessions in the first 30 minutes after SWS-onset in Context pre-exposure and immediate shock deficit fear conditioning experiment

Our predictions were as follows: Preexposure to context A would involve a contextual spatial learning for the rats in terms of the spatial organization of the room and the spatial organization of the chamber. Hence, we expected significant increases in spindle density to account for this spatial learning. Immediate shock in context B would involve the most significant learning in terms of association of the pre-exposure context to the shock which involves fear conditioning. Hence, we expected the most significant increases in spindle density to account for this associational and contextual fear learning. In addition, based on the observations of Rudy and O'Reilly, (Rudy & O'Reilly, 2001) and the behavioural analyses, we predicted increases in spindle density in the post-test sleep session correlative of memory retrieval when tested in context A, but not context B, along with possibility of reconsolidation of the memory trace. The results of freezing

behaviour in the contexts are reported in supplementary figure S17. As expected, rats displayed freezing in the original associated context and not in the actual conditioning context, strongly supporting the idea that the rats were conditioning to the memory representation active at the point of shock, and not to the physical cues.

Only the last day of home-cage baseline recording was considered for comparisons, like was used in earlier work (Eschenko et al., 2006). Differences in spindle density between the pre-task and post-task sleep were computed for the novel exposure session and compared to the spindle density difference (post-task minus pre-task) on the home-cage (HC) baseline days. HC-baseline was compared with each of the experimental days using a t-test for statistical comparison. No significant changes in spindle density were observed in the spindle density across the days of this experiment. Even though comparative spindle density increased during the post-exposure to context A, it failed to reach statistical significance. All the other test comparisons were non-significant. It is interesting to note that the analyses of the 30-60 minute window after SWS-onset partly was in line with our hypothesis, at least for the pre-exposure context and testing in the preexposure context (as demonstrated in supplementary figure S17), where the spindle density increases were statistically significant.

Task switch experiment:

In this task, the rats were trained in a cue-association task, in which the rats associated the reward to either an auditory or a visual cue during training and on the switch day, the rewarded cue was switched, called task-switch. A rest period was recorded after each session for about 30-40 minutes. It is worth noting that the post-task rest session recorded varied between days. So, we analysed the entire post-task epoch for spindle density analyses. The analyses were done for all rats with 2 switch days reported in the dataset. Switch-1 was the day before task switching and switch+1

was the day after task switching. The rats were also divided into young and aged rats (5 young; 11.4 months average age and 4 aged; 27.5 months average age) to study differences in age-related spindle density changes. We analysed the spindle density during SWS episodes during these post-switch task periods and compared the switch-1 day with the switch day and switch+1 day see if this task-switch learning would increase spindle density correlative of this learning experience. There were 5 young and 4 aged rats recorded in the task.

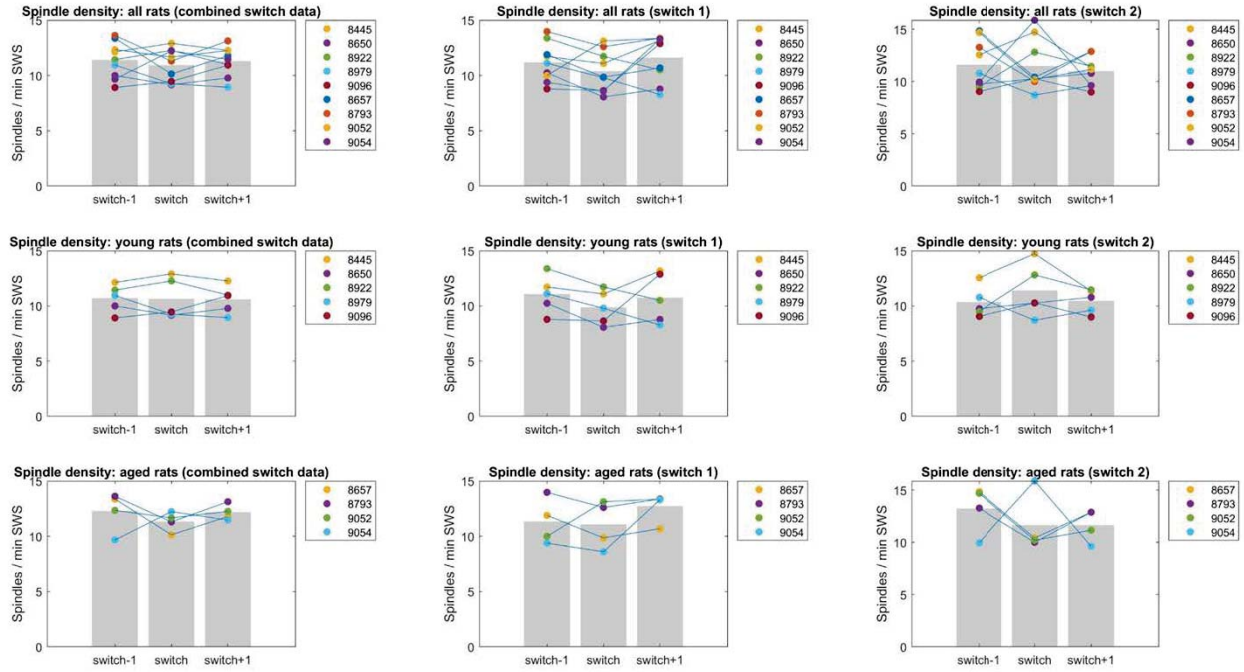


Figure 15: Changes in spindle density in the first 30 minutes after SWS-onset in task switch experiment.

Our predictions were as follows: increase in spindle density predictive and correlative of task-learning on the switch day switch+1 day. Switch day was compared with switch-1 day using a t-test for statistical comparison. No significant changes in spindle density were observed in the spindle density across the days of this experiment and even between rats. Even though comparative

spindle density decreased during the post-switch sleep in young rats after the first switch, it fell short of reaching statistical significance ($t(8) = 2.2467$; $p = 0.0574$ rounded to 0.06). All test comparisons were non-significant.

2.5 Discussion

As discussed in the introduction, several studies in humans and some studies in rodents have reported changes in spindle density as a result of learning. Firstly, we wanted to investigate if increases in spindle density are correlated with exploring a novel environment. Exploration of a novel environment involves spatial learning of the context, encoding of sensory information into the respective sensory brain regions and further processing of this encoded information which could very well be consolidated. Secondly, we wanted to investigate the changes in spindle density following other types of hippocampal-dependent learning not yet reported in rodent literature. To test whether learning could enhance spindles, we included a modified version of Morris Water task and a version of fear conditioning task called 'context pre-exposure and immediate shock deficit fear conditioning'. Finally, we wanted to test whether a cognitive demand in a familiar task might also lead to enhanced spindles. For this, we used a task switching behavioural paradigm which clearly involves some form of learning to switch behavior based on cues in an already learnt task. In the task driven experiments including water task, fear conditioning and task switch experiments, it is possible to relate the changes in spindle density to memory recall. We had speculated that spindle density would be increased during post-learning sleep in the first 30-min window after SWS onset as an indicator and predictor of learning in the investigated tasks.

For direct comparison between all the experiments, the most significant changes were observed within the first 30 minutes of SWS onset after the learning epoch as reported earlier

(Eschenko et al., 2006). For the discussion, relevant spindle density data investigations in the 30-60 mins time window after SWS onset will also be considered. Our primary measure is the increase in spindles from the pre-task sleep to the post-task sleep session. This helps eliminate changes in spindle density due to slight differences in electrode positions from day to day and thereby makes our results more reliable than previous studies, which compared post-task spindle density to a baseline sleep session recorded the previous day. Given that in earlier studies, spindle density after learning was usually compared to the activity from the preceding day without learning, we adopted a similar approach. For statistical analyses, we compared spindle density after learning with data from the preceding day, although we also collected and present data from several habituation days preceding the learning session(s).

Changes in spindle density in the post-learning sleep periods was consistent with our hypothesis, based on learning-dependent changes in spindle density observed mostly in human studies and in some rodent studies, as discussed in the introduction to this chapter. But it seems to be more nuanced than that. Increases in spindle density in the novel environment exploration task demonstrated that task-independent exploration involves a form of learning spatial context and environmental cues. Spindle density showed significant increase on day 2 in the water task while no significant changes were observed in the fear condition experiment and task-switch experiment. Interestingly, changes in spindle density in the 30-60 min window seemed to at least partly support our hypothesis both in the water task and fear conditioning, which will be discussed in detail in the following paragraphs. From the experimental results, it is evident that spindles could be enhanced by several different factors. The results of each of the experiments will be discussed in the light of our observations and factors influencing them from earlier reported studies.

Novel environment exposure experiments:

We had hypothesized that spatial and contextual learning happens even during exploration of a novel environment and that this experience could drive enhancement in spindle density. Even though learning the configuration of a room is an automatic process, it still involves considerable learning. Exploratory behaviour involves learning processes triggered by novelty, either of the context or elements within that context (Pisula & Modlinska, 2020). Exploration of a new environmental context and encounters with objects through the various sensory modalities are crucial for survival and adaptation and would conceivably involve several forms of spatial and sensory learning.

Several studies support our hypothesis, directly or indirectly. In mouse NREMS, the largest and fastest discrete spindles have been documented in barrel cortex, which is the primary region of the somatosensory cortex involved in active exploration and experience-dependent learning. (Fernandez et al., 2018). Enhancement of ripple-spindle correlations was found after exploratory behavior and preferential engagement of recently active CA1 units in SWRs and their phase-locking to spindles, suggesting that activation of thalamic cells and spindle cycles provide optimal temporal windows to promote synaptic plasticity and facilitate the integration of recently acquired memory traces into neocortical networks (Varela & Wilson, 2020). A novel spatio-tactile experience experiment in rats was found to induce sleep-dependent Arc gene expression in the cortex, but not in the hippocampus (Ribeiro et al., 2007), suggesting that the cortex undergoes plasticity during learning. IEG Arc expression has been reported as a result of active behaviour, such as exploring a novel environment with Arc and CaMKII acting synergistically to promote functional and/or structural synaptic plasticity modifications that accompany learning (Vazdarjanova et al., 2006). This indicates that exploration of a context can induce cellular changes.

Given these findings, the expectation that novel experience can enhance sleep spindles is not unreasonable.

Significant increase in spindle density were observed in experiment 1 after exploration of the novel environment. In addition to increases in spindle density withing the first 30 minute, in novel context exposure 1, significant increases in spindle density were also observed in the second 30 minute period of post-exposure sleep after SWS onset, as shown in supplementary figure S13. Thus, the elevated spindle activity appears to occur during the 1-hour period that others have reported for task-dependent spindle density increases (Eschenko et al., 2006).

Novel exposure experiment 2 was designed to study the effects of novelty over days of exposure to the same environment. We hypothesized that novelty-related learning would be the highest on day 1 of exposure and gradually decline as the rats explored the same environment over subsequent days. Even though this trend is not directly observed in our results considering the first 30 minutes, is was seen in spindle density within the 30-60 minute time window, with the spindle density gradually declining over all 3 days, as shown in supplementary figure S14. Referring back to the 0-30 minute window, we did observe significant changes on day 2 of the exposure. One possible reason for not finding the highest increases on day 1 is that learning possibly occurred the most on day 2. To address this possibility, we would have to further investigate learning in these tasks using various habituation measures derived from behaviour such as exploration time, rearing, extent of spatial exploration of the contextual cues and other measures (Pisula & Modlinska, 2020).

A social experiment was conducted which was hypothesized to maximize learning by exposure to a novel partner rat. The rats were allowed to interact with each other in a novel context along with several spatial and contextual cues. Consistent with our hypothesis, the social experiment showed the strongest increases on day 1 of exposure to the novel context, indicating that a social context, in addition to contextual spatial cues, could be a major driving factor and that

just a novel environment and contextual spatial cues could be a weaker driver of spindles. In fact, the increase in spindles seen after social experience was the highest of any of the tasks/experiences we studied.

In summary, spindles could be triggered by exposure to a novel environment, probably enhanced by memory representations of the exploration space and encoding of spatial and sensory information. When the novel experience involved a social experience, we saw a very robust increase in spindles. This suggests that the complexity of the novel context or the social component of the experience could be an important driving factor. In all of the above tasks, we did not score for learning so spindles could not be specifically related to the degree of learning.

Consistent with our hypothesis and contrary to what was reported in an earlier study (Eschenko et al., 2006), exposure to a novel environment did cause increase in spindle density in post-experience sleep and our results showed that this change could be dependent on the complexity of the experience. In the Eschenko et al study (2006), no enhancement of spindle density was reported as a result of an exploration task. Rats were allowed to explore the training box with the randomly distributed reward on the floor, with no specific odors. This could be considered as an exploratory task in food-restricted rats. However, given the lack of unique sensory stimulation, free foraging of food seems a rather trivial learning task. We speculate that this exploration did not involve learning enough to enhance spindles in subsequent sleep. In contrast, in our experiments, the later two novel environments presented learning opportunities due to novel cues and, in the last experiment, another rat. The encoding of all of this information would have possibly driven learning-dependent enhancement of sleep spindles in our studies. Of course, this doesn't explain why spindles in the 0-30 minute window were stronger in our first experiment, which involve no enrichment, simply a novel context. Perhaps our novel environment, a stainless steel alley, was sufficiently different than the rats home cage to trigger enhanced information-

gathering. It is also possible that the salience of stimuli can change over time. Perhaps prior experience with a novel chamber somehow influenced the response to the novel objects on day 1 of Experiment 2 (the novel object task).

Exploratory behaviour can help us better understand processing of novelty (Pisula & Modlinska, 2020). Encounters with new environmental contexts and novel objects are crucial for animal survival in terms of new opportunities or possible threats. A novel stimulus could be classified as high-intensity stimuli (of biological significance like food or predator; usually trigger stereotypical responses called 'species-specific defense responses') or low-intensity stimuli (stimuli which are not crucial for survival and are less easily observed) (Pisula & Modlinska, 2020). In the light of this understanding, the high degree of spindle increase in the social experiment suggests social experience is a high-intensity stimulus.

Water task:

In the water task experiment, spindles could represent encoding of learning and special information associated with memory consolidation. We hypothesized that there would be maximum learning of the context, task, and swimming as well as effects of stress on the first day of training in the water task. Hence, spindle increases were expected to be greatest on day 1 and lower on the following days. The increases in spindle density would be reflective of different combinations of learning in each of the conditions. A1 (first exposure to environment A) would involve a lot of learning for the rats in terms of the task, swimming in the water pool, the concept of finding a platform to escape, the spatial organization of the room and the spatial location of the escape platform with respect to extra-maze cues in the room, to name a few. Rev A required a reversal in room A with the platform position moved to the opposite quadrant to that during pretraining days. B refers to mass training in a new room B with different extra-maze contextual

cues and involves learning a new platform location in a new context, but in a familiar task. Comparison of A vs RevA isolates the effect of learning a new platform location in an already familiar context. Comparison of A vs B isolates new contextual learning from the effect of stress and task procedural learning. We expected to see elevated spindle density after mass training in room B, which would be the actual reflection of spatial learning of platform location in a novel context independent of task learning.

The sleep spindle density analyses within the first 30-minute window after SWS onset did not directly and clearly reflect our predictions. The first observation that came to our consideration was the disruption of sleep on day 1 of the water task, followed by modest disruptions on the other days. Rats were busy grooming and drying themselves for up to about 45 minutes after the task, delaying sleep and disrupting the reported window of consolidation related changes in sleep spindles reported in earlier studies. This could have confounded our results. Detailed analyses suggested a stress-related delay of enhancements of spindles, as seen in the 30-60 min window as shown in Figure S16, which most closely correlates with our hypothesis for this experiment. Most learning dependent increases in sleep spindles occurred on day 2 and 3 of training in context A, possibly due to high acute stress on day 1. A smaller increase was seen during this later window after mass training in room B.

When a rat performed water task behavior in 2 different rooms, activation patterns of neuron populations in the CA1 region of the hippocampus and in superficial layers of posterior parietal and granular insular cortices differed between the episodes, while activation patterns of neuron populations in deep layers of these cortical regions did not differ (Burke et al., 2005). Cells in superficial neocortical layers may be more sensitive to the spatial context compared with cells in deeper layers and this different sensitivity to context may originate from differential afferent signals from the hippocampus. Activation patterns of neurons in superficial layers of the neocortex

are more sensitive to spatial context than activation patterns in deep cortical layers. The laminar differences in activation or plasticity patterns could also be an intrinsic property of the neocortex (Takehara-Nishiuchi et al., 2013). It is not known how these changes in activation patterns involved in episode differentiation can influence spindle density in the water task.

The rise in GCs concentrations in a water maze experiment was found to be positively correlated with a memory of the platform location in a subsequent test performed one day, or one week later (Sandi, Loscertales, & Guaza, 1997). Preventing glucocorticoid receptor activity during water maze learning, pharmacologically in rats (Oitzl & De Kloet, 1992) or genetically in mice (Oitzl, Reichardt, Joëls, & de Kloet, 2001) reverses the GCs-mediated performance enhancement. Thus, stress induced glucocorticoid response is found to be important in water task spatial learning. But given the results in our experiments, it is conceivable that stress apparently interferes with consolidation-related spindle activity and could delay the spindle-elevation window.

Fear task:

We had anticipated the following results: Preexposure to context A would involve contextual spatial learning for the rats in terms of the spatial organization of the room and of the chamber. Hence, we expected significant increases in spindle density to account for this spatial learning. Immediate shock in context B would involve the most significant learning in terms of association of the pre-exposure context to the shock. Hence, we expected the most significant increases in spindle density to occur for this associational and contextual fear learning. We predicted increases in spindle density in the post-test sleep session predictive of memory retrieval when tested in context A, but not context B, along with possibility of reconsolidation of the memory trace. Contextual learning in the pre-exposure context would be predictive of learning-related sleep spindles after testing in the context which the rats associated with shock.

No significant changes in spindle density were observed across the days of this experiment. Even though comparative spindle density increased during the post-exposure to context A, it failed to reach statistical significance. All the other test comparisons were non-significant. Some investigation into the analyses showed enhances in the 30-60 min window, perhaps due to a stress-related delay of reactivation. From the fear experiment, it was clear that spindles could be triggered by the recall of a fear memory, as seen in the 30-60 min window after re-exposure to the original shock context without shock, as shown in Figure S17. This is consistent with our hypothesis for this experiment.

The lack of significant increase in spindle density during the 0-30 minute window in this fear task is puzzling, especially on the shock day, when we anticipated the highest changes in sleep spindles triggered by the fear conditioning. In previous studies, learning-related increase in sigma power and spindle density specifically in the 21-24-hour period, but not at any other time after the first training session, was positively correlated with shock avoidance task performance (SM Fogel et al., 2010; S. M. Fogel et al., 2009). Since this task was similar in the kind of stressor (i.e., foot shock), the observation of delayed enhancement of spindles would need further investigation in our fear conditioning paradigm as well. Perhaps shock is such a severe stressor that the learning-dependent neuronal reactivation window gets pushed way beyond the 1 hour window in which we looked.

Switch-task:

In the task-switch experiment, it is possible that spindles could be enhanced by learning required for the switch, specifically learning that reward was associated with a new arm of the maze with a new cue. Specific switching of task-learning could cause reorganization of neuronal networks to account for having to relearn or switch cue-reward association in an already learned

task. This could be perceived as a mild stressor and could put the brain in a state of confusion related to changes of the already encoded information and the new information which could be conflicting with the current information.

No significant changes in spindle density were observed in the spindle density across the days of this experiment, between the first and second switch or even between young and aged rats. Even though comparative spindle density decreased during the post-switch sleep in young rats after the first switch, it did not reach statistical significance. The decrease in spindle density after the first task switch could be related to the effect of stress related to task switching caused by confusion. We did not observe changes in spindle density after the second switch and this was surprising. This could either be due to the fact of being familiar with the concept of switching or to lower sensitivity to the second task switch. This reduction in spindles found in young rats and not in the aged rats, could indicate an age related difference in the involvement of spindles associated with task learning and task switching. As discussed earlier in section 1.11.3.6, spindle parameters demonstrate age-related changes. Age related changes in spindles including a reduction of prefrontal fast spindles and a disruption in SO-spindle coupling could impair memory consolidation. This factor could account for the observation of no changes in spindle density in older rats.

General discussion:

Briefly, spindles could be triggered by exposure to a novel environment, probably enhanced by memory representations of the explored space and encoding of spatial and sensory information, with very robust increase in spindles when the novel experience involved a social experience. This suggests that the complexity of the novel context could be an important driving factor, with social exposure presenting the most complex type of experience. With task learning in both the water task and fear conditioning, delayed enhancement of spindles in the 30-60 minute time window, but not

in the 0-30 min after SWS onset was observed. This was contrary to our initial predictions. No significant changes in spindle density were observed across the days of the task-switch experiment, between the first and second switch or even between young and aged rats. These results can be interpreted in the context of task-related factors including stress and the nature of the task, as discussed in the following paragraphs.

There is evidence that learning-induced reactivation occurs during sleep after learning (Euston et al., 2007; Stickgold et al., 2000; Tse et al., 2007; M. A. Wilson & McNaughton, 1994) and within the first hour (Eschenko et al., 2006; Gais et al., 2002; Iotchev et al., 2017). We looked for enhancement of sleep spindles within the first hour of SWS onset after a learning episode. Despite findings on learning-related increase in spindles in rats (Binder et al., 2012; S. M. Fogel et al., 2009), and dogs (Iotchev et al., 2017), this learning induced increase was not observed in some studies (Binder, Berg, et al., 2014). Earlier studies have also shown enhancement of spindles to be more pronounced in humans than in rats (Mölle et al., 2009).

Multi-level processing of information may influence the effects of a learning episode on brain activity during subsequent sleep. In the light of complex underlying neurophysiological processes and highly task-specific nature of sleep-dependent mnemonic processes in animal and human research (S. Diekelmann & Born, 2010; Susanne Diekelmann et al., 2009), we would need to look at several factors affecting the process. The effect of task learning on spindles appears to be more complex and dependent on several factors: task, circumstances and procedures of testing, hippocampal-dependence of the task during encoding, species, differences in brain regional connectivity between species, sensory modality, task difficulty, cognitive complexity, and motor demands (Mölle et al., 2009). Our results will be discussed in the light of many other conditions that are known to influence learning-dependent sleep spindles in the process of memory consolidation: timing of memory processing, effects of sleep stages, coupling with other relevant

brain oscillations, baseline spindle activity, task complexity, task-induced stress, inter-strain differences in animal experiments and specifics of the task itself.

Timing of memory processing during sleep stages could be a factor determining the enhancement of spindles. The consolidation window could depend on time after learning. Memory processing in sleep is cyclic in nature and its mechanism might depend more on timing than on the stage of sleep. Memory reprocessing was found to be the strongest around 3 to 6 hours after learning in one study, where instead of occurring during SWS throughout the night, memory reprocessing was detected during distinct times of the night, in stage 2 and stage 4 as well as REM sleep in the second 90-min period but not in the first or third (Schönauer et al., 2017). This was supported by experiments that found critical periods during memory consolidation during which memory is sensitive to disruption (Bourtchouladze et al., 1998). Inhibiting protein synthesis 15 min and 3 hours after learning, but not 1 hour after learning, impaired hippocampal one-trial avoidance learning (Igaz, Vianna, Medina, & Izquierdo, 2002). Learning-related increase in sigma power and spindle density specifically in the 21-24-hour period, but not at any other time after the first training session, was positively correlated with avoidance task performance (SM Fogel et al., 2010; S. M. Fogel et al., 2009). Our study was restricted to the first hour of recordings, the most common window during which spindle density was seen to increase due to learning (Binder et al., 2012; Eschenko et al., 2006; Iotchev et al., 2017; Mölle et al., 2009). However, it is conceivable to consider the possibility of delayed enhancement of spindles in the task-learning experiments.

The effects of learning-related sleep spindles could also be sleep stage specific. Learning-dependent increases in spindle density have been reported during SWS (Eschenko et al., 2006) in rats, during N2 (Meier-Koll et al., 1999) in humans and during SWS (Cox et al., 2012) in humans. However, since in most animals NREMS stages are not separated from each other (Genzel et al., 2014), these conditions cannot be tested in species other than humans. In our experiments, we

looked at spindles during NREM in rats, which has been reported in earlier studies (Binder et al., 2012; Eschenko et al., 2006; Iotchev et al., 2017; Mölle et al., 2009).

Even though spindles change with experience, it might be more nuanced than that with respect to interaction with other regional and global brain oscillations. Nested spindles may be more functionally relevant than independent spindles. This is consistent with the idea that spindle coupling to the SO is important to consolidation, as discussed in the introduction section 1.11.5 (J. Kim et al., 2019; Silversmith et al., 2020). This could be an influencing factor in our results. Increased spindle coupling to the up-state of the SO (Mölle et al., 2009), could be specifically important. It may be more relevant to study changes in spindle density that are classified as nested spindles, rather than overall spindle density as we have done here. Additionally, spindles that are temporally linked to ripples may be important markers of consolidation-related brain activity (as discussed in section 1.11.7; (Zsófia Clemens et al., 2011). Hence, given the spindle-ripple temporal coupling and SO-spindle-ripple triple coupling (as discussed in section 1.11.8), these factors would be better indicators of learning and memory related activation events during SWS, and this is worth investigating in task-related experiments.

Higher baseline sleep spindle activity, recorded from a resting brain without any task learning, correlated well with increase in post-learning spindle activity and memory recall in humans (Manuel Schabus et al., 2004), but this effect was found to be reversed in a rat study of an avoidance task (SM Fogel et al., 2010), where rats in non-learning condition had higher baseline sleep spindles which was unaffected by training, while learning rats had lower baseline sleep spindles which increased with learning. Baseline sleep spindle density can be a predictor of learning and may represent consolidation of maladaptive information through homeostatic regulation. Thus, baseline spindle activity could be a factor to be taken into consideration in our task-learning experiments.

Spindle density is also influenced by task complexity and integration of information into existing schema. Spindle density increases post-learning correlated with encoding difficulty (Schmidt et al., 2006), suggesting that the role of slow spindles becomes significant only above a certain level of task complexity. Correlation was also found between spindles and integration of new learned lexical information into existing neocortical networks indicating a role for spindles in how novel information processing takes place (Tamminen et al., 2010). Similar findings were obtained during the integration of memories into existing schema (Hennies et al., 2016); related to 'rapid consolidation' of schema related memories (L. R. Squire, 2007; Tse et al., 2007).

The interpretation of the results of our task-learning experiments can be influenced by stress. There is no study reported thus far as to the effect of stress on sleep spindle density. It is possible that stress can cause changes in spindle density in either direction or it can even delay the changes in spindle density. In the light of Roozendaal's model (discussed in section 1.6 of the introduction), stress could have differential benefits on learning and memory retrieval: an enhancement of consolidation with impairment of retrieval. As evidence, stress-induced cortisol blocks retrieval processes in favor of the consolidation of current memory encoding, by strengthening of synaptic connections involved in the memory formation of the events that led to their release, and impairment of long-term memory retrieval. Stress-induced cortisol effects on memory consolidation seem to be a multi-factorial and complex process: depending on the timing, intensity, and duration of stress in relation to a memory task, it can either enhance or impair memory (discussed in section 1.6 of the introduction). Acute stressors cause long-lasting HPA alterations in animal models and affect the circadian pattern of corticosterone, with marked alteration on day 1 post-acute stressor, less altered on day 3 and a return to normality on day 7 (Belda, Fuentes, Labad, Nadal, & Armario, 2020). Disruptions in the functioning of the HPA axis and elevated cortisol levels are some of the effects of exposure to stressors in rats (Starcevic et al.,

2016). Stress has also been shown impair optimal behavior in a water foraging choice task in rats (Graham, Yoon, & Kim, 2010).

Cortisol levels are under circadian influence, with low cortisol during SWS-rich early sleep and elevated cortisol levels during REM-rich late sleep (Born & Wagner, 2004). Increasing cortisol during early SWS-rich periods of nocturnal sleep impairs hippocampus-dependent declarative memory formation, without affecting procedural memory formation. Thus, the naturally-occurring inhibition of cortisol secretion (with the CNS glucocorticoid receptors being inactive) during early SWS-rich sleep in humans seems to be critical for hippocampus-mediated consolidation of declarative memories. On the other hand, preventing the natural increase in cortisol during REMS-rich sleep appears to enhance amygdala-dependent emotional memory, suggesting that the natural increase in cortisol during late sleep may diminish emotionality of memories. Thus, physiological cortisol feedback processes during early SWS-rich and late REM-rich sleep contributes to the differential effects of these sleep phases on memory formation. These findings are consistent with the view that cortisol, via activation of limbic glucocorticoid receptors generally diminishes memory consolidation in humans (Born & Wagner, 2004). Thus, changes in cortisol during early sleep can possibly disrupt sleep, sleep stages and sleep-dependent memory consolidation processes, partly explaining the surprising lack of learning-related spindle density increases in our water maze and fear conditioning experiments.

By affecting cortisol in both humans and rodent models, stress has been shown to affect several cognitive processes (Lynch, 2004; McEwen & Sapolsky, 1995), including memory (Diamond & Rose, 1994; Dominique, Roozendaal, & McGaugh, 1998; Joëls, Fernandez, & Roozendaal, 2011; Joëls et al., 2006; J. J. Kim & Diamond, 2002; J. J. Kim, Lee, Han, & Packard, 2001; Luethi, Meier, & Sandi, 2009; Lupien et al., 1997; Newcomer, Craft, Hershey, Askins, & Bardgett, 1994; Park, Zoladz, Conrad, Fleshner, & Diamond, 2008; Schwabe et al., 2012) and

decision-making (Graham et al., 2010; Koot et al., 2014; Starcke & Brand, 2012). Since our tasks were designed to measure both memory and decision making, the task-induced stress could be a confounding factor in our measurements of spindle density as a measure of task learning.

Corticosterone actions might be experience-dependent with regard to stimulus intensity, like water temperatures. Rats trained at 19°C showing a quicker rate of acquisition and better long-term retention than rats trained at 25°C, and this correlated with higher post-training corticosterone levels in the 19°C group than in the 25°C group (Sandi et al., 1997). Noradrenaline can enhance memories if increased during the consolidation period (A. K. Anderson, Wais, & Gabrieli, 2006; McGaugh, 2006). In addition, glucocorticoid effects on memory are not restricted to influences on consolidation, but also learning (Dominique et al., 1998; McGaugh & Roozendaal, 2002). Glucocorticoid administration shortly before retention testing can temporarily impair retrieval of memory for spatial/contextual information, an effect which dissipates within several hours after stress exposure or hormone injection (Dominique et al., 1998).

Furthermore, stress-associated neurobiological changes have been identified in hippocampus and medial prefrontal cortex, involved in different memory functions (Arnsten, 2009; J. J. Kim & Yoon, 1998). The effects on these critical memory-related brain regions could indirectly affect their electrophysiological activity including SWA, spindles and ripples. Additionally, the effects of stress on subsequent LTP and LTD appear to be mediated through the activation of the NMDA subtype of glutamate receptors (J. J. Kim, Foy, & Thompson, 1996) and since sleep spindles have been shown to mediate learning and memory through similar mechanisms (Rosanova & Ulrich, 2005; Steriade, 1999; Werk et al., 2005) (also reviewed in section 1.11.3.8 Spindles in network plasticity/LTP mechanisms), stress could affect information processing during sleep spindles in these mechanisms of memory encoding.

Experimental stress has been shown to decrease both SWS and REMS, interfere with sleep efficiency (SE) and lead to an increase in awakenings (E.-J. Kim & Dimsdale, 2007). The effects of stress and cortisol on sleep spindle density have not been studied to our knowledge, but this cannot be ruled out as a possible effect of acute stressors, given the reported findings of stress-related changes in several sleep parameters. It is reasonable to speculate that stress can affect sleep-dependent memory consolidation processes by affecting sleep electrophysiological phenomenon and could at least partly explain our results of no changes in spindle density within the first 30 mins of SWS-onset in experiments with particularly stressful tasks. In that direction of thought, we did find delayed onset of sleep and SWS in rats at least in the water task. Further, we observed variable times of sleep onset in the fear task experiment. This also raises the question about the suitability of water maze (which is an aversively motivated experience) and fear conditioning task for experiments on learning-related spindle enhancement. It is conceivable to consider other alternative tasks like Barnes maze (Pitts, 2018), which is a well-established alternative to the Morris Water task and has the advantage of not having potentially confounding influence of swimming behavior and the stress induced thereof.

Thus, in the light of direct effects of stress on cortisol and indirect effects on the brain at a cellular level, changes in spindle density that we observed in the water task and the fear task could be affected by stress-related cortisol or could delay it. It is conceivable that stress effects could follow an inverted U-shaped curve in their influence on learning-dependent plasticity mechanisms (Pavlides, Watanabe, & McEwen, 1993; Sandi et al., 1997), with the low stress of a novel environment just not enough to bring about changes in sleep spindles, moderate stress optimal to drive consolidation as in the social experiment and severe acute stress in the water and fear task disrupting the sleep parameters involved in encoding, learning and memory consolidation.

It is important to note, however, that given the observation that rats demonstrated learning and subsequent memory retrieval in both the Water task (Suppl Fig S11) and the fear conditioning experiment (Suppl Fig S12), stress may not have affected learning and consolidation. Enhancement of spindles was observed in the 30–60-minute window after SWS onset in the water task (Suppl Fig S16) and the fear conditioning task (Suppl Fig S17), consistent with our hypothesis. Thus, the most parsimonious explanation is that stress delayed the window for spindle-mediated memory consolidation.

Additionally, we would need to consider differences in the specifics of the task itself to investigate the effects it could have on post-task sleep spindle density, the measure of learning that we used. The earlier study (Eschenko et al., 2006) investigated changes in spindle density in an odor-reward association task in food-deprived animals. On one hand, food deprivation has been known to cause changes in sleep measures including SWS (B. L. Jacobs & McGinty, 1971). It is conceivable to think that the changes observed in the Eschenko study were influenced by food deprivation. On the other hand, food, being a high-intensity stimulus to food deprived animals (Pisula & Modlinska, 2020), could have could have facilitated the learning of food-location associations. In the light of this understanding, water task escape platform location and fear conditioning contextual encoding would very likely be considered high-intensity stimuli, important for survival and adaptive learning. We would speculate that if measures of spindle density are involved with consolidation of these memories, we would expect significant increases in spindle density after learning in these tasks; however, stress effects, as discussed in the preceding paragraphs, could also have influenced our results. In our tasks, stress is induced, while in the Eschenko task, stress is alleviated as they get food rewards. Hence the choice of task selected to study the effects of learning on sleep spindles may be an important consideration in the light of several factors that could be at play.

A novel stimulus could be classified as a high-intensity stimulus (of biological significance like food or predator; usually trigger stereotypical responses called 'species-specific defense responses') or low-intensity stimulus (stimuli which are not crucial for survival and are less easily observed) (Pisula & Modlinska, 2020). The social experiment may have represented a high-intensity stimulus, important for survival, and thus leading to the strongest learning and highest levels of consolidation-related brain activity.

In addition to the above discussed factors influencing task learning, we would need to consider differences in the strain of rats used in studies. Most of the earlier studies used Sprague-Dawley rats (Eschenko et al., 2006; SM Fogel et al., 2010; S. M. Fogel et al., 2009), while we used a hybrid Fisher-Brown Norway rats. Inter-strain differences in sleep measures and the specific electrophysiological measures may be important considerations, given possible differences in the baseline measures. The relationship between learning and spindle rate may also have a different slope in different rat strains.

No study has investigated how learning affects the duration of spindles. We did some preliminary investigations into the duration of spindles by classifying spindles into shorter spindles (200-500ms) or longer spindles (>500ms). We compared these with all the detected spindles between 200ms-2 sec duration. The changes in these categories of spindles were not uniform and it is possible that different kinds of learning, encoding and memories could be facilitated by different classes of spindles. Generally, we found more robust effects with the parameters chosen for analyses, but this might be an avenue for further exploration.

One of the limitations associated with this investigation that we would like to acknowledge is that the increase in family-wise error rate across the reported statistical analysis was not controlled for. Overall, we consider this research relatively preliminary with a need for replication with more rats.

Chapter 3 Effects of sinusoidal electrical stimulation on endogenous brain oscillations

3.1 Background information

Brain stimulation techniques have shown promising results in enhancing learning and memory as reported in several human and animal studies (reviewed in detail in earlier sections 1.12 and 1.13). Several stimulation techniques including transcranial electrical stimulation techniques like tDCs, SO-tDCs, tACs, TMS, TMR sensory stimulation, to name a few, have been demonstrated to have potential to improve memory in animals and health humans. These techniques may be of special importance in older adults and people with dementia. Compared to the human literature, very few studies in animals have demonstrated the efficacy of tDCs and SO-tDCs in memory improvement.

Animal models provide powerful tools in neuroscience research, especially in invasive procedures targeting specific brain regions. They have been used to investigate the behavioural and cognitive effects of tDCs and to identify the mechanisms by which tDCs modulates endogenous brain oscillation and neural network function underlying cognitive enhancements (Bennabi et al., 2014).

The first few studies in rats investigated the efficacy, mechanism, safety, and spatial extent of tDCs stimulation (Bennabi et al., 2014). tDCs was used in rats to evaluate the safety limits of cathodal stimulations (Liebetanz et al., 2009). Rats received single cathodal stimulations at 1-1000 uA for up to 270 min through an epicranial (situated on the cranium or skull) electrode and histological evaluation was performed 48 h later in order to calculate threshold estimate from volumes of DC-induced lesions. Current density of 142.9 A/ m^2 for durations greater than 10 min caused brain lesions with a linear increase in lesion size for current densities between 142.9 and

285.7 A/m^2. Brains stimulated below this current density threshold, including those stimulated over 5 consecutive days, were morphologically intact. Thus, current density is an important safety factor to be considered so that tDCs facilitates cognitive processes through neuronal activation without destroying local neurons.

Studies have also investigated the possible mechanisms of action of tDCs at a network level through the entrainment of neurons and endogenous oscillations and at a cellular level through cortical excitability and neuronal activation. Many of the studies can answer two very basic questions directly relevant in the context of our studies: Does DC stimulation change neuron activity? How long do stimulation effects last?

tDCs can mechanistically induce cortical excitability and neuronal activation. In order to map brain activation patterns using fMRI after tDCs in humans, a 400-μA anodal tDCs was applied over the frontal cortex (Takano et al., 2011). Significant increases of signal intensities were observed in the frontal cortex and nucleus accumbens, suggesting that the stimulation induces neuronal activation both in immediate cortical regions and functionally connected subcortical areas. Direct current stimulation has been demonstrated to increase cortical excitability during and after tDCs (Lauro et al., 2014) and increase neuronal activation in humans (Takano et al., 2011). DC stimulation has also been demonstrated to modulate transmembrane neuronal potential, level of excitability, firing rate of neurons and cortical function beyond the stimulation period (T. Wagner, Valero-Cabre, & Pascual-Leone, 2007).

While most of the effects of tDCs during stimulation seem to involve cortical excitability changes at the level of the membrane potential, the aftereffects of tDCs lasting beyond the duration of stimulation appear to be at the synaptic level (Stagg & Nitsche, 2011). At the cellular and molecular level, studies investigating mechanism of tDCs in rats have shown that anodal tDCs can increase calcium (N. Islam, Aftabuddin, Moriwaki, Hattori, & Hori, 1995), c-Fos expression

mediated by NMDA receptors (N. Islam, Moriwaki, et al., 1995), co-distribution of transduction proteins, protein kinase c gamma (PKC gamma), and c-fos protein in specific neurons (N. Islam, Moriwaki, A., & Hori, Y, 1995). Repeated anodal stimulation with 3 uA for 30 min caused calcium accumulation in the cerebral cortex, hippocampus and thalamus, with the degree and extent of accumulation greater in the hemisphere ipsilateral to the polarization than in the contralateral hemisphere. Calcium accumulation was detected after 24 hrs and remained constant up to 72 hrs after the last stimulation, suggesting that a long-lasting modulation of calcium levels is probably involved in the cortical plasticity changes as a result of anodal polarization (N. Islam, Aftabuddin, et al., 1995). Anodal DC to the surface of the unilateral sensorimotor cortex induced a massive increase in c-fos protein in neurons in widespread brain regions including cingulate, piriform, fronto-parietal cortices, and hippocampus ipsilateral to the polarization with effects being dependent on the duration and intensity of currents applied (N. Islam, Moriwaki, et al., 1995). The time-dependent induction of c-Fos was found to be maximal at 1 hr becoming weaker by 6 hrs, and eventually returned to baseline within 24 h following polarization. The rapid and transient c-fos activation was found to be mediated by NMDA receptors. Anodal direct current of 3 uA for 30 min to the surface of the left sensorimotor cortex resulted in increased co-distribution of both transduction proteins PKC gamma and c-fos protein ipsilateral to the polarization in cortical pyramidal cells, indicating that stimulation increased molecular mechanisms associated with cortical plasticity (N. Islam, Moriwaki, A., & Hori, Y, 1995).

In addition to the above studies directly measuring the time course of effects, changes in the expression of genes involved in synaptic plasticity are indirect measurements that allude to the effects lasting beyond the duration of stimulation. Anodal direct current stimulation has been shown to modify hippocampal synaptic plasticity-related proteins as measured by proteomic analysis (S. H. Jung et al., 2019), expression of immediate early genes *c-fos* and *zif268* in the

hippocampus (Ranieri et al., 2012), modification of synapses for associative learning *in vivo* (Márquez-Ruiz et al., 2012), LTP-like effects in cerebral cortex that are dependent on gene expression mediated by NMDA receptor activation and calcium (N. Islam, Aftabuddin, et al., 1995; N. Islam, Moriwaki, et al., 1995; Nitsche et al., 2008; Stagg & Nitsche, 2011), enhancement of BDNF-dependent synaptic plasticity (Fritsch et al., 2010; Podda et al., 2016; Rohan et al., 2015), LTP in CA1 neurons of rat hippocampus (Ranieri et al., 2012), modification of AMPA receptor phosphorylation and translocation in the rat hippocampus (Stafford, Brownlow, Qualley, & Jankord, 2018), modification the expression of genes related to serotonergic, adrenergic, dopaminergic, GABAergic, and glutamatergic signaling in the rat cortical transcriptome (Holmes et al., 2016), and regulation of neurotransmitter signaling of glutamatergic, GABAergic and cholinergic pathways (Giordano et al., 2017).

Several studies have investigated the mechanisms of transcranial direct current stimulation at a cellular level (S. H. Jung et al., 2019), but studies investigating the effects of the stimulation on endogenous brain oscillations are sparse and the findings are inconsistent. Entrainment of SO-frequency oscillations during the stimulation have been reported as a result of SO-tDCs application in rat studies (Fröhlich & McCormick, 2010; Ozen et al., 2010). Weak sinusoidal tDCs at SO frequency was found to enhance and entrain spontaneous SO-like physiological endogenous neocortical network activity patterns in ferret brains slice preparations. Entrainment of neocortical SO with an amplitude threshold within the range of *in vivo* endogenous field strengths was observed (Fröhlich & McCormick, 2010). In vivo extracellular and intracellular recordings from the neocortex and hippocampus of anesthetized rats and extracellular recordings in behaving rats were performed to investigate the effects of electric fields generated by sinusoidal patterns at slow frequency (0.8, 1.25 or 1.7 Hz) via electrodes placed on the surface of the skull or the dura (Ozen et al., 2010). Cortical unit activity during the TES revealed that stimulation reliably entrained

neurons in widespread cortical areas, including the hippocampus, and the percentage of TES phase-locked neurons increased with stimulus intensity and depended on the behavioral state of the animal. In this case, stimulation was most effective if the animal was in SWS. Intracellular recordings showed that both spiking and subthreshold activity were under the influence of the oscillatory stimulation and network activity.

Given the effects of tDCs at the cellular and network level, it is conceivable that they would have functional relevance in cognitive processing. Initial observations of tDCs-mediated memory enhancement were found in human studies and later were investigated in rodents. Repeated anodal tDCs (0.26 mA; over 30 min) bilaterally at fronto-cortical electrode sites during a retention period rich in SWS, enhanced retention of declarative paired associative word memory specifically and showed no effects on nondeclarative mirror tracing memory skills (Lisa Marshall et al., 2004). When applied during the wake retention interval, tDCs did not affect declarative and procedural memory. In addition, tDCs influenced sleep parameters and electrophysiological parameters. Anodal tDCs resulted in deeper sleep, with more time spent in SWS stages 3 and 4, toward the end of the stimulation period which continued during the subsequent 15 min period. Power spectra were also altered for periods of SWS and stage 2 sleep during the 30 min interval of stimulation. Stimulation-induced reductions of power in the lower β frequency range (15–20 Hz) during periods of stage 2 sleep and suppression of frequencies around the θ and lower α range (4–10 Hz) during periods of SWS were observed. Most relevantly, slow oscillatory and δ frequencies <3 Hz power was consistently increased during the 15-sec periods of acute anodal stimulation, compared with intervals of discontinued stimulation. Power enhancement in ~2 Hz frequency band in the frontal EEG and SO ~1 Hz frequency in parietal EEG were recorded. This observation showed promising declarative memory effects of tDCs mediated by enhancement of SWS-specific memory-related oscillations. Along with improvements in verbal memory by the specific application of SO-tDCs

(0.75Hz) and not of 5 Hz, during sleep, stimulation simultaneously and distinctly enhanced EEG power within the SO band (0.5–1.0 Hz) and slow spindle frequency range (8–12 Hz, peaking at 10.5 Hz) (Lisa Marshall et al., 2006). In contrast, 5Hz stimulation decreased SO and left declarative memory unchanged, indicating that the memory enhancing effects were restricted to SWA-related oscillations during SWS.

Very few animal studies have investigated the effect of tDCs on learning and memory processes (Bennabi et al., 2014; Campos-Beltrán & Marshall, 2017). Following up on their success in human studies showing memory improvement with tDCs (Lisa Marshall et al., 2004) and SO-tDCs (Lisa Marshall et al., 2006), the Marshall lab set out to establish a rat model of SO-tDCs for memory enhancement. They reported that transcranial SO stimulation during NREMS enhanced performance in rats (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014) and this effect was speculated to result from stimulation-mediated modulation of the endogenous SO through an interaction of tDCs with hippocampo-neocortical rhythms. Preference for the displaced object in the memory task was significantly greater than chance after a 24 h retention interval only in rats that received SO-tDCs, demonstrating that memory effects of SO-tDCs achieved in humans could be successfully transferred to a rodent model (Binder, Berg, et al., 2014). The stimulation protocol was as follows: trapezoid shaped current fluctuated between 0 and 9 μA (the frequency range of the sleep SO, 1.33–1.5 Hz) was applied bilaterally over the PFC with the oscillatory currents applied to both hemispheres in phase-synchrony. Stimulation started after the first occurrence of 60 sec of stable NREMS and lasted for 30 sec, followed by a stimulation free interval of 30 sec for as long as the rat remained in SWS, with stimulation discontinued if any changes in the sleep stage were detected or the animal awakened. Animals received, in total, 20 stimulations during NREMS according to the parameters described. Frequency overlap of stimulation and endogenous SO activity precluded analysis in the SO frequency range during acute SO-tDCs, but selective analyses

of power within the SO-tDCs frequency range showed that endogenous SO power (0.8–2 Hz) tended to be enhanced within the first 10 s of the stimulation-free interval right after cessation of SO-tDCs in the stimulation condition as compared to non-stimulation condition. Post-stimulatory enhancement of SO power was shown to be important in memory consolidation as animals that failed to show this effect also performed poorly in the memory test. Using a sophisticated mathematical model, they also estimated that electric field induced by the 9μA current was most pronounced directly beneath the electrodes in the PFC in rats. In another rat study (Binder, Rawohl, et al., 2014), SO-tDCs was shown to modulate both behavior and endogenous EEG activity similar to prior findings in human subjects (Lisa Marshall et al., 2006). Reduction in reference memory errors in radial maze task was observed across the 12-day experimental period of SO-tDCs application, evident as less re-entries into baited arms (Binder, Rawohl, et al., 2014). This suggested that a component of working memory was affected by stimulation. The stimulation protocol was as follows: sinusoidal constant current fluctuating between 0 and 5.6 μA at a frequency in the range of sleep SO (1.5 Hz), phase-synchronized bilaterally over the PFC. Stimulation started after the first occurrence of 60 sec of stable NREMS and lasted for 30 sec, followed by a stimulation free interval of 30 sec, alternating for as long as the rat remained in SWS, with stimulation discontinuation any changes in the sleep stage were detected or the animal awakened. Animals received stimulations during NREMS for 1 hr, starting with the time of the first stimulation, according to the parameters described. The SO-tDCs increased post-stimulation upper delta activity over all experimental days, but this increase was observed at the beginning of the 2-hr recording session following the first stimulation of the day, indicating that a temporal component within the recording session was involved in the task enhancement effects.

In addition to consolidation effects of tDCs, the stimulation could also facilitate encoding, when applied before a learning task, most likely through increased activity/plasticity, and not by

directly facilitating consolidation. tDCs was applied on the frontal cortex through skull electrodes, targeting the PFC at 200 μA for 30 min/day for 3 days before performance of a task involving visuospatial working memory and skill learning, called the Allothetic Place Avoidance Alternation Task (APAAT) task (Dockery et al., 2011). The stimulation had no measurable short-term effect on immediate working memory-related place avoidance learning, but the rats previously stimulated with cathodal (but not anodal) tDCs showed significantly more efficient place avoidance and skill retention compared to controls on day 21. Thus, frontal tDC stimulation could have long-term benefits of diminished excitability, modulating underlying neural network function for remote memory retrieval without affecting immediate working memory.

tDCs induces changes in neuronal membrane potentials in a polarity-dependent manner. tDCs applied to somatosensory cortex of behaving rabbits showed that the acquisition of classical eyeblink conditioning is either potentiated or depressed depending on stimulation polarity, anodal or cathodal tDCs respectively (Márquez-Ruiz et al., 2012). Thus, tDCs can modulate sensory perception and influence other memory types, such as associative learning processes. tDCs was demonstrated to modify thalamocortical synapses at presynaptic sites, as blocking the activation of adenosine A1 receptors prevented the LTD evoked in the somatosensory cortex after cathodal tDCs.

Repeated anodal tDCs at 0.2 mA for 20 min twice per day for 5 consecutive days over the left frontal cortex of the mouse lead to improvements in long-term spatial memory, as tested in Morris water maze, and working memory, as tested in an object recognition task (Pedron, Monnin, Haffen, Sechter, & Van Waes, 2014). The same stimulation did not affect locomotor activity and anxiety-related behaviors. In addition, abnormal behaviors associated with chronic nicotine exposure, such as depression-like behavior and increase in nicotine-induced place preference were found to be normalized by repeated tDCs. Thus, repeated tDCs in an animal model demonstrates

promise as a clinical tool to investigate mechanisms underlying the effects of tDCs on addiction and other psychiatric disorders. Effects of tDCs have also been investigated in alleviating cognitive memory dysfunctions. tDCs has also been shown to facilitate recovery from cognitive impairments induced by cerebral ischemic stroke in rats (Yoon, Oh, & Kim, 2012). DC of 200 μA for 20 min was applied once a day for 5 consecutive days. Both early (1 day) and late (1 week after ischemic injury) treatment had a beneficial effect on spatial memory evaluated in the Barnes maze test, without exacerbating ischemic volume and this effect began to appear 2 weeks after the stimulations and was maximal after 4 weeks. Cathodal tDCs was applied for 30 min per day for 2 weeks at 200 μA following pilocarpine-induced status epilepticus in immature rats to evaluate its effect on seizures and spatial memory deficits (Kamida et al., 2011). Repeated cathodal tDCs reduced seizures in the rat model of induced status epilepticus and reduced spatial memory impairments. In addition, the stimulation had detectable cellular effects including reduction of status epilepticus-induced hippocampal cell loss, and supragranular and CA3 mossy fiber sprouting. tDCs has yet to be tested for enhancing cognition in animal models of neurocognitive and neuropsychiatric disorders (Bennabi et al., 2014).

3.2 Study Objectives

Cortical slow-oscillatory stimulation is thought to contribute to memory consolidation by modulation of SWA. As discussed above, human and rodent studies have shown memory enhancing effects of tDCs and SO-tDCs correlating with the modulation of SWS-specific memory-related oscillations. Transcranial electrical stimulation across the frontal cortex in both humans and rats has been shown to enhance both SWA and subsequently tested memory performance (Binder, Berg, et al., 2014; S. Binder, J. Rawohl, J. Born, & L. Marshall, 2014; Marshall, Helgadottir, Molle,

& Born, 2006; Marshall, Molle, Hallschmid, & Born, 2004). Further, in aging individuals, subjects with greater mPFC atrophy had less SWA and reduced memory performance (Mander et al., 2013). This could support the hypothesis that frontal cortex could contribute to driving SWA in the rest of the cortex which in turn enhances memory consolidation. The present study sought to test this by electrically stimulating the frontal cortex during SWS with slow oscillating direct current stimulation to investigate if the stimulation leads to any changes in the power of endogenous brain activity lasting beyond the duration of the stimulation itself. We wanted to investigate the effects of different stimulation parameters on the modulation of different frequency bands, especially power in the slow-wave frequency range. In addition, we sought to assess the effect of stimulation on spindle density, as this seems to correlate with memory consolidation. Both are important markers of SWS in rats. Do the effects of stimulation on delta power and spindle density persist during the post-stimulation period? How is the strength of this effect influenced by the frequency and amplitude of the sinusoidal stimulation?

3.3 Methods and study design

Animals and handling:

The data was collected from 7 male Fisher/Brown Norway hybrid rats, which were bred in-house within the University of Lethbridge animal facility. The rats were kept on a 12 h light / 12 h dark cycle with lights ON from 0730-1930 and lights off from 1930-0730. Water and food were available *ad libitum in their standard* clear plastic cages. Rats were housed in pairs before surgery and handled daily after transfer to the experimental protocol. In addition, before starting the experiments, animals were handled daily for 10 min in the preceding week to accustom the animals to the experimenter and recording conditions. This presumably reduces stress during behavioral

testing. The rats were housed individually after electrode implantation surgery, with *ad libitum* food and water access while being kept on the same 12 h light-dark cycle. They continued to be handled at regular intervals between experiments. They were also habituated regularly (as described in more detail below under the section Electrophysiological Sleep Recordings) to the recording chamber, recording room, recording conditions and procedures for a week before the first experiment and then for a few days before each experiment. Behavioural experiments and recording sessions took place during the light phase. All the animal protocols were approved by the Animal Welfare Committee of the University of Lethbridge. All experimental procedures were performed in accordance with the Canadian Council of Animal Care and the Animal Welfare Committee guidelines at the University of Lethbridge (national and institutional regulations respectively for care and use of laboratory animals).

Electrodes

LFP electrodes: Teflon-insulated annealed (AN) stainless steel wire 0.002 inch diameter (A-M systems, Sequim, WA, USA; 793600) was used for all cortical and hippocampal LFP recording electrodes, which were manufactured in-house. This was made into a twisted pair of electrodes with the help of a magnetic rotator and the exposed ends stripped of insulation. The ends were offset by 0.5 mm to act as a bipolar electrode pair.

Ground screw electrodes: The base of a torx 000-120 screw head was soldered to a 0.005 inch stainless steel insulated wire half-hardened (HH) (A-M systems, Sequim, WA, USA; 791500) as customized ground recording screw electrodes.

Stimulation screw electrodes: The base of the torx 000-120 screw head was soldered to a 0.005 inch wire stainless steel insulated wire (HH) or 0.003 inch stainless steel insulated wire (HH)

(A-M systems, Sequim, WA, USA; 791500) as customized stimulation screw electrodes for both source and return.

EMG electrodes: The methodology for implanting EMG electrodes was devised specifically for our surgical and recording purposes. A 20 cm multistrand stainless steel wire (Cooner wire Inc, Chatsworth, CA, USA; AS632) was knotted about 3-5 times in the center. At a distance of about 1 cm from the knot on one side, the insulation was carefully removed for around 1mm length of the wire, for muscle electrical activity recording. The electrode wire was then doubled over and twisted for 25-30 rotations using a magnetic rotator by holding the other end with a clamp. The wires were then briefly heated using a heat gun so that the insulation would stick together and prevent the electrode from unwinding. Insulation was also removed from the free ends. These ends were inserted into a 21G 2-inch, or 22G 1.5-inch needle tip cut off from the bases, soldered and secured. This needle tip was slightly bent to aid in insertion and threading of the wire from the neck to the skull (as described below).

Electrode implantation surgical procedure and recovery:

Fisher 344/Brown Norway hybrid rats were between 4-8 months old and weighed between 350-450g at the time of surgery. All stereotaxic electrode implantations were performed under isoflurane anesthesia. Hair on the head (for stereotaxic electrode implantation) and about an inch bilaterally on the neck (for EMG electrode insertion) was shaved using standard clippers. Animals were anesthetized with isoflurane (1-1.5 % by volume at oxygen flow rate of 1.5 L/min). The animal was secured in the stereotaxic apparatus with ear bars. Anaesthesia was maintained with isofluorane delivered via a stereotaxic-mounted nosepiece. A nose cone in the front and ear bars on the sides restrained the head. Bregma and Lambda were levelled by adjusting the height of the stereotaxic nosepiece. A thermostat-controlled heating pad regulated the animals body temperature

during surgery. A rectal temperature probe, heart rate monitor and oximeter were connected to the animal. The eyes were covered with ophthalmic ointment. A thick piece of aluminum foil cut in the shape of eye shades was secured on the eyes to protect them from the lights used to illuminate the surgical field. Throughout the surgical procedure, fluid support was provided with subcutaneous (SC) pre-warmed sterile isotonic saline (0.9% NaCl) at a volume of 5 ml rats at regular intervals. After sterilizing the surgical area with chlorohexidine and alcohol, lidocaine (0.1 ml of 2 %: 20mg/mL) with Epinephrine was injected SC along the midline of the skull under the planned incision site as a local anesthetic. The epinephrine is a vasoconstrictor and so helps to reduce bleeding, while lidocaine is an analgesic. The incision was then made along the midline, extending from roughly 5 mm in front of bregma to the occipital ridge at the back of the skull. The exposed skull was cleared of fascia. Holes were drilled at the appropriate stereotaxic locations with respect to bregma for the insertion of ground screws, anchor screws, as well as stimulating and recording electrodes.

Recording electrodes were surgically implanted bilaterally in the motor cortex, hippocampus, and neck for continuous chronic recordings of LFP and EMG, respectively. More specifically, four bipolar LFP electrodes, two EMG electrodes and two ground electrodes were implanted at appropriate locations according to coordinates obtained from the rat stereotaxic atlas (Paxinos & Watson, 2006). Bipolar twisted-pair LFP recording electrodes were implanted bilaterally above the motor cortex (anterior 1.6 mm from bregma, lateral +/- 1.5 mm for bilateral implantation, at a depth of 1.8 mm to 2 mm from skull surface) and hippocampus (posterior 3.5 or 4 mm from bregma, lateral +/- 2.5 mm for bilateral implantation at a depth of 2.5 mm from surface of dura or 3.5mm from skull surface) under microscopic guidance. The bilateral positioning of stimulation screw electrodes was as follows: anterior 3.0 mm from bregma, lateral +/- 2 mm with respective bilateral cerebellar return electrodes.

Two EMG electrodes were implanted bilaterally in the neck muscle using a specialized method. An incision was made in the skin with a scalpel on either side of the back of the neck about an inch from the back of the skull incision. The curved needle tip attached to the customized EMG electrode was inserted through the skin incision and advanced until the tip protruded through the skull-top incision. The knot at the end of the EMG electrode helped secure the electrode in the neck muscle and the skin incision was closed with tissue glue. This ensured that the exposed part of the electrode was secure in the neck muscle to record myocardial activity. Once the EMG electrode projected out of the head region, the needle tip was slipped off leaving the exposed end of the EMG electrode to be eventually connected to the vias of the custom-designed printed circuit board (PCB) connected to a Mill-max connector for recording purposes.

Four anchor screws were placed at the edges of the surgical field as shown in the figure. The electrodes were carefully connected into the vias of the custom-designed printed circuit board by putting a drop of solder into each via with the inserted electrode tip. Vias have respective connections through wires to the pads which were soldered to the Mill-Max Mfg. Corp. connectors. The connector was secured to the skull by dental acrylic, with a visible bump moulded around the circumference, to provide purchase for the tissue tape used to secure the recording headstage (Neuralynx HS27) during recordings.

Following surgery, the animals were housed individually with food and water available ad libitum. They were handled daily during the following recovery week for post-operative monitoring and injections, body weight checkup, and familiarization with experimenter. Antibiotic Baytril (5mg/ml 10.0 mg/kg SC) was administered every 24 hours for 5 days and analgesic Meloxicam (Metacam) (5mg/mL 5-10 mg/Kg SC) was administered every 24 hours for 3 days during post-operative care to control inflammation, pain, and infections. The rats were allowed to recover for 7 days from the surgical procedure before any experiments and procedures.

Electrophysiological sleep recordings/Data acquisition:

After recovering from surgery, the animals were placed in a recording chamber located in a quiet, dimly lit room daily for about 3 hours for 3 days. The purpose was to habituate them to the experimenter and recording conditions. They were also habituated to the process of attaching recording cables to the skull connector for an additional week before the first experiment. On experiment days, rats were taken to the recording room, connected to the recording system, and placed in the recording chamber where they rested. The vivarium maintained a 12-h light cycle (7:30 a.m. on/7:30 p.m. off) and the animals were tested in the same order every day such that recordings on different days occurred at approximately the same time (±1 h).

Recording was done with a Neuralynx data acquisition system (Digital Lynx SX, Neuralynx, Boseman, Montana). The electrophysiological recordings were acquired in a dimly lit room as the rats rested in a glass box with metal rims (16-inch cube box). The movements of the rats were tracked by a standard video camera mounted to the ceiling directly above the recording chamber connected to the data acquisition system. The rat was connected to the amplifier by a cable allowing free movement within the box. LFP and EMG was recorded with a unity-gain headstage, HS-27 (Neuralynx, Bozeman, MT) and multi-wire tether cables (TETH-HS-27-3M, Neuralynx, Bozeman, MT) and a 128 channel commutator (PSR-36-4, Neuralynx, Bozeman, MT). A counter-weight system was customized to reduce the weight of the headstage and tethers for the rats to relax and rest. The counter-weight was attached to the tether cable above the headstage using fishing line running through a plastic pulley attached to the bottom of the commutator. Data was recorded using Digital Lynx neurophysiology data acquisition system and Cheetah data acquisition software. The signals were sampled at 2 kHz. All signals were amplified, digitized and bandpass filtered between 0.01 and 1000 Hz via a Digital Lynx system (Neuralynx, Bozeman, MT) and

referenced to a ground screw electrode. Two standard computers running Microsoft Windows 7 were used: one for running the Cheetah data acquisition software and one running the MATLAB (MathWorks, Natick, Massachusetts) software for real-time detection of sleep stages.

Electrophysiological LFP and EMG recordings were used to score sleep-wake and different sleep stages to analyze specific brain oscillations, as described elsewhere (M. J. Eckert et al., 2021). Briefly, the awake state was identified by the presence of low-amplitude fast activity in the EMG associated with muscle tension. SWS was identified by continuous high-amplitude slow activity and high sigma power (power overlapping in the spindle frequency band) in the cortical electrode combined with low EMG activity. REMS was characterized by strong theta activity in the hippocampus and an absence of EMG high frequency activity. A real-time sleep state detector was written in MATLAB (MathWorks, Natick, Massachusetts). More specifically, a 3-second buffer of LFP recording (Local Field Potential; updated every 500 ms) was used to calculate the Fourier transform of the hippocampal and EMG signals. EMG power spectra was used for automated scoring of sleep-wake episodes. For the EMG signal, the ratio of power in the high (100-300) to low (10-20) frequencies was calculated. The EMG signal was categorized automatically into wake and rest epochs using a manually set threshold. The ratio of cortical delta (1-4 Hz) to hippocampal theta (6-10 Hz) power was calculated. Power spectra of delta (0-4 Hz) and theta (5-10 Hz) frequency bands from the LFP traces were used to further classify rest epochs as SWS or REM. The hippocampal delta/theta ratio was divided by the EMG power (delta power / theta power / EMG power) to obtain a real-time SWS score. Higher values on this metric were indicative of SWS while lower values indicated either REM or wakefulness. For each animal, the threshold value, above which the SWS score was classified as SWS, was determined during a habituation session prior to the start of the experiment. Periods of SWS were determined by finding peaks greater than 2 SD from the mean in the power signal of the SWS power from the previously detected sleep

periods, to take into account differences in LFP signal amplitude from one recording session to the next. This was also visually verified with Neuraview (Neuralynx, Bozeman, MT) software to validate the automated detection.

Experiments used to investigate the effects of stimulation were based on a rationale that we would need to compare the stimulation session with a sham session recorded earlier on the same day. Each session of a chosen stimulation parameter was preceded by a sham stimulation session for comparison, during which the stimulation times were marked by our automated SWS detector without the actual deliverance of stimulation. The approach helped to study the changes in stimulation-related changes in oscillatory activity during stimulation session compared to baseline sham stimulation controls. Accordingly, each recording session consisted of two sessions: a baseline sham stimulation recording and a stimulation recording session. The procedure was repeated for all the chosen stimulation parameters. The stimulation parameters selected were used to test frequency-dependence and amplitude-dependence of effects.

Bilaterally synchronized stimulation was delivered using two separate circuits, one for the right side of the brain, another for the left, each with a separate stimulus isolator (A365, World Precision Instruments, Sarasota, FL) under the control of an Arduino microprocessor (Arduino Mega 2560, https://www.arduino.cc/) with the code defining the stimulation amplitude and frequency. The output of the SIU was unipolar varying between 0 and the chosen amplitude. We also used a custom made sine wave generator with a well-known low pass filter (Chebyshev low pass filter) with the following components in the circuit: Resistor: 2x 270Ohm, Capacitor: 2x 47nF, Capacitor: 1x 100nF, Inductor: 2x 4.7mH, Trimmer/preset potentiometer: 1x 100K (circuit diagram as represented in the Suppl Fig S19). This converted the SIU output to a graded value by regulation of the duty cycle to modulate the output smoothly between 0 and whatever value we wanted.

Stimulation during SWS was delivered by a real time detection of SWS periods. The stimulations were delivered during SWS in 30 sec blocks with an inter-stimulation interval of 30 sec, with the chosen stimulation parameter. The system was set up to continue the stimulations as long as the rat remained in SWS, with the stimulation discontinuing with any state changes. This method yielded a list of timestamps indicating the start and end timepoints of each stimulation.

Experimental design: Stimulation parameters tested

Experiment 1:

Stimulation parameters	# of stimulations	# of sessions	# of rats
0.8 Hz; 9 uA	100	1	4
0.8 Hz; 36 uA	100	1	4
1.6 Hz; 9 uA	100	1	4
1.6 Hz; 36 uA	100	1	4
12 Hz; 9 uA	100	1	4
12 Hz; 36 uA	100	1	4

Table 7: Sine wave stimulation parameters and details of stimulation for experiment 1

In this experiment, the polarity of stimulation was positive to the anterior cortical electrode and negative to the posterior cerebellar electrode.

Experiment 2:

Stimulation parameters	# of stimulations	# of sessions	# of rats
0.5 Hz; 9 uA	100	2	3
0.5 Hz; 36 uA	100	2	3
1 Hz; 9 uA	100	2	3
1 Hz; 36 uA	100	2	3

1.5 Hz; 9 uA	100	2	3
1.5 Hz; 36 uA	100	2	3

Table 8: Sine wave stimulation parameters and details of stimulation for experiment 2

The polarity of stimulation was reversed with negative to the anterior cortical electrode and positive to the posterior cerebellar electrode in a different set of rats from experiment 1.

For both Experiment 1 and 2, each stimulation parameter was preceded by a sham stimulation session for comparison, with the higher amplitude stimulation session following the lower amplitude session for a given frequency, with a rest day in between each. The 30sec inter-stimulation periods were analyzed for any changes in delta power (0.5-4 Hz) beyond the stimulation periods. They were normalized with sham stimulation sessions during which a similar stimulation was timestamped in the recording without actually delivering the stimulation.

Experiment 3:

For this experiment, 2-hr pre-stim sleep and 2-hr post-stim sleep was recorded before and after the stimulation session respectively, to study the effects of stimulation beyond the stimulation session in a different set of rats from experiment 2. In this experiment, the polarity of stimulation was positive to the anterior cortical electrode and negative to the posterior cerebellar electrode.

Stimulation parameters	# of stimulations	# of sessions	# of rats
12 Hz; 9 uA	100	1	4
12 Hz; 36 uA	100	1	4

Table 9: Sine wave stimulation parameters and details of stimulation for experiment 3.

The 30sec inter-stimulation periods were analyzed for any changes in spindle density beyond the stimulation periods. Each stimulation parameter was preceded by a sham stimulation session for comparison, with the higher amplitude stimulation session following the lower amplitude session for a given frequency, with a rest day in between each. They were normalized with sham stimulation sessions during which a similar stimulation was timestamped in the recording without actually delivering the stimulation. The post-stimulation sleep recording was also analyzed for long-term changes in spindle density beyond the stimulation periods.

Data processing and statistical analysis:

Offline electrophysiological data processing was performed using MATLAB software. The 30sec inter-stimulation periods were analyzed for any changes in either delta power (0.5Hz - 4Hz) or spindle density beyond the stimulation periods. They were normalized with sham stimulation sessions during which a similar stimulation was timestamped in the recording without actually delivering the stimulation. Only the 30sec inter-stimulation periods when the rat continued to remain in SWS from the corresponding periods from sham and stimulation session were used for analyses. The power in the post-stimulation periods were converted to z-scores using the mean and standard deviation of the corresponding sham stimulation sessions.

Signal artefacts due to the sinusoidal pattern of stimulation and the frequency overlap of stimulation and endogenous SO activity, precluded EEG analysis in the SO range during acute stimulation periods. A spectrogram was created for all the selected inter-stimulation intervals. Spectral power in the 0.5-4 Hz frequency range was calculated for the 30-second inter-stimulation intervals, by averaging the corresponding bins from the spectrogram. The first 4 second window was discarded due to effect of electrical transients that occurred right after the stimulation was turned off. That gives us a 26-second time interval to analyse any changes right after the stimulation

is turned off. The power in the 0.5-4 Hz frequency range for the post-stimulation periods were averaged and an average z-score was calculated for post-stimulation periods using the mean and standard deviation of the corresponding sham periods. Spectral power in the 0.5-4 Hz frequency range during the analyses period calculated for the sham is hereby called 'baseline SO-power' and the spectral power in the 0.5-4 Hz frequency range calculated for the 30-sec post stimulation period is hereby called 'stim SO-power'. The stim SO-power was z-scored over the mean and standard deviation of the baseline SO-power, and then averaged to get an average 'stim SO-power' z-score for each rat for every stimulation parameter relative to the baseline. For experiment 2, each stimulation parameter was delivered over 2 sessions and the z-score computations on both sessions were averaged.

For spindle detection, the cortical LFP was bandpass filtered between 10-20 Hz, then squared and smoothed (175 ms rectangular window, sliding with step size 1 sample) to obtain a power signal. Periods of significant spindle activity were determined by finding peaks greater than 2 SD in the power signal to take into account considerable differences in LFP signal amplitude, based on the 30-sec post-stimulation intervals across the entire test session. The start/end timestamps were found by measuring the power before/after the peak until the power fell to 0.75 times the peak value: an upward-rising crossing defined the spindle onset, and the next falling crossing defined the end of the spindle. The start/end time stamps marked all the threshold crossings of the root mean square signal. This method yielded a list of timestamps indicating the start and end timepoints of each spindle. Detected events shorter than 200 ms and longer than 2 sec were discarded. Threshold values for automated detection of peak spindle power was done while visually inspecting the sham recording. Once set, the same thresholds were used for stimulation recording sessions. The spindle analysis was restricted to SWS episodes with continuous spindling activity (≥1 spindle per 10 s), a sleep state closely analogous to spindling

activity in human NREM2 (De Gennaro & Ferrara, 2003; Eschenko et al., 2006). Spindle density was calculated as the number of spindles per minute of SWS. Reliability of SWS and spindle-detection algorithm was verified by visual observation.

3.4 Results

Experiment 1:

The chosen frequency and amplitude of stimulation for this experiment were as follows: 0.8Hz/9uA, 0.8Hz/36uA, 1.6Hz/9uA, 1.6Hz/36uA, 12Hz/9uA and 12Hz/36uA. The polarity of stimulation was positive to the anterior cortical electrode and negative to the posterior cerebellar electrode. The 30sec inter-stimulation periods were analyzed for changes in delta power (0.5 Hz - 4Hz) beyond the stimulation periods. The procedure was repeated for all the chosen stimulation parameters. The 'stim SO-power' in the 0.5-4 Hz frequency range was averaged for the selected periods and an average z-score was calculated for post-stimulation periods over the mean and standard deviation of the corresponding sham periods ('baseline SO-power') and then averaged to get an average 'stim SO-power' z-score for each rat and for every stimulation parameter.

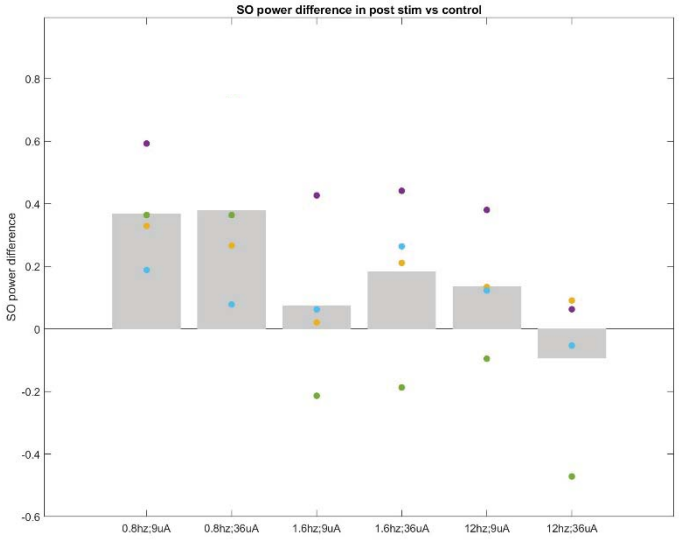


Figure 16: z-score of delta power in the post-stimulation periods compared to sham-stimulation periods with the polarity of stimulation positive to the anterior cortical electrode and negative to the posterior cerebellar electrode.

The calculated z-scores did not reflect a significant change (a z-score of greater than 1.98 would be equivalent to a probability significance value of $p>0.05$) during the inter-stimulation intervals compared to the corresponding baseline sham periods with any of the chosen stimulation parameters. The corresponding spectrogram from a sample rat is represented in Suppl fig S20 to show the trend of changes in lower frequencies without affecting higher frequencies.

Experiment 2:

This experiment was conducted to study the difference between anodal and cathodal stimulation in the frontal cortex for changes in SWA in the post-stimulation periods. The chosen frequency and amplitude of stimulation for this experiment were as follows: 0.5Hz/9uA, 0.5Hz/36uA, 1.0Hz/9uA, 1.0Hz/36uA, 1.5Hz/9uA and 1.5Hz/36uA. The polarity of stimulation was reversed with negative to the anterior cortical electrode and positive to the posterior cerebellar electrode. The analyses were exactly the same as experiment 1. The ‘stim SO-power’ in the 0.5-4 Hz frequency range was averaged for the selected periods and an average z-score was calculated for post-stimulation periods over the mean and standard deviation of the corresponding sham periods (‘baseline SO-power’) and then averaged to get an average ‘stim SO-power’ z-score for each rat and for every stimulation parameter.

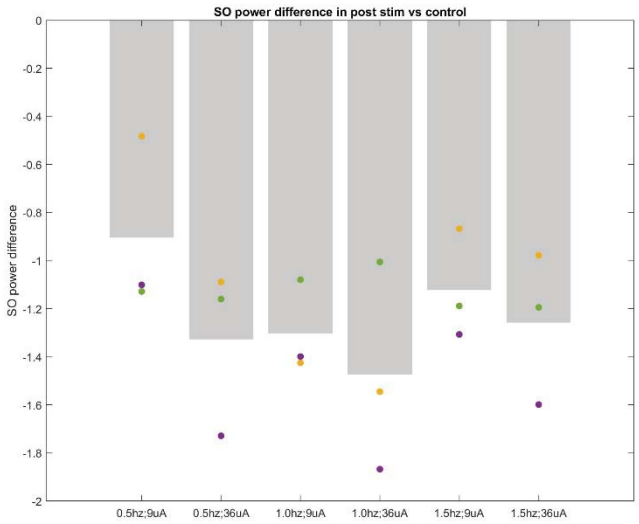


Figure 17: z-score difference of delta power in the post-stimulation periods compared to sham-stimulation periods with the polarity of stimulation reversed, negative to the anterior cortical electrode and positive to the posterior cerebellar electrode

The calculated z-scores did not reflect a significant change (a z-score of greater than 1.98 would be equivalent to a probability significance value of $p>0.05$) during the inter-stimulation intervals compared to the corresponding baseline sham periods with any of the chosen stimulation parameters. It is worth noting that the effect size was stronger than in experiment 1, and surprisingly, all effects were in the negative direction.

Experiment 3:

This experiment was designed to study if an oscillatory stimulation in the endogenous spindle frequency range would increase spindle density during the post-stimulation 30-sec periods and/or have long duration effects in spindle density in the post-stimulation recording session and to see if the changes were amplitude-dependent. The chosen frequency and amplitude of stimulation for this experiment were as follows: 12Hz/9uA and 12Hz/36uA. The polarity of

stimulation was positive to the anterior cortical electrode and negative to the posterior cerebellar electrode. For this experiment, 3 recording sessions were conducted: pre-stimulation 2-hr recording session (sham stimulation session, during which the stimulation times were recorded without actually delivering the stimulation), stimulation session (stimulation was delivered during SWS in 30-sec blocks with an inter-stimulation interval of 30 sec for as long as the rat remained in SWS) and finally a post-stimulation recording session (sham stimulation session, during which the stimulation times were recorded without actually delivering the stimulation). The stimulation frequency was specifically chosen to overlap with the spindle frequency range of 10-20 Hz, to study the changes in spindle density in the inter-stimulation intervals and in the post-stimulation sleep period compared to corresponding periods in the pre-stimulation session. The 30sec inter-stimulation periods were analyzed for changes in spindle density beyond the stimulation periods.

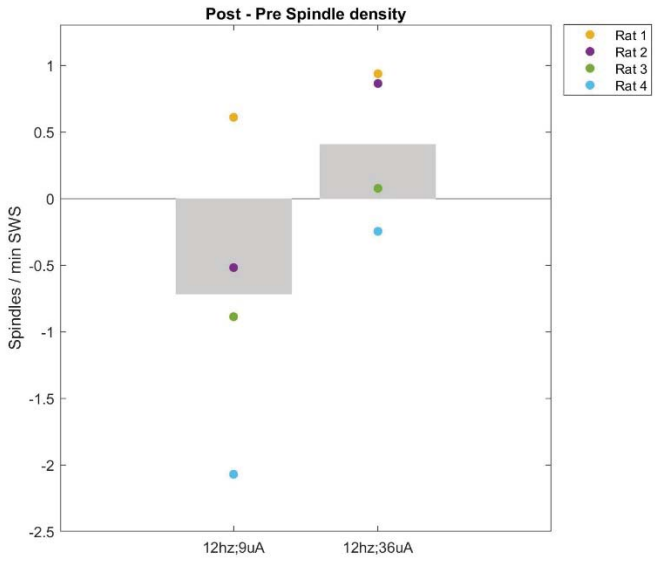


Figure 18: Difference of spindle density during the 12Hz stimulation session compared to baseline with the polarity of stimulation positive to the anterior cortical electrode and negative to the posterior cerebellar electrode.

Spindle density (spindles/min of SWS) was calculated for the 30-second inter-stimulation intervals when the stimulation or sham stimulation continued to occur during SWS. The procedure was repeated for all the chosen stimulation parameters. They were normalized with corresponding sham stimulation sessions. Spindle density during the stimulation session for the selected periods and an average z-score was calculated for post-stimulation periods over the mean and standard deviation of the corresponding sham session spindle density and then averaged to get an average z-score for each rat and for every stimulation parameter relative to the baseline.

The calculated z-scores did not reflect a significant change (a z-score of greater than 1.98 would be equivalent to a probability significance value of $p>0.05$) during the inter-stimulation intervals compared to the corresponding baseline sham periods with any of the chosen stimulation parameters (Figure 18).

In the same experiment 3, spindle density was also calculated during post-stimulation session compared to corresponding pre-stimulation session in 30-min intervals from the onset of SWS. The calculated z-scores of spindle density in the post-stimulation session also did not reflect a significant change (a z-score of greater than 1.98 would be equivalent to a probability significance value of $p>0.05$) during the post-stimulation session compared to the pre-stimulation session with any of the chosen stimulation parameters (Figure 19).

Sinusoidal stimulations in the 12Hz range from surface electrodes did not show significant increases in spindle density either right after the stimulation was turned off and even during the post-stimulation sleep session SWS.

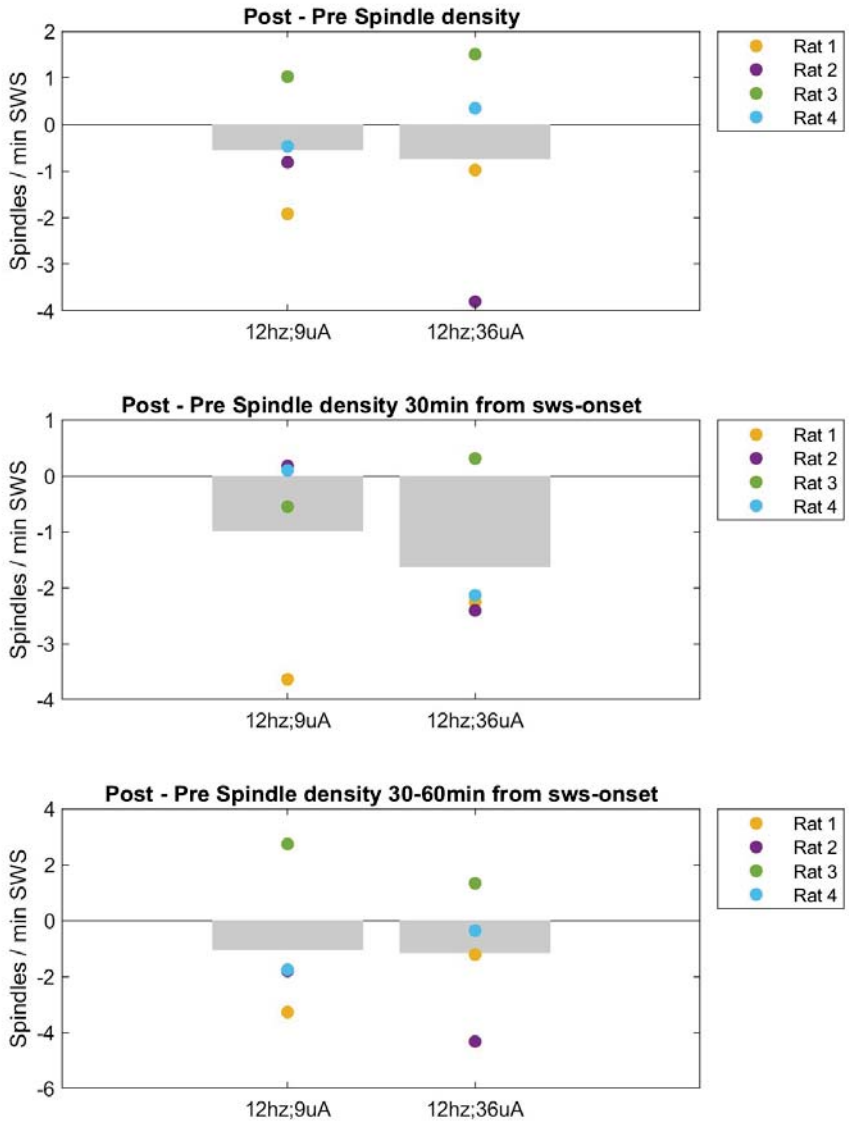


Figure 19: Difference of spindle density during the 12Hz post-stimulation sleep session compared to pre-stimulation sleep session

Top panel: Difference in spindle density between post- and pre-stimulation session during the entire 2-hour window of the post stimulation session
Middle panel: Difference in spindle density between post- and pre-stimulation session during the first 30 minutes after SWS-onset
Bottom panel: Difference in spindle density between post- and pre-stimulation session during the 30-60 min period after SWS-onset

3.5 Discussion

The primary objective of this study was to determine whether a 30-sec stimulation during SWS could induce changes in brain oscillations that could be detected beyond the stimulation window. In addition, we sought to test a range of stimulation parameters to determine which ones might give the strongest stimulation effects in the SO frequency range and spindle frequency range, both prominent endogenous brain rhythms during SWS. The SO (0.5-2.0 Hz) and upper delta band (2.0-4.0 Hz) together represent slow wave activity (SWA). Some scientists have used SO of below 2 Hz in rats (Ozen et al., 2010; Vyazovskiy, Riedner, Cirelli, & Tononi, 2007) and they have analysed their data accordingly, but we have used the full range of SWA 0.5-4 Hz (Vyazovskiy et al., 2007) for the entire delta frequency band.

In our first experiment, we tested three frequencies and two levels of intensity (9uA and 36uA), all with anodal current delivered to motor cortex. Two of these frequencies were chosen to be in the SO range (0.8 and 1.6 Hz) with the idea that this frequency would entrain and strengthen on-going SO activity. The third frequency was chosen at 12 Hz. The stimulation frequency was specifically chosen to overlap with the spindle frequency range (10-20 Hz), another prominent SWS oscillation. Overall, sinusoidal stimulations in the SO range from surface electrodes with the polarity of stimulation positive to the anterior cortical electrode and negative to the posterior cerebellar electrode produced slight increases in delta power, but these increases did not reach significance. Sinusoidal stimulations in the 12Hz range from surface electrodes also did not show significant increases in delta power.

In our second experiment, we tested three frequencies and two levels of intensity (9uA and 36uA), all with cathodal current delivered to motor cortex. The frequencies were chosen to be in the SO range (0.5 Hz, 1.0 Hz and 1.5 Hz) with the idea that this frequency would entrain and strengthen on-going SO activity. Interestingly, sinusoidal stimulations in the reverse polarity

(negative to the anterior cortical electrode and positive to the posterior cerebellar electrode) in the SO range from surface electrodes resulted in consistent decreases in delta power, but these decreases also did not reach statistical significance. The polarity of stimulation seems to affect the directionality of the effect.

Experiment 3 was conducted to include the two-hour window before and after stimulation so that we could see whether there were any longer-lasting effects of stimulation on spindles. In this experiment, we tested 12 Hz stimulation with two levels of intensity (9uA and 36uA), all with anodal current delivered to motor cortex. The stimulation frequency was specifically chosen to overlap with the spindle frequency range (10-20 Hz). Our dependent measures were the changes in spindle density in the inter-stimulation intervals and/or in the post-stimulation sleep period compared to corresponding periods in the pre-stimulation session. This experiment was designed to study if an oscillatory stimulation in the endogenous spindle frequency range would increase spindle density during the post-stimulation 30-sec periods and/or have long duration effects in spindle density in the post-stimulation recording session and to see if the changes were amplitude-dependent. A non-significant trend was seen in which the higher amplitude stimulation increased spindle density more than the lower amplitude stimulation in the post-stimulation periods. During the post-stimulation sleep session, on the other hand, higher amplitude stimulation seems to cause a small (but non-significant) reduction in spindle density compared to the lower amplitude stimulation. Sinusoidal stimulations in the 12 Hz range from surface electrodes also did not yield significant increases in spindle density either right after the stimulation was turned off and even did not show significant changes in spindle density in a post-stimulation sleep session SWS.

Rat models of tDCs (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014) suggest that focal electric field effects on fontal/prefrontal brain regions from surface electrodes in the motor cortex are comparable to those predicted in human studies, which have successfully used

stimulation to enhance memory (Lisa Marshall et al., 2006). The difference between the stimulations in humans and rat studies was that the human study used a trapezoidal SO-tDCs, while the rodent models used sinusoidal stimulation signal.

Slow oscillatory stimulation was found to enhance the SO band (0.5-1 Hz) (Antonenko et al., 2013; Lisa Marshall et al., 2006) or upper delta (2-4 Hz) activity (Binder, Rawohl, et al., 2014), but, in both cases, this effect was strongest only within the first post-stimulation interval of the day and not across all the inter-stimulation periods. In contrast, another study (Binder, Berg, et al., 2014) showed only a trend toward a transient enhancement of endogenous SO EEG power in the lower delta range (0.8-2 Hz) within the first 10 s of the stimulation-free interval in the stimulation condition and this did not change from the first to last stimulation event. With similar stimulation parameters, we found a trends in which SO power increased with anodal stimulation and decreased with cathodal stimulation. One difference in our study was that the electrodes were in contact with the surface of the brain, while in the earlier studies (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014), the stimulation electrodes were epicranial (in contact with the skull). A trend towards an increase in the SO power in our results of experiment 1 are also similar to some observations of SO enhancement observed in animals in-vitro (Fröhlich & McCormick, 2010) and in-vivo (Ozen et al., 2010). Overall, the effects of stimulations are weak and inconsistent between studies in rodents in terms of the frequency band influenced and the stimulation period affected. Some studies have also questioned whether transcranial stimulation can entrain SO as they found no entrainment of SO as a result of low frequency transcranial electrical stimulation (Lafon et al., 2017). SO-tDCs did not enhance sleep spindle density during NREM during the 30 s post-stimulation intervals nor during NREM after the end of last stimulation for the rest of the recording session (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014). This is comparable to our findings where we found no

significant changes during the inter-stimulation periods nor the sleep session following stimulation (at least with 12 Hz stimulation in expt 3).

A human study has shown that weak electric field stimulation may increase firing rates during the UP-state only if the oscillatory stimulation is synchronized with the endogenous brain slow-waves (Reato et al., 2013). Hence the effects of stimulation in entrainment/enhancement of endogenous oscillations may be complex and state-dependent. Some human studies have shown that SO-tDCs applied to the cortex significantly increases frontal SWA and spindle activity (Koo et al., 2018; Ladenbauer et al., 2016; Lisa Marshall et al., 2006; Paßmann et al., 2016), even though the effect of SO-tDCs on learning and memory enhancements were contradictory between these studies. This is contradictory to our findings in rats where we did not find significant changes in either SO power or in spindle density with the stimulation parameters tested during the post-stimulation periods. In addition, a recent study in rodents and human cadaver brains showed that, for current applied to the surface of the scalp, attenuation due to soft tissue and skull account for about a 75% reduction in signal intensity as measured within the brain (Vöröslakos et al., 2018). They speculated that the effectiveness of the externally applied field depends on several factors including neuronal density and geometry, organization of axons and dendrites relative to the induced electric field, ion channel types, ion channel distribution in the neurons, amount of myelination, and density of glial cells, affecting the electric shunting effect of the extracellular matrix. The study also calculated that a minimum voltage gradient of ~1 mV/mm was necessary to directly affect neuronal circuits. This meant at least 4-6 mA of current was needed to be applied to the scalp to generate those current density fields, which is higher than what is used in most conventional TES experiments.

The analyses of the 36uA stimulation parameter with each frequency tested suggests that larger stimulation might be more effective. This is possibly due to the electric field distributions

that were found to be amplitude-dependent, in terms of the depth of spread in a modeling study (Binder, Berg, et al., 2014). Higher amplitude stimulation can generate larger electric fields that have the potential to penetrate deeper layers of the cortex and even into subcortical structures. Thus, current density can affect neural tissue, and this would need to be tested with different amplitudes of stimulation in different cortical sites to study the effects on modulation of endogenous oscillations. This can explain the inconsistent and weak effects observed in studies depending on stimulation parameters. Overall, the post-stimulation increase in SO power observed in humans (Antonenko et al., 2013; Lisa Marshall et al., 2006) compared with the small enhancements reported in rodent data (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014) suggest that stimulation effects are greater in humans. This could be due to differences in the current densities used in rodents and humans. Taken together, from the modeling study (Binder, Berg, et al., 2014) and the human cadaver study (Vöröslakos et al., 2018), stimulation parameters that were used seem be below the threshold to enhance endogenous brain oscillations. In the light of this, the physiological effect that mediates memory effects seen in the human (Lisa Marshall et al., 2006) and rodent (Binder, Berg, et al., 2014) study needs further investigation.

On another note, it is possible that the SO transcranial direct current stimulation can bring about a change in the power of SO if the stimulation was done in phase with the ongoing endogenous oscillations. Closed-loop auditory stimulation in phase with the ongoing SO was shown to reliably boost SO (H.-V. V. Ngo et al., 2013), resulting in increased slow wave amplitude as well as temporal alignment of sleep spindles. Thus, stimulation delivered in phase with the endogenous brain activity may be important in entrainment/enhancement of brain oscillations lasting beyond the duration of the stimulation itself. The stimulation in our experiments was not delivered phase locked to the ongoing stimulation.

A recent study showed that the efficacy of stimulation on declarative memory retention was associated with nesting of slow spindles to SO troughs (UP to DOWN state transitions) (Dehnavi et al., 2021). Anodal theta-tDCs applied on the fronto-lateral locations in humans during NREMS caused a global reduction in SWA, decreased SO power and decrease in frontal slow spindles with a reduced performance in a declarative but not a procedural memory task (Marshall et al., 2011). In our experiments, the amount of increment in SO-power in the inter-stimulation periods with 12 Hz 9 uA stimulation was almost half the amount of increment seen in 0.8Hz stimulation and this increment was further decreased and in fact was in the negative direction with a 12Hz 36uA stimulation. Thus, the effects of stimulation could be frequency dependent, with frequencies that do not match the endogenous SO range having an inhibitory effect.

Anodal and cathodal tDCs have been shown to have opposing effects. Anodal tDCs over target brain regions has been shown to increase excitability of the underlying cortical tissue while cathodal stimulation is known to decreases excitability (Bestmann, de Berker, & Bonaiuto, 2015; Bindman, Lippold, & Redfearn, 1962; Creutzfeldt, Fromm, & Kapp, 1962). We found a trend in our result as in which the polarity of stimulation seems to affect the directionality of the effect. Cathodal sinusoidal SO direct current stimulation of anterior cortex yielded a non-significant increase in delta power, while anodal sinusoidal SO direct current stimulation of the same region slightly decreased delta power.

In contrast to tDCs, oscillatory forms of stimulation like tACs and oscillatory tDCs are thought to be more effective in entrainment of brain oscillations through phase-dependent frequency-specific amplification of endogenous rhythms by interacting with the ongoing oscillations (Paulus, 2011). tDCs and tACs have been speculated to affect the brain through different mechanisms (Zaghi, Acar, Hultgren, Boggio, & Fregni, 2010). Direct current appears to modulate membrane potential and spontaneous neuronal firing rates in a polarity-dependent

fashion. The site-specific effects of the stimulation are propagated to other underlying regions of the brain via networks of interneuronal circuits, and thereby affecting higher-order cortical processes like decision making and memory. Alternating current on the other hand, has been shown to have only modest effects on cortical excitability, but it has been shown to induce changes in brain EEG activity. Thus, tACs stimulation may not excite brain tissue, but the rhythmicity of stimulation can synchronize and enhance the efficacy of endogenous electrophysiological brain activity (Zaghi, Acar, et al., 2010). tACs studies have demonstrated that enhancement on endogenous brain oscillations are frequency specific (Ali, Sellers, & Fröhlich, 2013; Moliadze et al., 2010; Neuling et al., 2013; Reato, Rahman, Bikson, & Parra, 2010; Tino Zaehle et al., 2010; Zaghi, Acar, et al., 2010; Zaghi, de Freitas Rezende, et al., 2010). Studies which varied the phase of tACs stimulation showed entrainment of endogenous network activity within a few seconds of stimulation initiation regardless of the initial stimulation phase, but the phase was observed to determine the time it took for the network to phase lock to the stimulation (Ali et al., 2013). Hence, the phase of stimulation can determine entrainment effects. In addition, the effects of tACs seems to be frequency specific in terms of the endogenous activity that it can influence. Reduction in delta activity across the whole scalp was observed when a 12-Hz spindle-like tACS waveform was applied to frontal regions phased locked to the spindles during SO-spindle coupling in SWS (Lustenberger et al., 2016). Slow frequency tACs of 0.7 - 1.7 Hz to frontal cortical regions in anesthetized and naturally sleeping rats entrained neurons in the cortex and hippocampus in a state-dependent manner, with the effects restricted to SWS and this entrainment was proportional to stimulation intensity (Ozen et al., 2010). In fact, a couple of studies have shown promising effects with tACs (Ketz et al., 2018; Lustenberger et al., 2016) in terms of the modulation of endogenous oscillatory activity. Transient slow oscillatory tACs triggered by matching the frequency and phase of stimulation to endogenous SO was found to increase slow-wave power and coupling with

spindles (Ketz et al., 2018). The most robust increase in endogenous brain activity caused by a frequency-dependent stimulation was observed in feedback-controlled closed-loop tACs in the frontal regions locked to real-time detection of endogenous spindle activity during SO-spindle coupling in SWS (Lustenberger et al., 2016). The stimulation delivered was a 12-Hz spindle-like alternating current waveform instead of a constant frequency stimulation. They observed a general increase in spindle activity during N2 NREM compared to sham. This indicates the robustness of not just tACs, but more specifically a spindle-like waveform to enhance spindle activity. These studies using tACs suggest directions for future attempts to enhance SO and spindles using stimulation. Hence, given the differences between tDCs and tACs, it is more likely that we can induce entrainment and enhancement of endogenous brain oscillations with tACs rather than with tDCs. This can explain the effects in our experiments with tDCs, which may not be robust enough to enhance frequency-matched oscillatory activity.

In the light of discussion in the preceding paragraphs, a fruitful avenue for further research would be to try SO-tDCs stimulation phase-locked to the endogenous SO. In addition, the possibility of using alternating current stimulation (tACs) might be more promising to entrain endogenous brain oscillations.

Chapter 4 Reliable induction of sleep spindles with intracranial electrical pulse stimulation

Chapter published as:

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Given the importance of sleep spindles (discussed in section 1.9.4) and its role in memory processing (discussed in section 1.11.3) and the different ways to manipulate spindles (from section 1.13 of this thesis), the current study was designed.

4.1 Abstract

Neocortical sleep spindles have been shown to occur more frequently following a memory task, suggesting that a method to increase spindle activity could improve memory processing. Stimulation of the neocortex can elicit a slow oscillation (SO) and a spindle, but the feasibility of this method to boost SO and spindles over time has not been tested. In rats with implanted neocortical electrodes, stimulation during SWS significantly increased SO and spindle rates compared to control rest periods before and after the stimulation session. Coordination between hippocampal SWR and spindles also increased. These effects were reproducible across five consecutive days of testing, demonstrating the viability of this method to increase SO and spindles.

4.2 Introduction

Neocortical sleep spindles are prominent oscillations during SWS (A. Kandel & Buzsáki, 1997; Steriade & Deschenes, 1984; Mircea Steriade, David A McCormick, et al., 1993) that play a role in consolidation of both declarative (Gais et al., 2002; Manuel Schabus et al., 2004; Schmidt et al., 2006) and nondeclarative memories (Barakat et al., 2011; Johnson et al., 2012; Nishida & Walker, 2007; Peters et al., 2008). Spindles are preferred times of memory reactivation in the cortex during sleep, suggesting that reactivation during spindles contributes to memory performance (M. Eckert, McNaughton, & Tatsuno, 2020; Peyrache et al., 2009; Ramanathan et al., 2015).

Based on this evidence, a method to induce spindle oscillations may prove beneficial to memory consolidation. Recent optogenetic experiments have shown that it is possible to induce cortical spindles by activating the thalamic reticular nucleus (Halassa et al., 2011; A. Kim et al., 2012), and that these induced spindles improve memory performance (Charles-Francois V Latchoumane et al., 2017). Given the large gap between optogenetic manipulation and clinical practice, a more practical technique for enhancing spindles would be desirable. Electrical stimulation of the neocortex with an implanted electrode can evoke a slow oscillation (SO) and a spindle, but the reliability and longevity of this effect are unknown (D Contreras & Steriade, 1995; Steriade & Deschenes, 1984; Vyazovskiy, Faraguna, et al., 2009). Here we show that electrical pulse stimulation reliably evokes spindles for the duration of a 1-h stimulation session, resulting in a significant increase in SO and spindle density.

Methods

Four adult male Fisher–Brown Norway rats had recording electrodes implanted bilaterally in the motor cortex, hippocampus, and neck. The stimulating electrode was implanted in the deep motor cortex of one hemisphere adjacent to one of the cortical recording electrodes (see

Supplemental Fig. S1 for details, as well as the Supplemental Material for greater detail of all methods). After recovering from surgery, the animals were habituated to a quiet, dimly lit room and recording box for 3 d. All procedures and recordings were performed during the animal's light cycle. The vivarium maintained a 12-h light cycle (7 a.m. on) and the animals were tested in the same order every day such that recordings on different days occurred at approximately the same time (±1 h). On experiment days, rats were taken to the recording room, and they rested in a small cage after being connected to the recording system. The recording consisted of three consecutive 1-h sessions: a baseline recording with no stimulation, a recording with repeated single pulse electrical stimulation, and a final recording session with no stimulation. The procedure was repeated on five consecutive days to test the reliability of the method.

Recording was done on a Cheetah system (Neuralynx) and all signals were sampled at 2 kHz. Stimulation was delivered by a constant current stimulus isolation unit that was controlled by an Arduino microcontroller. Delivery of stimulation pulses was targeted to SWS using a real-time sleep state detector written in MATLAB. A 3-sec buffer of recording was used to calculate the ratio of δ (1–4 Hz) to θ (5–10 Hz) in the hippocampal LFP as well as the amount of EMG activity. SWS detection occurred when there was a high δ/θ ratio and low EMG activity. For each animal, the threshold value for the SWS detection was determined during a habituation session prior to the start of the stimulation experiment. Once SWS was detected, a single biphasic pulse (150 μS per phase, 500 μA) was delivered every 3 sec for as long as the animal remained in SWS (Fig. 20A). The real-time detection corresponded well with offline sleep structure analysis (Supplemental Fig S3).

Spindles, SO, and SWR were detected automatically based on thresholded power signals of filtered LFP recordings (SO 1–4 Hz, spindle 10–20 Hz, SWR 100–250 Hz) (see the Supplemental Material for details). Threshold values for automated detection of LFP features was

done while visually inspecting the prestimulation recording from day 1, and then verifying that they were appropriate by checking the prestimulation session from days 2–5. Once set, the same thresholds were used for all recording sessions on all days. Spindles that occurred within 750 msec of a SO (measured from the peak of the hyperpolarization), SWR, or stimulation pulse were considered "coupled" to the SO/SWR or "evoked" by the stimulation pulse. SO within 500 msec of a stimulation pulse were considered "evoked" (Charles-Francois V Latchoumane et al., 2017). For triple coupling of SO, spindles, and SWR, the SO was taken as time zero and then both a spindle and SWR had to occur within 750 msec of the SO. Although SOs, spindles and SWR appear similar in mice and rats (Csernai et al., 2019; Mölle et al., 2009; Niethard et al., 2018), differences in detection methods and parameters can give rise to different event timings. Furthermore, definitions of "coupling" can vary across studies, with gaps of 2 sec between SO and spindles accepted as coupled (Kam, Pettibone, Shim, Chen, & Varga, 2019). Because of these issues, we tested several gap durations where we report significant coupling to ensure the coupling is not specific to a particular gap.

As previously reported (Vyazovskiy, Faraguna, et al., 2009), brief stimulation pulses delivered to the corpus callosum during SWS evoked SO and spindles in the motor cortex (Fig. 20A). Spindle oscillations occurred reliably following the SO throughout the 1-h stimulation session, an effect that occurred ipsilaterally as well as contralaterally to the stimulation electrode.

4.3 Results

Other than the presence of a stimulation artifact, which was removed with an automated algorithm (Supplemental Fig. S2), stimulation-evoked spindles appeared qualitatively similar to spontaneously occurring spindles (Fig. 20B). Examination of overlaid average waveforms of spindles from the prestim and stimulation sessions revealed that evoked spindles were larger in amplitude (Fig. 20C). Measuring the peak amplitude showed a significant increase in the amplitude of evoked spindles compared to spontaneous spindles in the prestim session ($t_3 = 5.16$, $P < 0.05$) (Fig. 20D). This effect did not last beyond the stimulation session, and spindle amplitude in the post-stim session was not significantly different than prestim amplitude ($t_3 = 0.47$, $P = 1$). We also compared frequency and duration of evoked and spontaneous spindles. Detected spindles were 400–600 msec in duration on average, which is in the range normally reported for spindles (Gardner et al., 2013; Charles-Francois V Latchoumane et al., 2017), and stimulation did not alter the duration (Supplemental Fig. S4). Similarly, spindle frequency was not changed by stimulation (Supplemental Fig. S4). Like evoked spindles, the amplitude of evoked SO was significantly larger compared to spontaneous SO from the prestim session (Supplemental Fig. S5). Unlike spindles, the amplitude of spontaneous SO in the post-stim session, although smaller than evoked SO, remained significantly larger than the prestim SO (Supplemental Fig. S5).

Importantly, stimulation did not affect the rats' sleep. They spent most of the time sleeping, and the amount of sleep was similar between the stimulation session and the post-stimulation session (Supplemental Fig. S6A). The amount of sleep in the prestim session was less than the other sessions, likely because the rats were still aroused after being transported to the recording room. However, the proportion of SWS was similar between all epochs, indicating that stimulation did not significantly alter the sleep structure (Supplemental Fig. S6B).

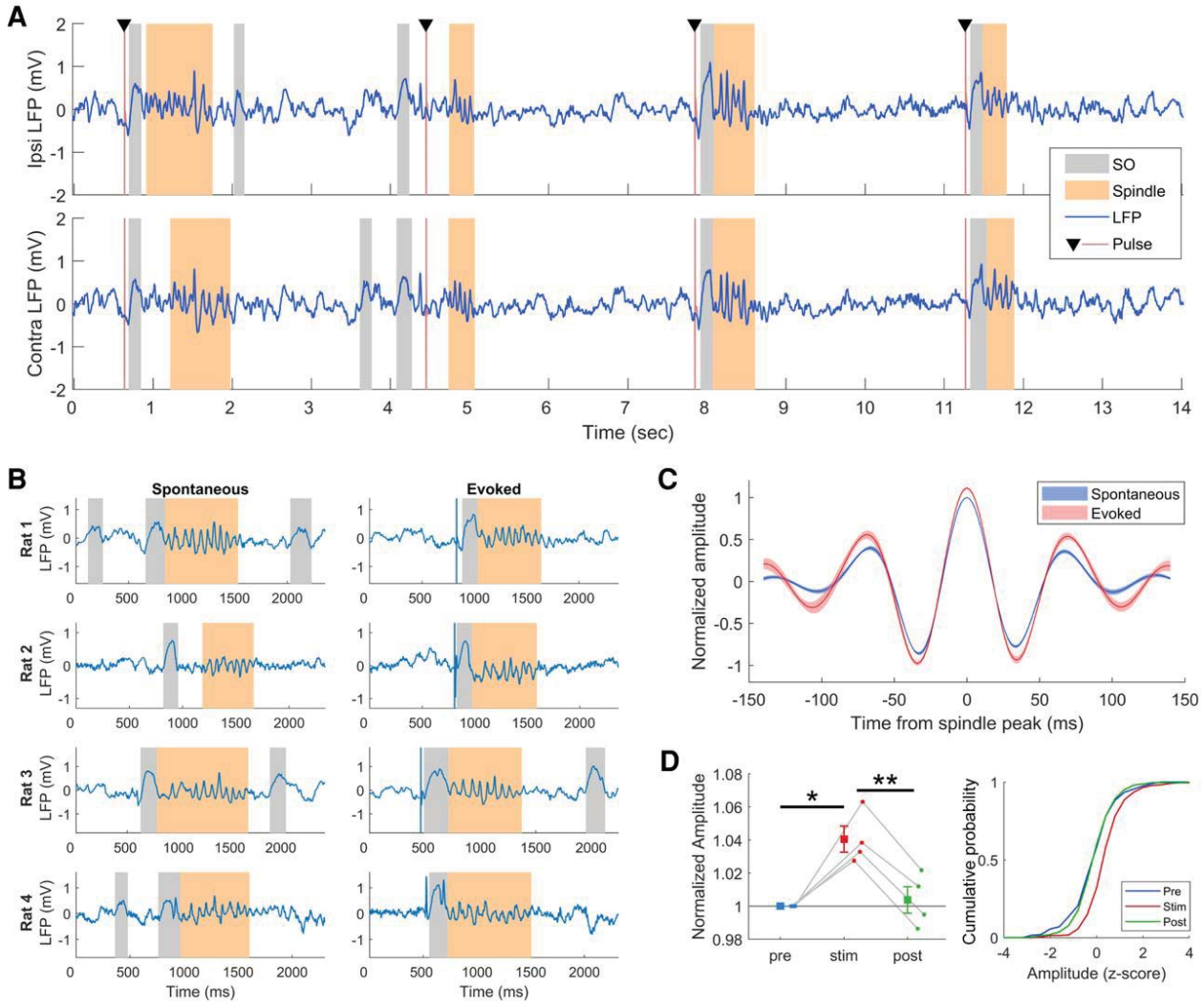

Figure 20: Neocortical stimulation during SWS reliably evokes spindles.

(*A*) Segment of LFP from SWS showing SO and spindle oscillations occurring following stimulation pulses. Colored patches indicate offline detected SO and spindles. (*B*) Comparison of spontaneous and evoked spindles from each rat. (*C*) Average waveforms of spontaneous and evoked spindles showing larger amplitude of evoked spindles. Waveforms are aligned to the peak amplitude of the spindle and normalized by the amplitude in the prestim session. Shaded region is SEM of $n = 4$ rats. (*D*) Quantification of spindle amplitude across prestim and post-stim recording sessions. Amplitude is normalized to the spindle peak of the prestim session. For the distributions, prestim spindles were used to standardize the amplitude. (*) $P < 0.05$, $t_3 = 10.45$; (**) $P < 0.01$, $t_3 = 5.16$. Error bars are SEM of $n = 4$ rats.

SO and spindles occurred reliably following stimulation in both hemispheres. On average, 76% of pulses were followed by a SO and 59% were followed by spindles (Supplemental Fig. S7).

To quantify the increase in the number of SO and spindles events over the duration of the 1-h

stimulation session, we compared the SO and spindle density (number per minute; averaged across hemispheres) of the prestim and post-stim sessions. SO and spindle density both increased significantly during the stimulation session, SO by an average of 30% (range 18%–45%) (Fig 21 A1), and spindles by an average of 37% (range 23%–54%) (Fig 21 B1; individual hemispheres in Supplemental Fig. S8). Temporal coupling of SO and spindles was previously identified as important for memory (Charles-Francois V Latchoumane et al., 2017). Using a similar measure, we classified spindles as coupled to a SO if they occurred within 750 msec. Despite the significant increase in both SO and spindles, the SO-spindle coupling was not increased by stimulation (Supplemental Fig. S9). Furthermore, stimulation did not appear to cause any lasting effect on SO or spindle density as both SO and spindle density in the post-stim session were comparable to the prestim session. We also calculated the peristimulus time histogram (PSTH) of SO and spindle occurrences and found a significant increase in SO and spindle rate following stimulation pulses (Fig 21 A2, B2). To test the reproducibility and longevity of the stimulation-induced increase in SO and spindles, we repeated the experiment on five consecutive days and observed similar increases in SO and spindle density on each day (Fig 21 C, D).

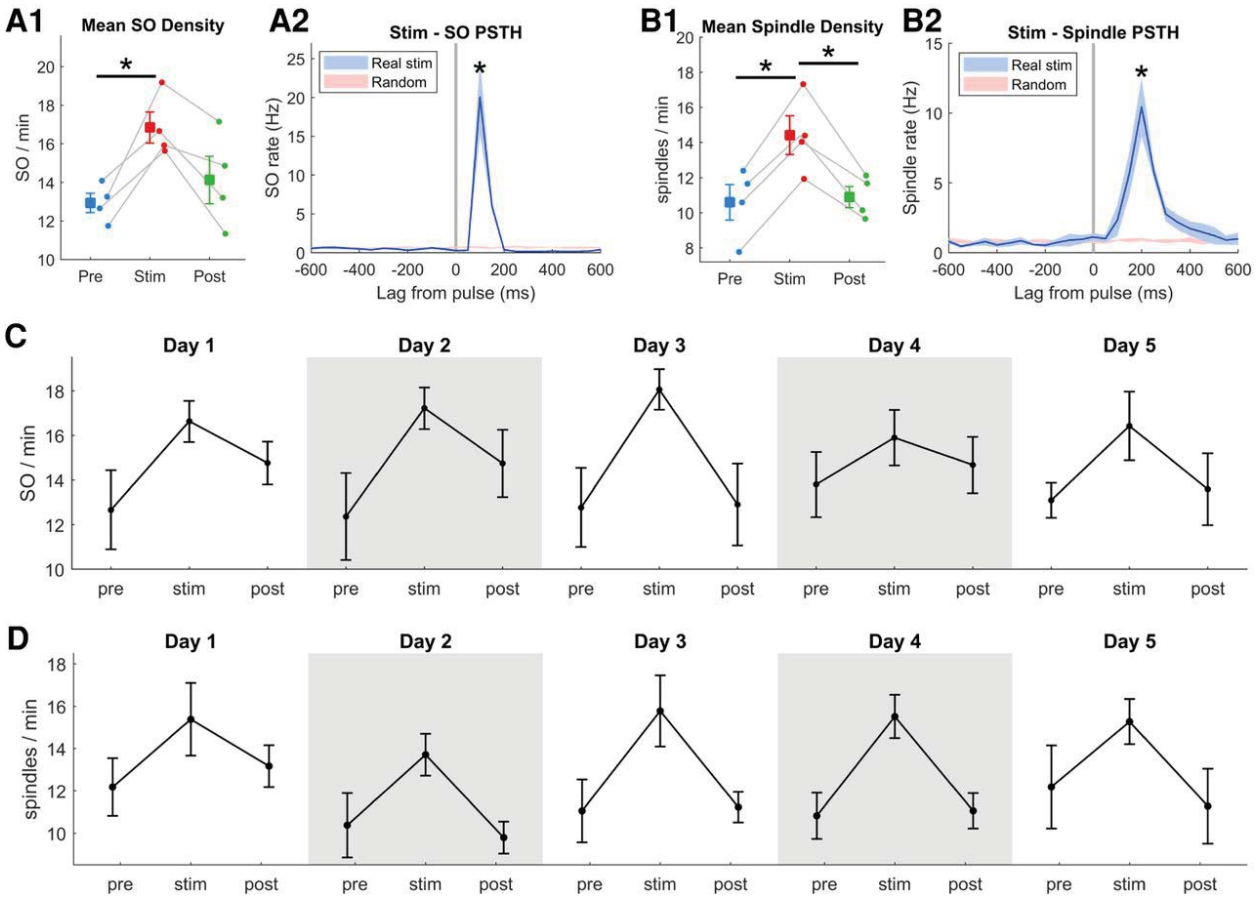

Figure 21: One hour of stimulation reliably increases SO and spindle rate.

(*A1*) Average SO density across prestim and post-stim sessions ($t_3 = 5.19$). (*A2*) Peristimulus time histograms (PSTH) of SO relative to stimulation pulses or randomly distributed pulse times ($t_3 = 3.86$). (*B1*) Average spindle density across sessions (prestim: $t_3 = 8.05$; stim-post: $t_3 = 4.88$). (*B2*) PSTH of spindle occurrences relative to stimulation pulses ($t_3 = 4.71$). (*C*,*D*) Stimulation-induced increase in SO and spindle rate is consistent across five consecutive days. In all panels, (*) $P < 0.05$, error bars are SEM of $n = 4$ rats.

We next examined the possible effect of cortical stimulation on hippocampal sharp-wave ripples (SWR). Unlike spindles, stimulation did not affect the overall rate of SWR occurrence (Fig 22A). However, the timing of SWR was affected by stimulation. SWR were more likely to occur within 300–400 msec following a cortical stimulation pulse, and were less likely to occur at other latencies (ANOVA $F_{(19,114)} = 6.7$, $P < 0.01$) (Fig 22 B). The increased tendency of SWR within this

time window led to increased coupling between SWR and spindles ($t_3 = 4.72$, $P < 0.01$) (Fig 20C). To verify that the increased coupling was not due to the definition of coupling (750-msec gap), we tested a range of gap durations (250–2000 msec) and found the coupling to be robust (Supplemental Fig. S10). The increased SWR-spindle coupling was only present during the stimulation session, and there was a decrease in coupling in the post-stim session such that there was no difference between the prestim and post-stim coupling. Finally, we tested for possible triple coupling of SO, spindles, and SWR. Although triple coupling increased in all four rats during the stimulation session, it failed to reach statistical significance ($t_3 = 2.10$, $P = 0.63$) (Fig 20D).

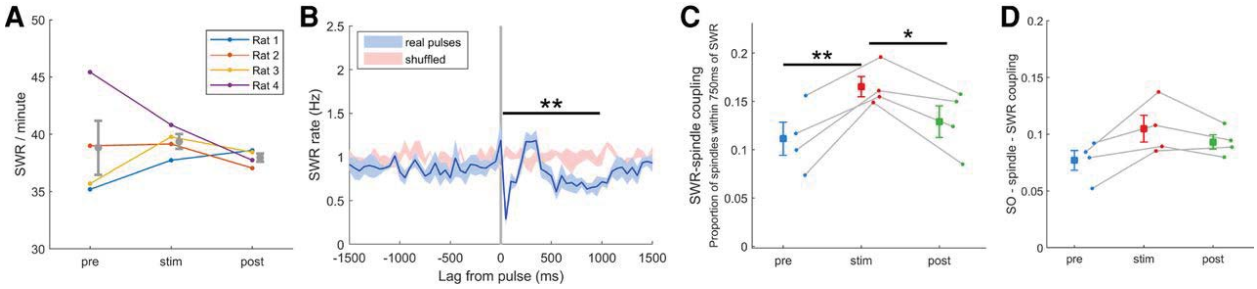

Figure 22: Effects of cortical stimulation on hippocampal sharp-wave ripples (SWR).

(*A*) The rate of SWR in the prestim and post-stim sessions were similar, indicating that stimulation did not affect the overall rate of SWR. (*B*) Stimulation altered the timing of SWR. The PSTH of SWR relative to stimulation pulses shows an overall suppression of SWR following the pulse, with a grouping of SWR occurring 300–400 msec after the pulse. (**) $P < 0.01$, ANOVA $F_{(19,114)} = 6.7$. (*C*) Stimulation caused an increase SWR-spindle coupling. Compared to the prestim session, there was a significant increase in the proportion of spindles that occurred within 750 msec of a SWR during the stim session. (**) $P < 0.01$, $t_3 = 4.72$. The increase in SWR-spindle coupling during the stim session did not persist into the post-stim session. (*) $P < 0.05$, $t_3 = 2.49$. (*D*) Triple coupling of SO, spindles, and SWR increased in all four rats during the stimulation session but failed to reach statistical significance ($t_3 = 2.10$, $P = 0.063$).

4.4 Discussion

In summary, our results show that spindles can be evoked reliably by single-pulse electrical stimulation of the neocortex for the duration of a 1-h stimulation session, resulting in a significant increase in SO and spindle density, and an increase in SWR-spindle coupling. One potential limitation of our study is the within animal design. Because the same parameters were used to detect spindles and SO, the observed increase in spindles and SO could be due to an endogenous increase that occurs over time. While we cannot strictly rule out this possibility, it is worth noting that the increase in spindle and SO density decreased significantly in the post-stim session, so the increase cannot be due to a progressive increase in spindles and SO. Together with the very strong cross-correlation of stimulation pulses and spindles/SO, the evidence favors the view that the increase in spindle/SO density was indeed due to the stimulation. Furthermore, this increase is reproducible across five consecutive days of stimulation, suggesting that this method is viable as a deep-brain stimulation implant for increasing SO and spindles.

Previous attempts to boost spindle expression during sleep have used different methods with mixed results. Playing auditory stimuli during sleep is a minimally invasive method that has shown promise in altering SWS oscillations. In the context of a TMR experiment, when the auditory stimulus was previously paired with a learning task, tones presented during sleep increased spindles whose content was related to the memory task (Cairney et al., 2018). Even when auditory tones are used in non-TMR experiment (i.e., the tone was not previously paired with a learning task), tones played during sleep increased spindle power and improved memory performance (H.-V. V. Ngo et al., 2013). However, a recent study showed a similar increase in spindle power following tone presentation, yet there was no corresponding memory improvement (Henin et al., 2019). Furthermore, the practical use of auditory stimuli outside of a clinical setting has been

questioned because it is less reliable and subject to interference from other sounds (H.-V. V. Ngo et al., 2015).

Initial studies of transcranial electrical stimulation (TES) used mild oscillating currents during SWS and showed that SO and spindles were increased, and that memory performance was improved (Lisa Marshall et al., 2006). Subsequent studies using similar protocols yielded mixed results, and a recent study showed that currents typically used in TES studies are too weak to affect neural activity (Lafon et al., 2017). Our more invasive method of implanting a stimulating electrode in the cortex and delivering brief pulses of current was first shown to be effective at evoking spindles many years ago in anesthetized cats (D Contreras & Steriade, 1995; Diego Contreras & Steriade, 1996; Roy, Clercq, Steriade, & Deschênes, 1984; Steriade & Deschenes, 1984). Since then, an increase in spindle power has been observed following electrical stimulation of the neocortex in sleeping rats (Vyazovskiy, Faraguna, et al., 2009), or by transcranial magnetic stimulation in humans (Til O Bergmann, Mölle, Schmidt, et al., 2012; Marcello Massimini et al., 2007). Despite these results, it has not been shown that repeated cortical stimulation is capable of increasing spindle rates, and our current results show that stimulation pulses through implanted electrodes can be used to evoke spindles reliably, not only during a 1-h recording session, but across multiple days.

A recent optogenetic study showed that thalamic reticular activation increased the coupling of spindles and SO and improved contextual fear memory (Charles-Francois V Latchoumane et al., 2017). Interestingly, they did not report a net increase in spindle density, so the improved memory was attributed to the increased coordination between SO, spindles, and SWR. We observed a significant increase in SWR-spindle coupling, as well as a strong trend toward increased triple coupling of SO, spindles, and SWR, although this failed to reach statistical significance. The weaker triple coupling is possibly due to the fact that our stimulation pulses occurred randomly

with respect to SO, whereas the reticular stimulation in (Charles-Francois V Latchoumane et al., 2017) was triggered by a SO. If our electrical stimulation was triggered by SO, then it is possible the triple coupling would be increased as well, although this remains to be tested. Another critical next step is to determine if the substantial stimulation-induced increase spindle and SO density, as well as the increased SWR-spindle coupling, is sufficient to improve memory.

Chapter 5 General discussion and future directions

The primary motivation for the studies presented in this thesis were the promising results of enhancement of memory with electrical brain stimulation during SWS from earlier work, first reported in humans (Lisa Marshall et al., 2006; Lisa Marshall et al., 2004), followed by a couple of studies trying to replicate the findings in rats (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014). The potential therapeutic implications of enhancing memory using SO-tDCs during sleep reported in the studies seemed promising. We wanted to replicate the memory enhancement studies of SO-tDCs in rats in other hippocampal-dependent tasks not investigated earlier. The earlier studies had used an object-place recognition task (Binder, Berg, et al., 2014) and radial arm maze reward-place association task (Binder, Rawohl, et al., 2014). We wanted to test the efficacy of SO-tDCs stimulation in different tasks and for that purpose we chose a modified version of the Morris Water task, and a modified version of fear conditioning called the "context pre-exposure and immediate shock deficit" paradigm. Both the tasks have been shown to be dependent on the hippocampus for spatial memory and contextual memory respectively (Redish & Touretzky, 1998; Rudy, Barrientos, & O'reilly, 2002; Rudy & O'Reilly, 2001).

Both SO and spindles individually and in temporal association have been demonstrated to be indicators of memory processing from several human and rat studies. Learning was found to be associated with task-related increased SO amplitude in both humans and rats (Mölle et al., 2009), increased SO coherence across cortical regions (Mölle et al., 2004) during post-learning sleep. In addition, increases in SWA EEG power as a reflection of SO during post-learning sleep were reported to be correlated with memory retention in humans in a visuo-motor spatial task (Huber et al., 2004), declarative word list and procedural motor skill learning (Holz et al., 2012). Some of the mechanisms that have been proposed for the memory effects of SO by enhancing neocortical-

hippocampal communication are: coordinated inter-regional neuronal spiking activity (Ji & Wilson, 2007), SO-ripple temporal coupling through interaction with hippocampus (Todorova & Zugaro, 2020), SO-spindle coupling through interaction with thalamic nuclei (Lisa Marshall et al., 2006; H.-V. V. Ngo et al., 2013; Steriade, 2006), and SO-spindle-ripple triple coupling through interaction with thalamus and hippocampus (Zsófia Clemens et al., 2007; Mölle & Born, 2011; Sirota & Buzsáki, 2005; Sirota et al., 2003). In addition to SO, spindles have also been implicated in memory processing. Learning of a novel information or task has been shown to enhance subsequent spindles during sleep in both humans (R Bódizs et al., 2008; Zofia Clemens et al., 2005; Zsófia Clemens et al., 2006; Gais et al., 2002; Hoedlmoser et al., 2014; Meier-Koll et al., 1999; Mölle et al., 2009; Manuel Schabus et al., 2004; Schmidt et al., 2006) and rats (Eschenko et al., 2006; S. M. Fogel et al., 2009). In addition to increases in post-learning spindle activity, studies in humans (Zofia Clemens et al., 2005; SM Fogel et al., 2002; S. M. Fogel & Smith, 2006, 2011; S. M. Fogel et al., 2007; Gais et al., 2002; Holz et al., 2012; Mölle et al., 2009; Manuel Schabus et al., 2004; Van Der Helm et al., 2011) as well as in rodents (Eschenko et al., 2006) have shown positive correlations between spindle activity and later memory retrieval as recorded during subsequent memory tests. Spindles can be involved in sleep-dependent memory consolidation in two ways: local reactivation of task-specific cortical networks and functionally connecting these cortical regions with relevant sub-cortical networks of the hippocampus, putamen and thalamus (Boutin et al., 2018). These studies clearly indicate that the role of sleep spindles during post-learning encoding help consolidate memories for retention and retrieval.

Therefore, given the involvement of spindles and SO in post-learning memory processing, manipulating the brain to modulate these particular SWS oscillations was hypothesized to modulate memory. In line with this reasoning, memory improvements have been found to be correlated with enhancement of SO power during post-learning sleep through tDCs in humans (Lisa Marshall et

al., 2006; Lisa Marshall et al., 2004) and rats (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014), and closed loop phase-locked auditory stimulation mediated enhancement of SO (H.-V. V. Ngo et al., 2013). This has been interpreted as supporting a causal role for SO in memory consolidation as the stimulation boosted SO power related to SWA and correlated with better memory recall. Experimental disruption of spindles have provided additional evidence for the functional involvement of spindles in memory processing, as deficits in learning and memory can be induced by interfering with spindle activity and/or coupling of spindles with associated oscillations (Binder et al., 2019; Katsuki et al., 2017; Marshall et al., 2011). Given these observations, stimulating the brain to enhance these particular SWS-specific oscillations was hypothesized to enhance memory.

Before we embarked on the studies with a large cohort of rats, we wanted to explore whether the stimulation we planned to use even had an effect in terms of modulating the endogenous brain oscillatory activity and if this effect lasted beyond the timeframe of the stimulation itself. To test this, we used a range of stimulation parameters from brain surface electrodes in the slow-wave frequency range during SWS to see if it would entrain the endogenous sleep slow oscillation in the post-stimulation intervals. We also investigated if stimulation in the 12Hz frequency range would enhance spindle density in the post-stimulation intervals. In addition, we explored if brief pulse stimulation with intracortical electrodes could trigger SWS-specific oscillations like k-complex and spindles.

We also wanted to explore what types of tasks lead to strong memory processing (as indicated by spindle density increases), and to tap into the results to enhance these changes when they show up. This set of studies was designed to follow-up on the observation by (Eschenko et al., 2006) that task-learning in odor-reward association correlated with enhancement of spindle density within the first hour of post-learning sleep and predicted retrieval. In addition, the study

showed that this correlation was specific to task learning; they reported no enhancement of spindles associated with an exploration task. We intended to address the broader question: "Does learning enhance spindles?". A follow-up question was, "Do we really need a task to drive consolidation-related brain activity?". In other words, would novel environment exploration be sufficient to increase spindle activity? The rationale was that encoding of sensory and spatial information happens during exploration and not necessarily only during a learning task. To test whether encoding of a novel environment might be sufficient to lead to enhanced spindles, we compared two environments with enhanced learning potential (novel objects and novel social experience with novel objects). Secondly, in addition to exploration of novel environments, and building upon the few rodent studies reporting correlation between increase in spindles and task learning (Eschenko et al., 2006), we wanted to investigate the changes in spindle density following other types of hippocampal-dependent learning not yet reported in rodent literature. To this end, we included a modified version of the Morris Water task and a fear conditioning task. Additionally, to test whether a cognitive demand in a familiar task, one which clearly involves some form of learning, might also lead to enhanced spindles we used a task switching behavioural paradigm.

Based on the described speculations, our hypotheses were that: a) spindle density would increase during post-learning sleep in the first 30-min window after SWS onset as an indicator of learning and predictor of future performance; b) SO-tDCs during SWS would enhance the endogenous slow wave activity in the post-stimulation periods; and c) intra-cortical stimulation would evoke k-complex and spindles.

In Chapter 2, we investigated the effects of active exploration and task dependent learning paradigms on sleep spindle density occurring immediately after learning during the first hour from the onset of SWS. The results from our experiments showed that spindles could be triggered by exposure to a novel environment, probably enhanced by memory representations of the exploration

space and encoding of spatial and sensory information, with very robust increase in spindles when the novel experience involved a social experience. This suggests that the complexity of the novel context and intensity of stimulus could be an important driving factor. With task learning in both the water task and fear conditioning, delayed enhancement of spindles in the 30-60 minute time window, but not in the 0-30 min after SWS onset was observed, in line with our predictions. No significant changes in spindle density were observed across the days of task-switch experiment, between the first and second switch or even between young and aged rats.

Based on prior findings and theories, spindle density changes could be related to post-behavior encoding and processing of memories; however, the factors underlying sleep spindle density increases appear to be more nuanced than previous experiments might suggest. For one, changes in sleep spindle density seem to be extremely sensitive to the task at hand. Task-related factors that are known to influence learning-dependent sleep spindles in the process of memory consolidation include task complexity, task-induced stress, and specifics of the task itself. The increases in spindle density found in the novel environment exploration experiments (incidental learning in a non-stressful environment) followed a similar time course (when, during sleep, the peak in spindle density occurs) as those reported in a prior study (Eschenko et al., 2006). But our results from the water task and fear conditioning experiments (motivated learning in a stressful environment) showed that increases in spindles as a function of learning could be delayed and we speculate that this delay could be induced by task-induced stress-related hormones. Despite findings on learning-related increase in spindles in rats (Binder et al., 2012; S. M. Fogel et al., 2009), this learning induced increase was not observed in some studies (Binder, Berg, et al., 2014). Earlier studies have also shown enhancement of spindles to be more pronounced in humans than in rats (Mölle et al., 2009). In addition, we will need to investigate which time frame is most likely to lead to success in stimulation-dependent enhancement of memory. It is possible that the time of

memory encoding and consolidation can differ based on the task. This raises questions about the suitability of the water maze (which is an aversively motivated experience) and fear conditioning tasks for experiments on learning-related spindle enhancement, especially if we want to look for learning-related enhancement in spindles in the first 30 mins of SWS onset. If using stressful tasks, we will need to investigate which time frame (when, during sleep, the peak in spindle density occurs) is most likely involved in learning-related spindle enhancement. It is possible that the time frame of learning-related sleep spindle enhancement is task-dependent. Since these changes in sleep spindle density are very sensitive to the task, we would need to test this on different simpler tasks that can be directly related to learning mediated changes. It would also help to differentiate tasks based on whether they are appetitive or aversive and based on task difficulty. The choice of the selected task to study the effects of learning on sleep spindles may be an important consideration in the light of several factors that could be at play. It is conceivable to consider other alternative tasks, for example, the Barnes maze, which is a well-established alternative to the Morris Water task and has the advantage of not having potentially confounding influence of swimming behavior and the stress induced thereby.

In Chapter 3, experiments were designed to investigate the effects of cortical slow oscillatory sinusoidal direct current stimulation on changes in endogenous brain oscillatory activity during the periods between stimulations (i.e., the inter stimulation intervals) to see if the effects of stimulation last beyond the duration of the stimulation itself. We used similar stimulation parameters as tested in earlier studies that have shown stimulation-induced learning enhancement (Binder, Berg, et al., 2014; Binder, Rawohl, et al., 2014). Overall, sinusoidal stimulations in the SO range with positive polarity over frontal regions lead to a slight increase in delta power, but these increases did not reach significance. Interestingly, sinusoidal stimulations in the reverse polarity in the SO range from surface electrodes showed decreases in delta power, but these

decreases also did not reach significance. Hence, the polarity of stimulation seems to affect the directionality of effect. Sinusoidal stimulations in the 12Hz range from surface electrodes also did not show significant increases in delta power. In addition, we also tested if sinusoidal direct current stimulation at a 12 Hz frequency could enhance spindle density during the post stimulation periods. Sinusoidal stimulations in the 12Hz range from surface electrodes also did not show significant increases in spindle density either right after the stimulation was turned off and even did not show significant changes in spindle density during SWS in a post-stimulation sleep session.

From earlier studies, it is evident that the effects of tDCs stimulations are not consistent between studies in rodents in the frequency band influenced and the durability of the effect. Slow oscillatory stimulations were found to enhance the SO band (0.5-1 Hz) (Antonenko et al., 2013; Lisa Marshall et al., 2006) or upper delta (2-4 Hz) activity (Binder, Rawohl, et al., 2014), but this effect was strongest only within the first post-stimulation interval of the day in both the cases and not across all the inter-stimulation periods. Another study (Binder, Berg, et al., 2014) did not show these effects and showed only a trend toward a transient enhancement of endogenous SO EEG power in the lower delta range (0.8-2 Hz) within the first 10 s of the stimulation-free interval in the stimulation condition and this did not change from the first to last stimulation event. Some studies have also questioned whether transcranial stimulation can entrain SO as they found no entrainment of SO as a result of low frequency transcranial electrical stimulation (Lafon et al., 2017). Overall, the prior attempts to demonstrate the effects of SO stimulation on the brain are weak and hence, consistent with the lack of significant effects in our experimental analyses.

Several factors like the phase and type of stimulation may play an important role in the efficacy of brain stimulation. Stimulation delivered in phase to the endogenous SWA may have the potential to better enhance delta power in the post stimulation periods. In addition, transcranial alternating current stimulation seems to be more efficient in entraining endogenous brain

oscillations (A. P. Jones et al., 2018; Ketz et al., 2018) rather than transcranial direct current stimulation that we used in our experiments. These parameters could be tested in the future to better enhance the power of SWA and/or induce increases in spindle density in the post stimulation periods. It is also possible that slow-oscillation stimulation does enhance spindles, but only during stimulation. While this possibility cannot be tested in the slow-wave frequency range due to the technical limitations in recording during stimulation, it might be possible to explore this for the spindle frequency range.

The findings from the stimulation experiments conducted in Chapter 3 led us to rethink the stimulation parameters that would best enhance memory-related SWS-specific brain oscillations. In Chapter 4, we investigated whether brief biphasic pulsatile stimulation might be useful for triggering consolidation-related oscillations in the cortical local field potential (LFP) and how evoked responses varied as a function of sleep state. Intra-cortical pulse stimulation via bipolar electrodes located in deep layers of dorsal medial frontal cortex reliably triggered an evoked response in the cortical LFP. During SWS, the stimulation robustly evoked both k-complexes and low-voltage spindles and the stimulation-evoked LFP was visually similar to naturally occurring k-complexes followed by spindle activity. Quantitatively, we found that the rate of spindle occurrence during stimulation periods was significantly higher than during equivalent slow-wave periods in the pre-stim period before stimulation began. The results show that stimulation over frontal cortex with brief pulses can increase spindle density in a state-dependent manner along with coupling with other sleep-dependent memory-associated oscillations of SO and ripples. The observation that spindle density was enhanced during the stimulation session, but not in the post-stimulation recording session, may indicate that the effects of electrical stimulation may be restricted to a brief window The stimulation does not appear to trigger plastic mechanisms which might lead to long-term effects lasting beyond the duration of acute stimulation period. Another

very intriguing finding from qualitative observations was that the evoked response effects of pulsatile stimulation were state-dependent. Stimulation during waking states did not trigger spindles while stimulation during the transition from waking to SWS reliably triggered high-amplitude spindles, a separate phenomenon from the low-amplitude spindles associated with memory consolidation (supplementary figures S18).

The pulsatile stimulation method may be useful for future studies which attempt to boost memory via transcranial or intracranial stimulation. In order to enhance the effects of pulse stimulation, we could test different frequencies and amplitudes of stimulation to study which parameters are the best in triggering memory-related oscillations. In addition, we could also test whether stimulation delivered in specific layers of the cortex might have different effects. Further, we could test whether specifically targeting the networks that were activated by the experience in the recent past might increase memory enhancement, as these neuronal presumably are the ones most important to the consolidation of the specific memory. It would also be interesting to test the effects of stimulation specifically synchronized, via closed loop systems, to brain events such as UP-states, spindles and ripples. These stimulation parameters which may have a better potential to enhance spindle-SO coupling, spindle-ripple coupling and spindle-ripple-SO temporal coupling, which have each been individually and in combination shown to play a critical role in memory processing during SWS, specifically in hippocampal-neocortical communication during memory consolidation.

The ultimate purpose of the stimulation experiments was to study the enhancement of memory with electrical brain stimulation during SWS in a memory task specifically designed to test this phenomenon. In order to best investigate the anatomical and functional significance of electrically stimulating the brain during SWS, we would need to design experiments to fine tune different stimulation parameters of pulse stimulation, like the target region, frequency, amplitude

and phase with respect to endogenous brain activity. In addition, we would need to investigate which behavioural tasks could be best suited to study the effects. Perhaps a hippocampal-dependent, appetitively-motivated task, at least for rats, may be the best choice.

Modulating sleep spindles may be important in several situations as a therapeutic strategy to treat underlying cognitive deficits. Properties of sleep spindles have been shown to be modulated and can be a promising biomarker in several neurodevelopmental disorders (autism-spectrum disorders or attention-deficit hyperactivity disorders), neurocognitive disorders (mental retardation, cognitive impairments), neuropsychiatric disorders (schizophrenia, depression, mood and anxiety disorders), age-related neurodegenerative disorders (Alzheimer's, Parkinson's, Huntingtin's, dementia), sleep disorders (insomnia, sleep-related movement disorders) and epilepsy (Fernandez & Lüthi, 2020). Sleep spindles have been found to be elevated in patients with insomnia (Spiegelhalder et al., 2012) and disrupted in schizophrenia (Göder et al., 2015). Spindle activity during NREM2 correlates well with behavioural recovery in stroke and traumatic brain injury (TBI) patients. In stroke patients, increases in the power and coherence of sleep spindles was observed during chronic recovery (Gottselig et al., 2002). In TBI patients, there is a correlation between sleep spindles measured by EEG and higher levels of recovery measured by fMRI and PET neuroimaging techniques (Forgacs et al., 2014; Urakami, 2012). Increased oscillatory frequency of sleep spindles has been reported in PTSD, without any changes to the amplitude, duration, or density of slow or fast spindles (C. Wang et al., 2020). Impaired spindle-SO coupling is predictive of cognitive decline and pathological features of Alzheimer's disease (Winer et al., 2019). Spindles change across the lifespan from early development to adolescence and aging. Spindle amplitude, density and duration are higher in the early years and steadily decline with age (Clawson et al., 2016). Disruption in slow-wave spindle coupling correlated with cognitive decline and medial frontal atrophy (Helfrich et al., 2018). Aging-related reduction of prefrontal fast sleep

spindles correlates with poor episodic learning and impaired hippocampal activation during recall (Mander et al., 2014). It is conceivable to predict the therapeutic potential of brain stimulation in terms of enhanced memory for the aging population and people with brain damage, dementia and other developmental, cognitive or psychiatric disorders. Studies like these shed light on mechanism of memory that may lead to more dramatic treatments for memory impairment in the future.

While generalized stimulation of the brain may have some advantage in overall memory processing during post-experience states, the optimal stimulation methods would be able to activate specific memories of learning episodes for better consolidation, without generalized activation of neuronal networks. Electrical stimulation of the brain does not offer very good spatial resolution in terms of the effect on localized brain tissue (Jakobs et al., 2019). Electric current affects all the neural elements around the electrode tip including extracellular matrix, cellular membranes, axons, dendrites and soma depending on the parameters of electric current stimulation like frequency, amplitude, and pulse width (Dostrovsky & Lozano, 2002; Herrington et al., 2016). Whether this leads to an activation or inhibition of neural networks depends on the type of axon being activated, the effects on neuromodulators and neurotransmitters being released at the axonal terminals and the balance between excitation and inhibition caused by the complex interactions between orthodromic and antidromic action potentials (Jakobs et al., 2019). The methodologies to enhance memory seek to increase inherent adaptive processes underlying memory consolidation while suppressing maladaptive processes. For example, the synaptic homeostasis hypothesis (Tononi & Cirelli, 2003, 2006) proposes an adaptive process of synaptic tagging. However, with electrical stimulation, it is not clear how a molecular-level processes such as this could be selectively influenced. Approaches that are more targeted, with a high spatial and temporal resolution, like optogenetics, may be more useful as a manipulative tool to enhance specific memories. Recent evidence has shown that defined cell populations can form a cellular basis for memory engrams

(X. Liu et al., 2012; Ramirez, Tonegawa, & Liu, 2014; Tonegawa, Pignatelli, Roy, & Ryan, 2015). A robust demonstration of this phenomenon was provided by selective labelling of neuronal population activated by a fear conditioning experiment in mice. Subsequent optogenetic activation of this memory engram of hippocampal cells (specifically dentate gyrus) that were selectively labelled during encoding of fear conditioning elicited freezing behaviour (X. Liu et al., 2012), indicative of fear memory recall. This demonstrated that memory engram cells have causal contributions to behaviour and that direct activation of the subset of cells involved in memory formation (memory engram) was sufficient to induce the behavioural expression of that memory in the form of freezing. The precise tagging of relevant cells opens avenues for future manipulation by targeting specific brain regions, specific neuronal networks of cell populations and specific cell types, involved in a particular memory. Thus, high spatial and temporal resolution provided by optogenetic manipulations provide a promising potential in targeting behaviorally-relevant cell ensembles and manipulating specific memories without generalised manipulations over large neuronal networks.

Is there a cost to enhancing memories in general? More specifically, in the context of this thesis, is enhancing memory by eliciting spindles even a good idea? This question remains to be answered after investigating the memory enhancing effects of stimulation. Baseline sleep spindle activity correlated well with increased post-learning spindle activity and memory recall in humans (Manuel Schabus et al., 2004), but this effect was found to be reversed in a rat study of avoidance learning (SM Fogel et al., 2010). Non-learners in the avoidance task had higher baseline sleep spindles which was unaffected by training, while learning rats had lower baseline sleep spindles which increased and correlated with learning. Thus, sleep spindle density can be a predictor of learning and in some cases, higher spindle activity may represent consolidation of maladaptive information and suppression of adaptive processes through homeostatic regulation. It is possible

that spindles are subject to homeostatic regulation, put in place to compensate for other physiological changes occurring during normal aging and in other neurodevelopmental, neurocognitive and neuropsychiatric disorders and there would be a cost to enhancing them. Further, non-pathological or natural forgetting in healthy brains seems to suggest that it may represent an important feature of normal memory functioning and could offer potential adaptive value rather than being a maladaptive process (Ryan & Frankland, 2022). The methodologies involved in enhancing memories, could counteract natural processing in the brain, which could come at a cost.

Overall, brain stimulation techniques are powerful tools that have shown promising impact through their neuromodulation effects in enhancing memory in humans and animal models. They have advanced our understanding of memory consolidation processes in brain networks, including interaction between global and regional brain oscillations facilitating the process and investigation of underlying pathophysiology. Research to improve the stimulation parameters for maximum efficacy for the enhancement of endogenous oscillations and coupling with regional brain oscillations, can help the adaptation of this technology in the development of novel therapeutics for the safe and effective treatment of several brain dysfunctions.

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APPENDIX

Supplementary material (specific to Chapter 4; up to Fig S10):

All procedures were performed in accordance with the Canadian Council for Animal Care guidelines as well as University of Lethbridge guidelines. Four adult male Fisher-Brown Norway rats had electrodes implanted in the cortex, hippocampus, and neck for recording. The stimulating electrode was implanted in the deep cortex adjacent to one of the cortical recording electrodes (Fig S1 for details).

Prior to recordings, the animals were habituated to a quiet, dimly lit room and recording box for 3 days. On experiment days, rats were taken to the recording room and they rested in a small cage after being connected to the recording system. The recording consisted of three consecutive 1-hour sessions: a baseline recording with no stimulation, a recording with single pulse electrical stimulation, and a final recording session with no stimulation.

Recording was done with Cheetah data acquisition software (Neuralynx, Boseman, Montana). Signals were filtered between 0.1 and 500 Hz, sampled at 2 kHz, and referenced to a skull screw above the cerebellum. Biphasic pulse stimulation (150 uS per phase, 500 uA) was delivered by a constant-current stimulus isolation unit that was controlled by an Arduino microcontroller. Delivery of stimulation pulses was controlled by a real-time sleep state detector written in MATLAB (MathWorks, Natick, Massachusetts). A 3-second buffer of LFP recording (Local Field Potential; updated every 500 ms) was used to calculate the Fourier transform of the hippocampal and EMG signals. For the hippocampal LFP, the ratio of delta (1-4 Hz) to theta (6-10 Hz) power was calculated. For the EMG signal, the ratio of power in the high (100-300) to low (10-20) frequencies was calculated. The hippocampal delta/theta ratio was divided by the EMG value to obtain a SWS score. For each animal, the threshold value for the SWS score was determined during a habituation session prior to the start of the stimulation experiment. The

threshold was adjusted so that there was good correspondence between the online SWS detector and the offline sleep structure analysis. Once SWS was detected, a single pulse was delivered every 3 seconds for as long as the animal remained in SWS (Figure 1A), and the timestamp of the pulse was logged in the recording system.

Data analysis

The EMG signal was thresholded manually to segment the recording into epochs of rest and movement. Rest epochs were further classified as SWS or REM based on the method used in the real-time detection of SWS (delta power / theta power / EMG power). For the stimulation sessions, stimulation artifacts were removed from the LFPs prior to analysis (Fig. S2 for details). Stimulation evoked SOs and spindles in both hemispheres, so we averaged measures from both hemispheres to obtain a single value for a rat. In one rat, SOs were not detectable in one hemisphere because of electrode placement so that hemisphere was excluded (only for SO; spindles were detectable and included in the average). SOs were detected using a time-shifted difference signal. To generate the difference signal, the raw signal was subtracted from a time-shifted (35 ms) version of the signal. This difference signal emphasized large amplitude fluctuations on a timescale similar to SOs. Peaks in the difference signal greater than 5 SD were considered SOs. For spindle detection, the cortical LFP was filtered between 10-20 Hz, then squared and smoothed (175 ms rectangular window, step size 1 sample) to obtain a power signal. Periods of significant spindle activity were determined by finding peaks greater than 2 SD in the power signal, and then the start/end timestamps were found by measuring the power before/after the peak until the power fell to 0.75 times the peak threshold. This method yielded a list of timestamps indicating the start and end timepoints of each spindle. Detected events shorter than 200 ms and longer than 2 sec were discarded. For SWR, the signal was filtered between 100-250 Hz, then squared and smoothed (20

ms rectangular window, step size 1). Peaks greater than 3 SD were considered potential SWR. Start/end timestamps were found by measuring the power before/after the peak until it fell to 0.5 times the peak threshold. Events shorter than 15 ms and longer than 300 ms were discarded. Threshold values for automated detection of LFP features was done while visually inspecting the pre-stimulation recording from day 1, and then verifying that they were appropriate by checking the pre-stimulation session from days 2-5. Once set, the same thresholds were used for all recording sessions on all days.

The following spindle measures were calculated: density, amplitude, frequency, and duration. Density was calculated as the number of spindles per minute. Amplitude was calculated as the maximum of the Hilbert transformed LFP within the start/end timestamps. Frequency was calculated as the average of the instantaneous peak-to-peak frequency. Duration was calculated as the difference in the start/end timestamps. For each SWS epoch, the average of these measures was calculated to obtain a single value for that epoch. The average of all SWS epochs in the 1-hour recording session was used to determine statistical significance between sessions.

SO-spindle coupling was quantified by two measures. First, the proportion of spindles occurring within 750 ms was calculated for each session (Charles-Francois V. Latchoumane, Hong-Viet V. Ngo, Jan Born, & Hee-Sup Shin, 2017). Second, the PSTH of spindle occurrences relative to SOs was calculated.

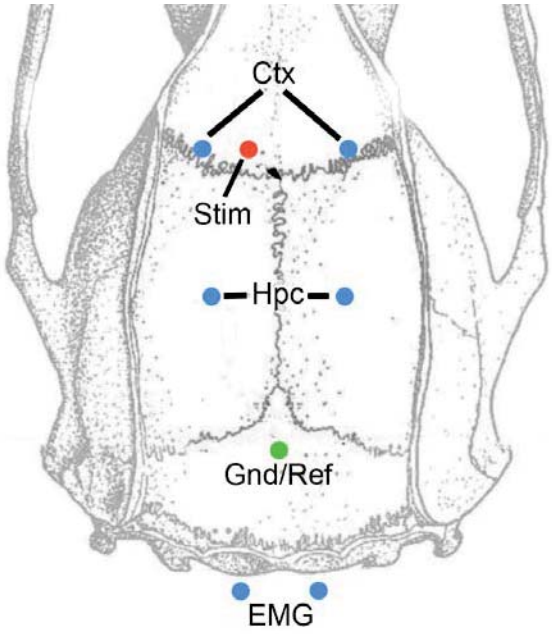


Figure S 1: Surgery methods.

Twisted bipolar electrodes (Teflon-insulated stainless steel, ID: 76 um) were implanted bilaterally in the motor cortex for recording (1.0 mm anterior, 3.0 mm lateral), in the corpus callosum adjacent to the motor cortex for stimulating (1.0 mm anterior, 1.5 mm lateral), in the hippocampus for recording (3.5 mm posterior, 2.6 mm lateral, 2.6 ventral). Multi-strand stainless steel wire was inserted in the neck muscle to record EMG activity.

Fisher 344/Brown Norway rats were between 4-8 months old and weighed between 350-450g at the time of surgery. They were housed individually in a vivarium on a 12 h light cycle (7am lights on), and they had free access to food and water. Following surgery, rats were given Metacam for 3 days as an analgesic, and Baytril antibiotic for 5 days. They were allowed 1 week of recovery prior to the start of experiments. Recordings were done during the light cycle.

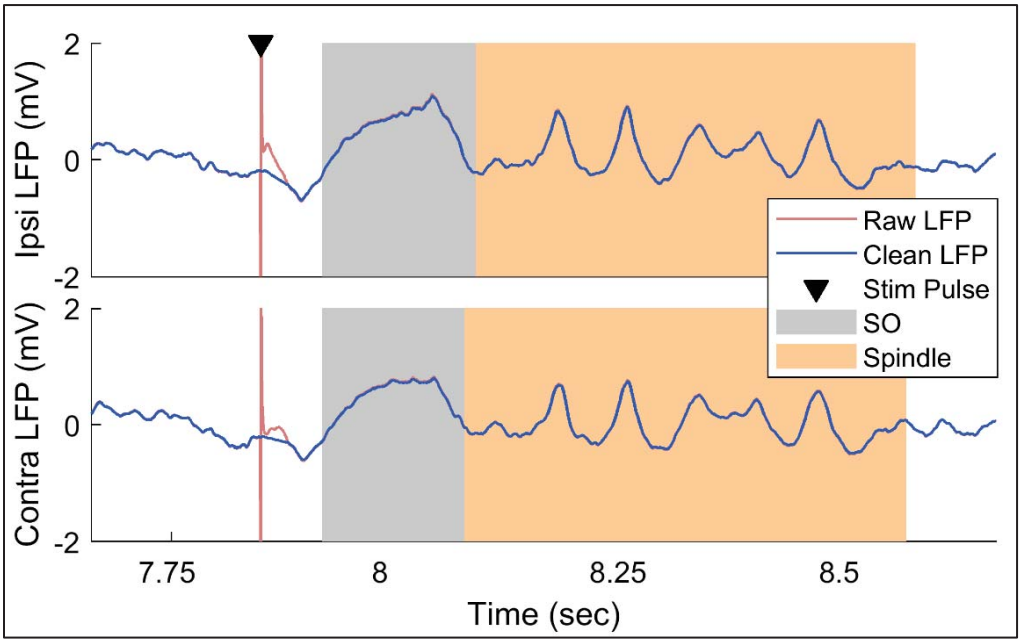


Figure S 2: Removal of artifact caused by the stimulation pulse (enlarged from Fig. 1A).

Artifact times were identified by finding the 99.95 percentile of the first derivative of the LFP. The first part of the artifact, containing the largest deflections, was removed by reducing the amplitude with an inverted Blackmann window (width 6-9 ms). Subsequent slower electrical transients were removed by subtracting a smoothed signal (2.5 ms rectangular window, step size 1 sample) from the raw signal, effectively high-passing the signal with ½ amplitude frequency of 90 Hz. The duration of the second part of the correction ranged between 25-27 ms and did not impact SO or spindle detection.

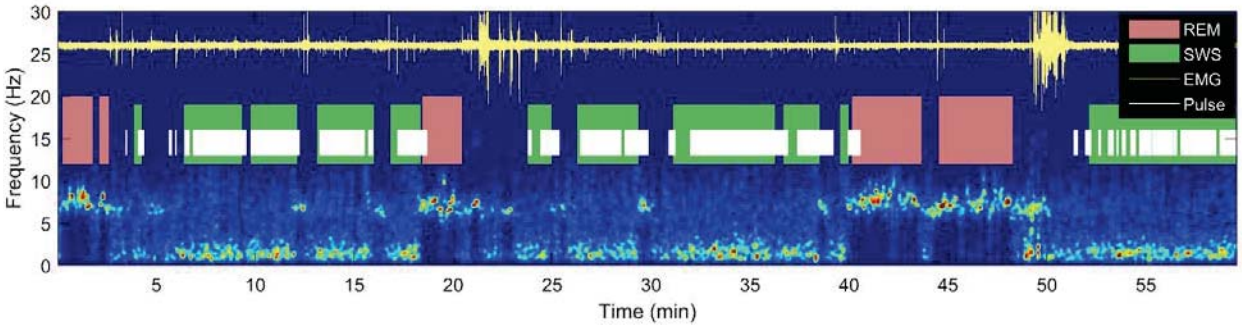


Figure S 3: Real-time detection of SWS and delivery of stimulation pulses during a representative stimulation session.

Background image shows the spectrogram of the hippocampal LFP, and the EMG trace is in yellow at top. Red/green patches show offline detection of REM and SWS. White patches show times when SWS was detected in real-time and stimulation pulses were delivered.

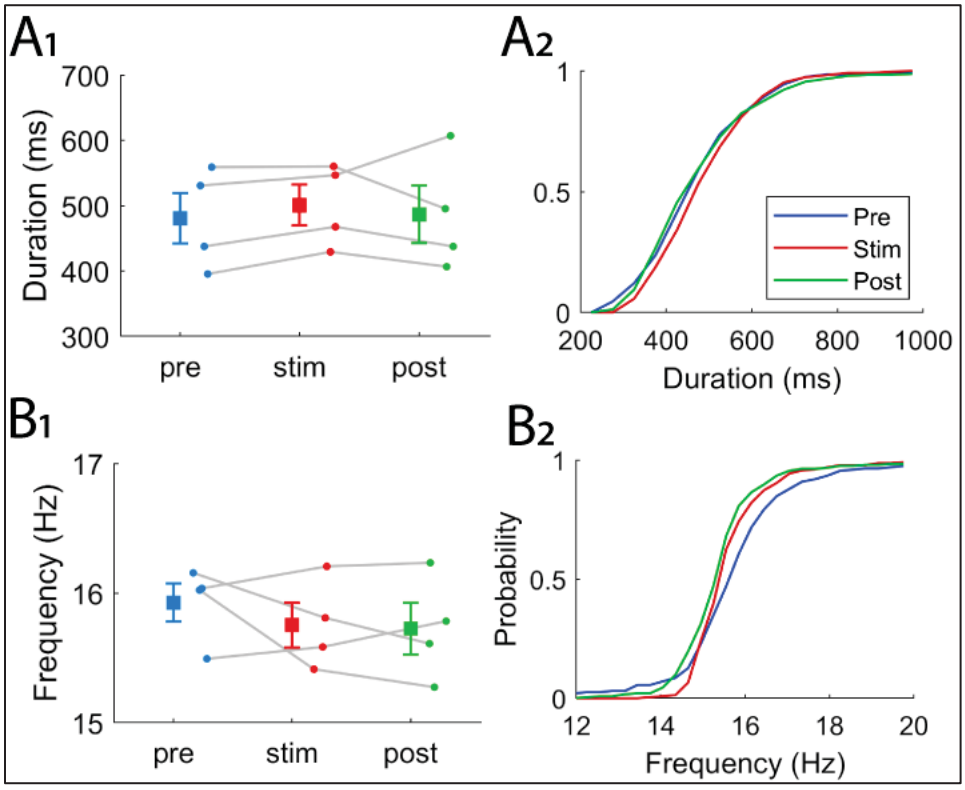


Figure S 4: Stimulation does not alter duration (A1,2) or frequency (B1,2) of spindles.

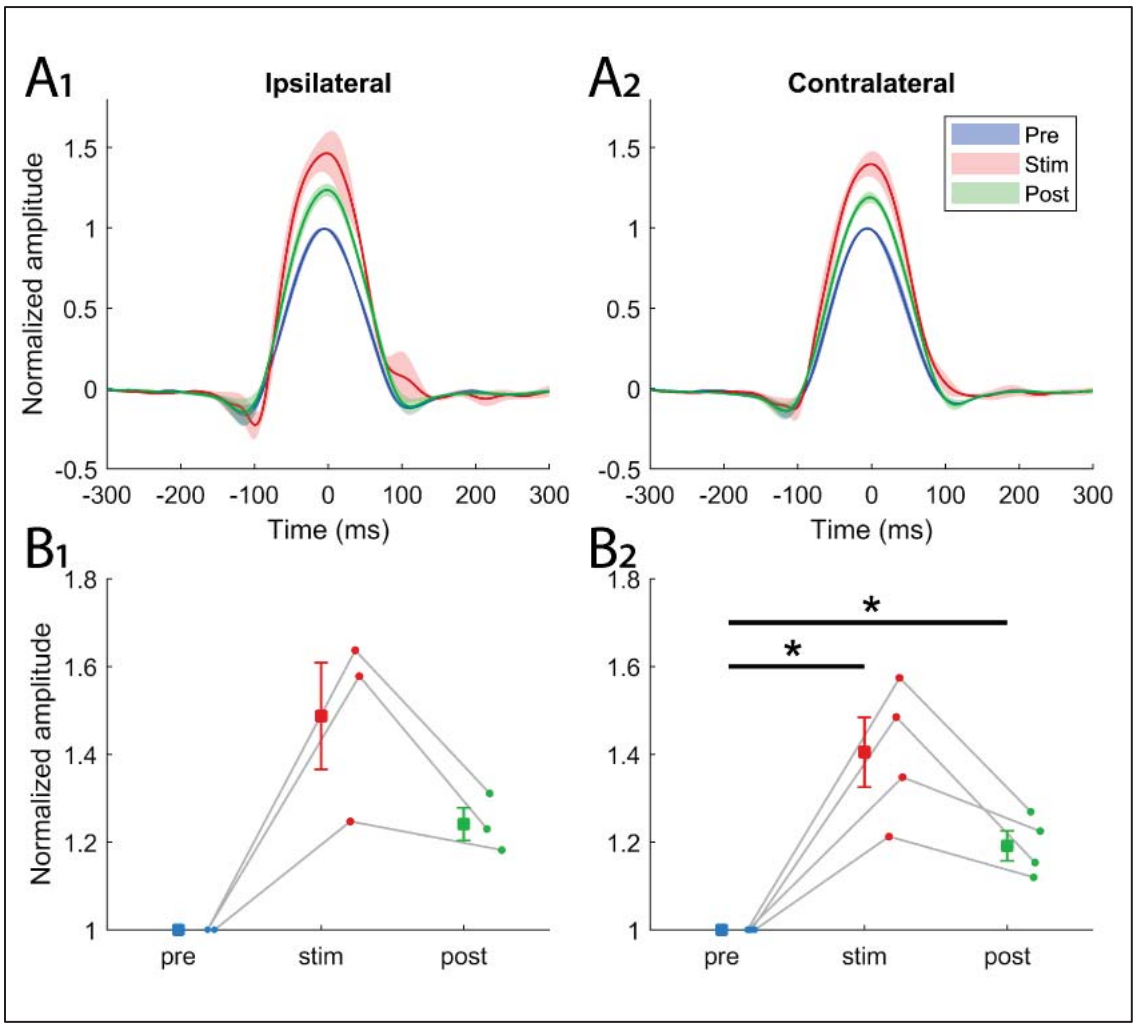


Figure S 5: Comparison of spontaneous and evoked SOs.

(A1,2) Average waveforms of spontaneous and evoked SOs. Amplitude is normalized to peak of spontaneous SO in each rat. Shaded region is SEM of n = 3 (Ipsilateral) or 4 (Contralateral) rats. (B1,2) Quantification of amplitude across recording sessions (*$p < 0.05$, Bonferroni corrected; pre-stim $t_3 = 5.12$, stim-post $t_3 = 5.68$).

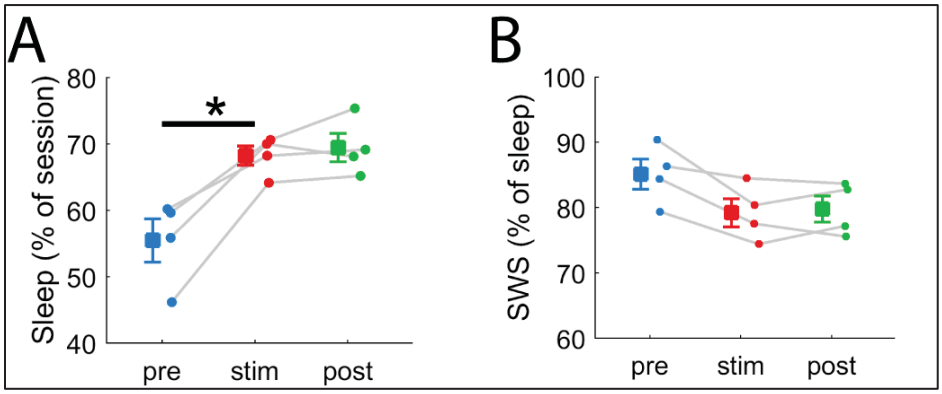


Figure S 6: Sleep structure across recording sessions.

(A) Percent of time spent sleeping was less during the pre-stim epoch, likely because the rat had been transported from the colony room and was still aroused (*$p < 0.05$, $t3 = 5.98$, Bonferroni corrected). The amount of sleep during the stim and post-stim sessions was similar. (B) The proportion of SWS was not significantly different across sessions, indicating that stimulation did not alter sleep structure significantly.

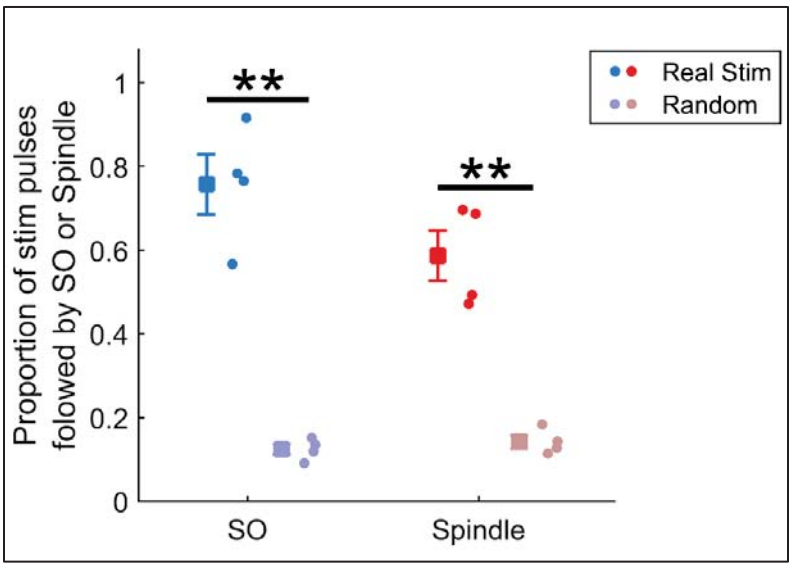


Figure S 7: Reliability of stimulation measured by the proportion of pulses that evoke SOs or spindles.

The proportion of SOs and spindles following real stimulation pulses (SO: < 500 ms; Spindle: < 750 ms) was significantly greater than an equivalent number of randomly distributed times during SWS (**$p < 0.01$; SO: $t3 = 8.20$; spindle: $t3 = 7.61$, $p < 0.01$).

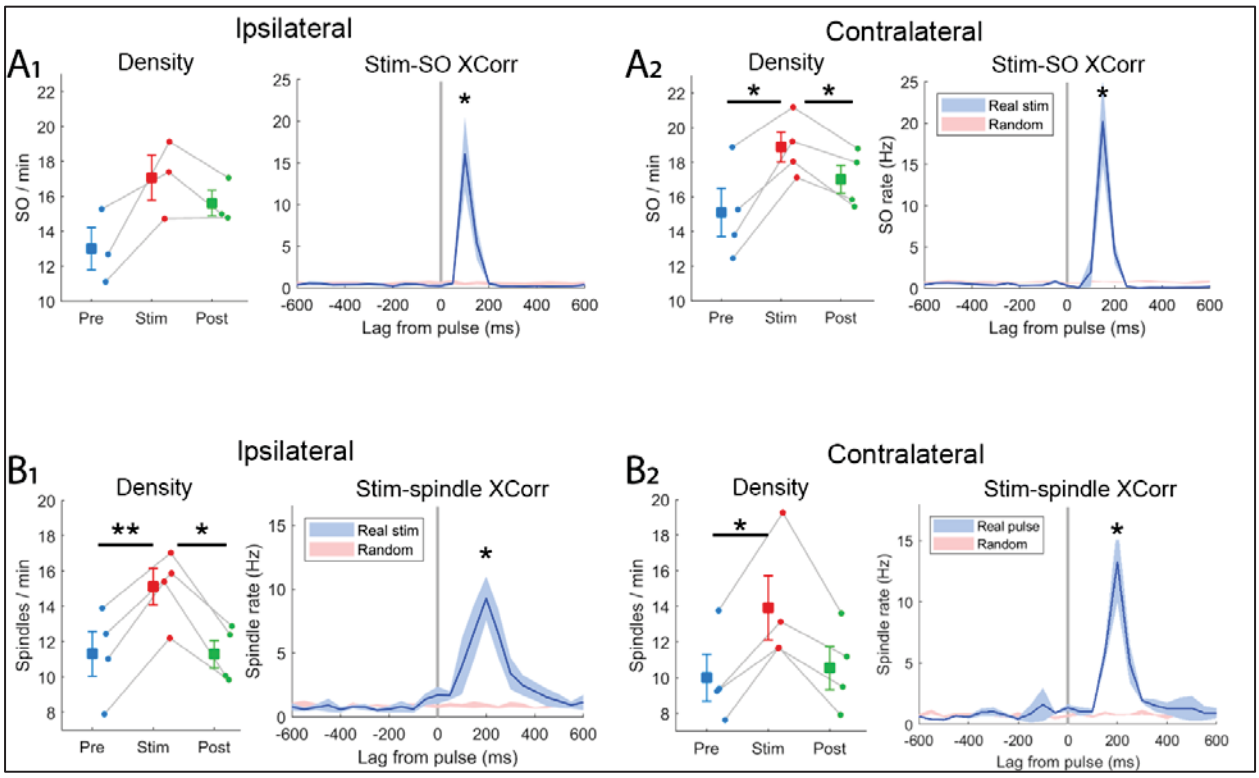


Figure S 8: SO and Spindle measures by hemisphere (relative to stim electrode).

(A1) Ipsilateral SO density (# / min) and cross correlation with stimulation pulses ($t_2 = 5.23$; n = 3 because one rat did not have measurable SO's). (A2) Contralateral SO density (pre-stim $t_3 = 5.10$, stim-post $t_3 = 5.16$) and cross correlation with stimulation pulses ($t_3 = 3.67$). (B1) Ipsilateral spindle density (pre-stim $t_3 = 12.4$; stim-post $t_3 = 5.56$) and cross correlation with stimulation pulses ($t_3 = 3.40$). (B2) Contralateral spindle density ($t_3 = 6.26$) and cross correlation with stimulation pulses ($t_3 = 5.75$). All panels: *$p < 0.05$, **$p < 0.01$, p-values in density panels are Bonferroni-corrected for 3 comparisons.

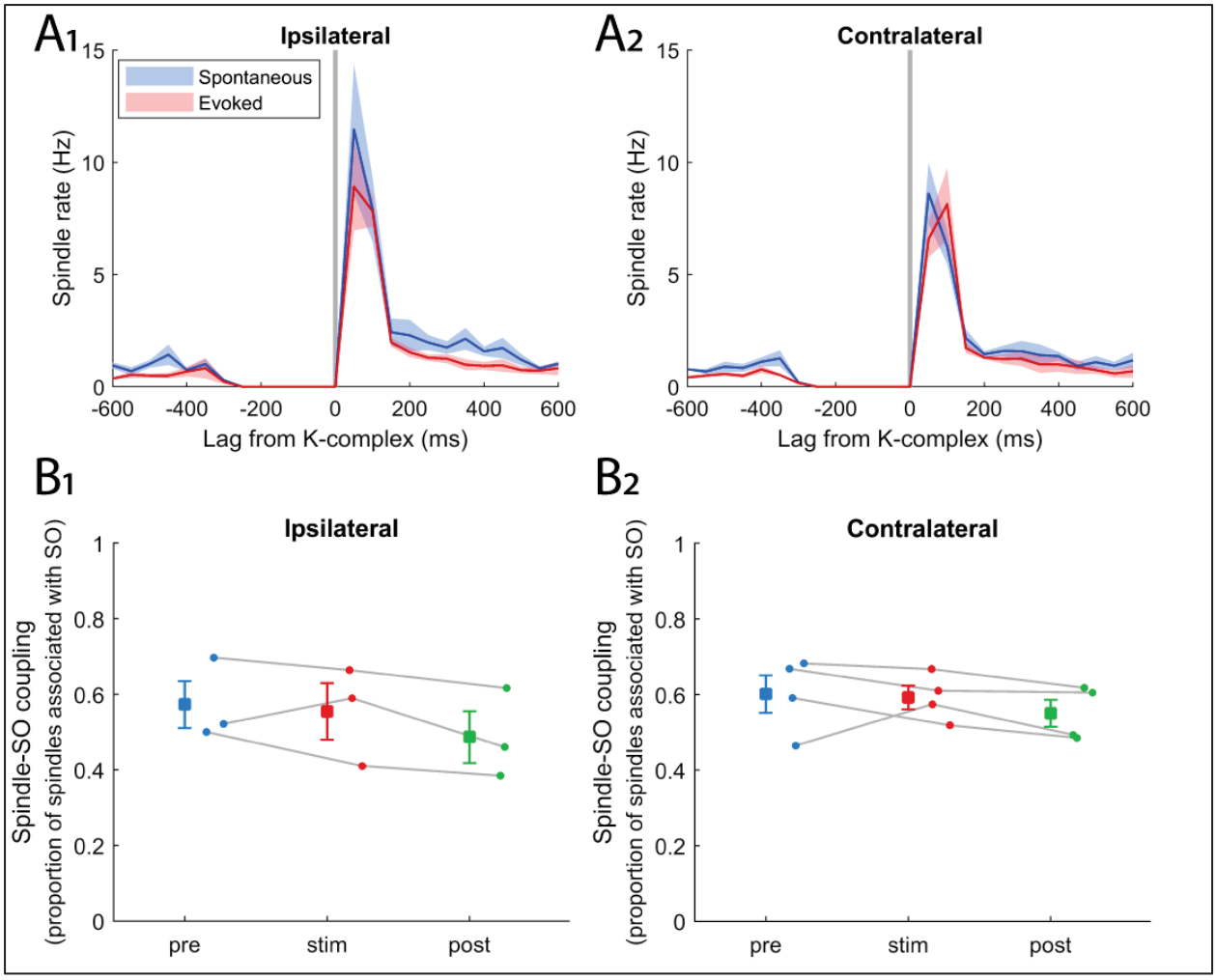


Figure S 9: Stimulation does not affect coordination between SOs and spindles.

(A 1,2) PSTHs of spindles locked to SO time for ipsilateral (n =3), and contralateral (n=4) hemispheres. (B 1,2) Spindle-SO coupling (proportion of spindles that occur within 750 ms of a SO) is not changed by stimulation.

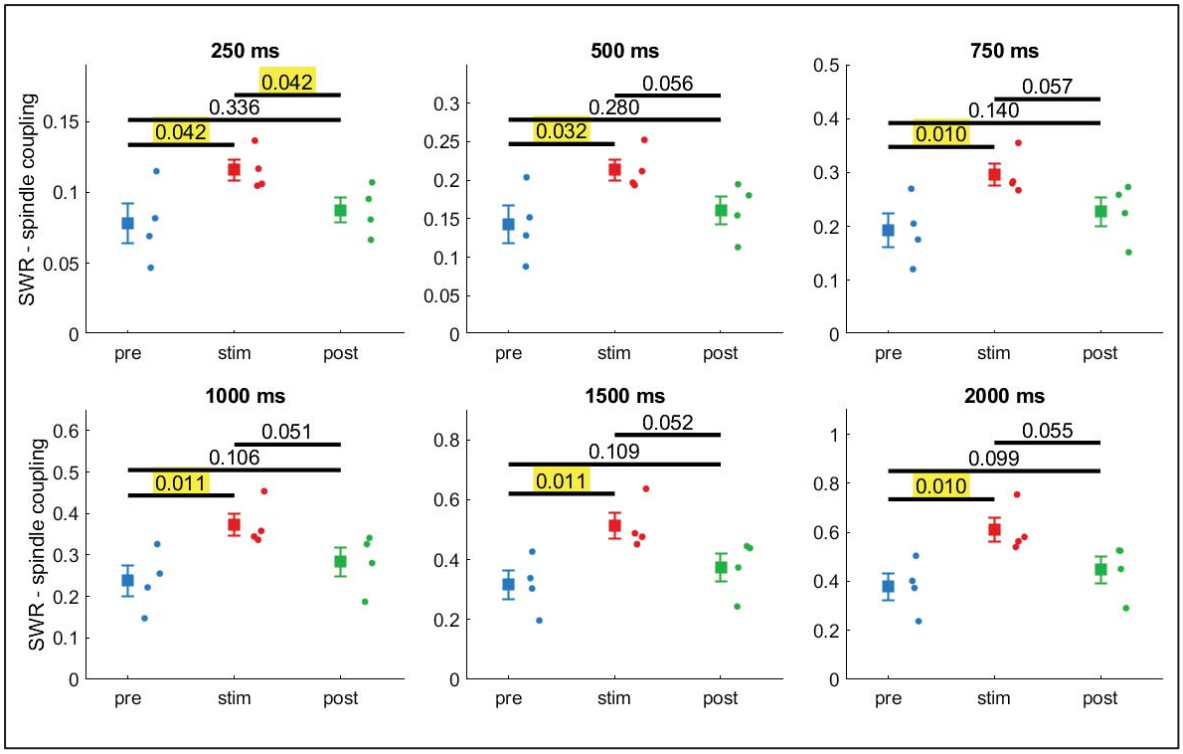


Figure S 10: Increased SWR-spindle coupling is significant when a range of inter-event gaps is tested (250 – 2000 ms).

The title of each panel shows the maximum duration between a SWR and spindle for the spindle to be considered coupled to the SWR, and the values indicate the p-value for the comparison indicated by the bar. In all cases, the SWR-spindle coupling increases significantly during the stimulation session.

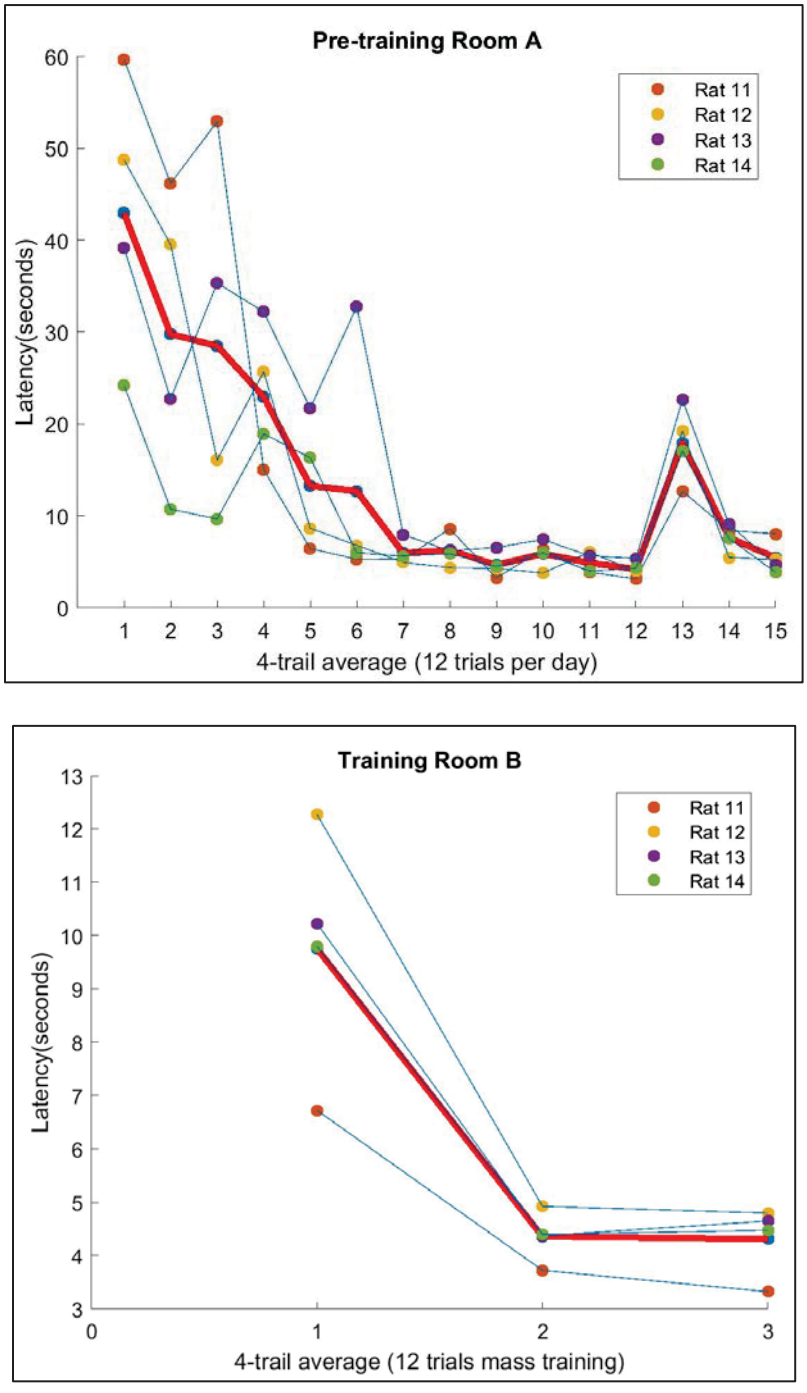


Figure S 11: Modified Morris Water Task behavioural result

(A) Pretraining in room A. Four trial averages of the latency in seconds to find the platform over training 4 days/12 trials per day and reversal training. **(B)** Mass training in room B. Four trial averages of the latency in seconds to find the platform over training. The solid red line is the group average.

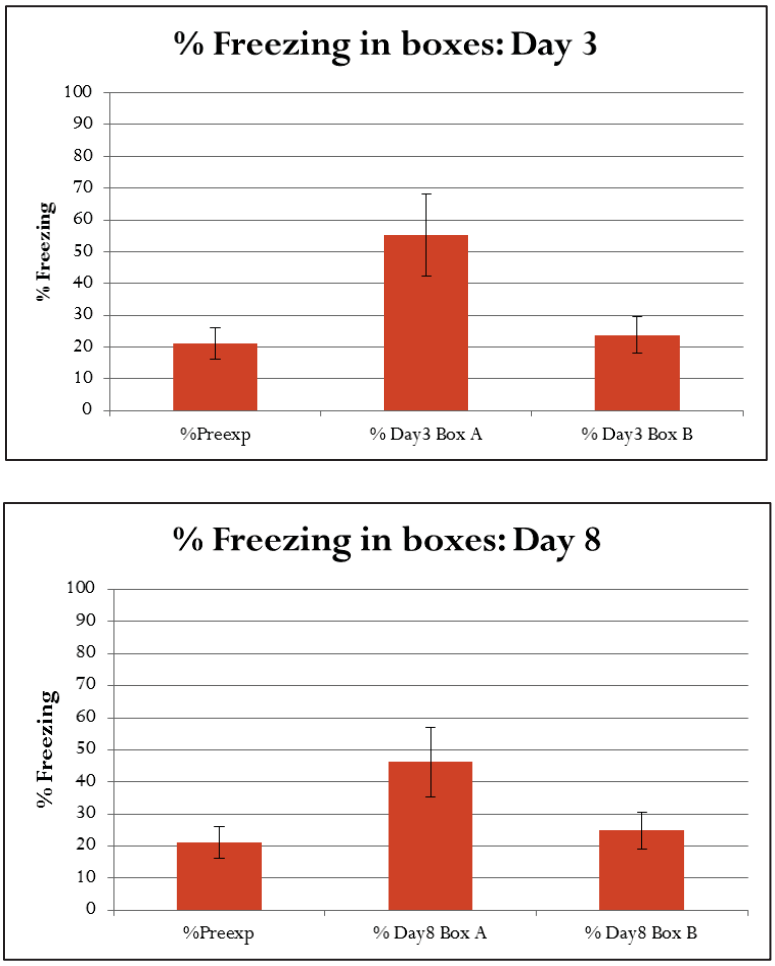


Figure S 12: Context pre-exposure and immediate shock deficit fear conditioning experiment behavioural results

Percentage freezing in original, associated context (context A) and actual conditioning context (context B). A transport–context association is established through preexposure (context A) and then this transport context is used to bring rats into a different context (context B) for immediate-shock conditioning. Rats were tested in the original, associated context and the actual conditioning context. As expected, rats displayed freezing in the original associated context and not in the actual conditioning context, strongly supporting the idea that the rats were conditioning to the memory representation active at the point of shock, and not to the physical cues.

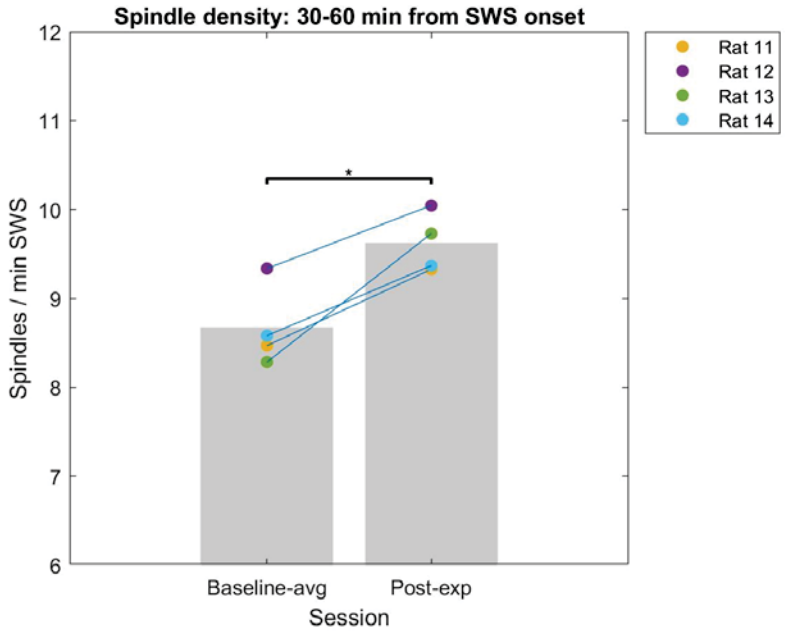


Figure S 13: Spindle density in the 30-60 minute time window after SWS onset novel exposure experiment 1

The bar with asterisk indicates the comparison reached statistical significance ($p<0.05$).

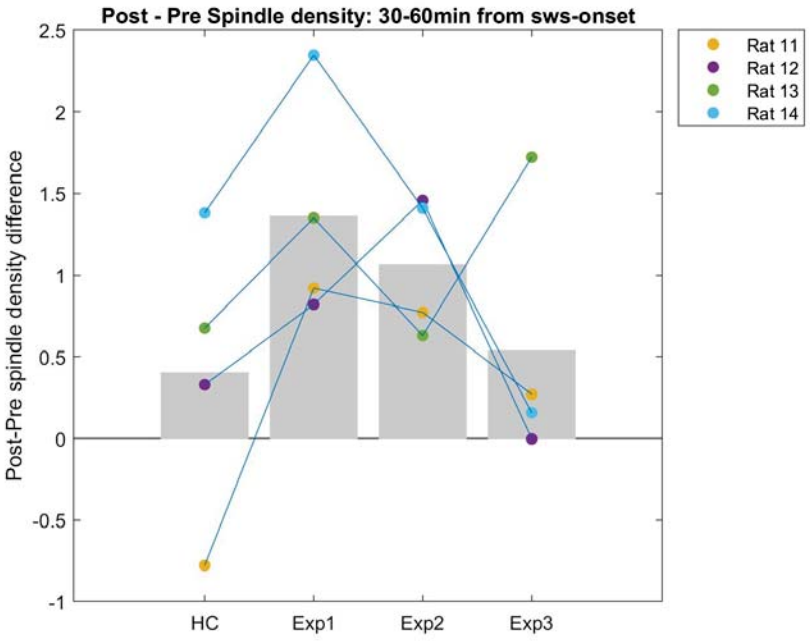


Figure S 14: Difference in spindle density between post-experience and pre-experience sessions in the30-60 minute time window after SWS onset in novel exposure experiment 2

The bar with asterisk indicates the comparison reached statistical significance ($p<0.05$).

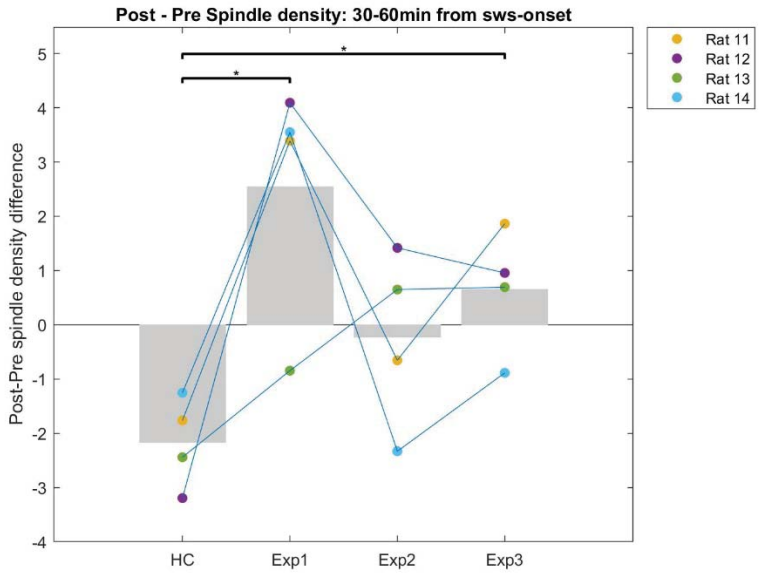


Figure S 15: Difference in spindle density between post-experience and pre-experience sessions in the 30-60 minute time window after SWS onset in novel exposure experiment 3

The bar with asterisk indicates the comparison reached statistical significance ($p<0.05$).

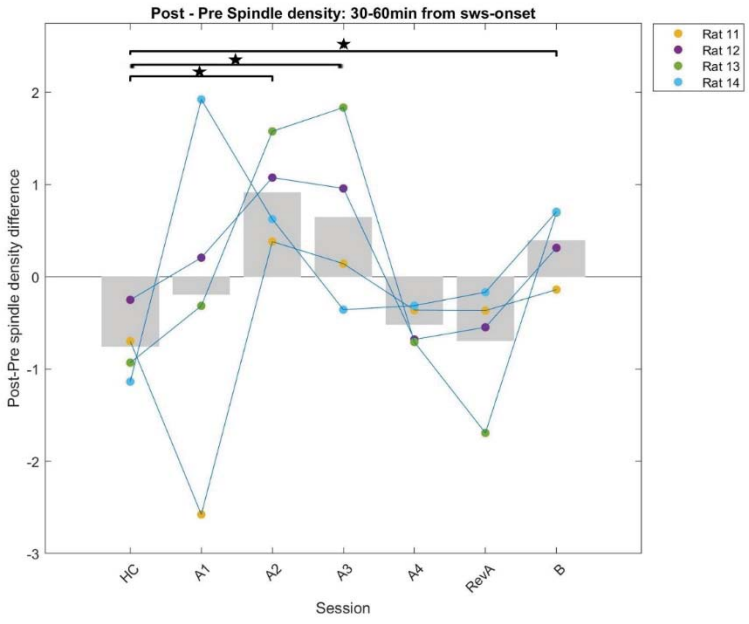


Figure S 16: Difference in spindle density between post-experience and pre-experience sessions in the 30-60 minute time window after SWS onset in the water task experiment.

The bar with asterisk indicates the comparison reached statistical significance ($p<0.05$).

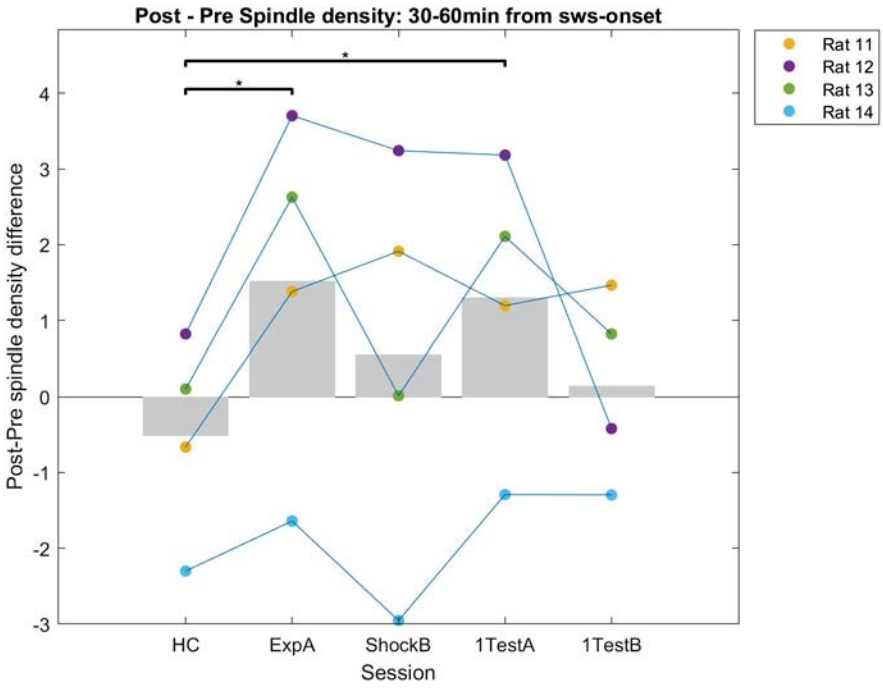
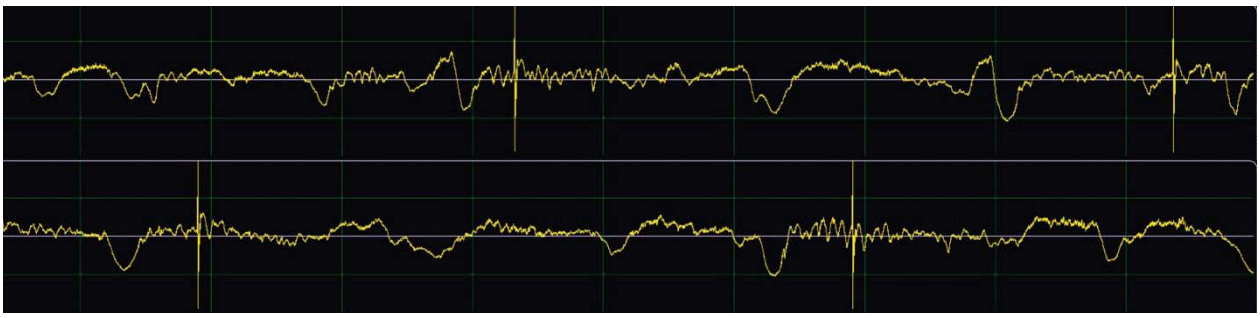


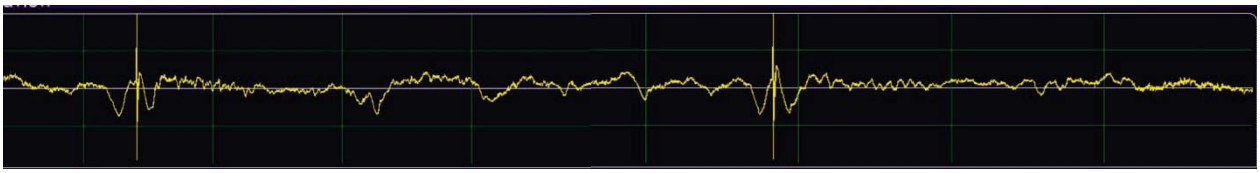
Figure S 17: Difference in spindle density between post-experience and pre-experience sessions in the 30-60 minute time window after SWS onset in the fear experiment.

The bar with asterisk indicates the comparison reached statistical significance ($p<0.05$).

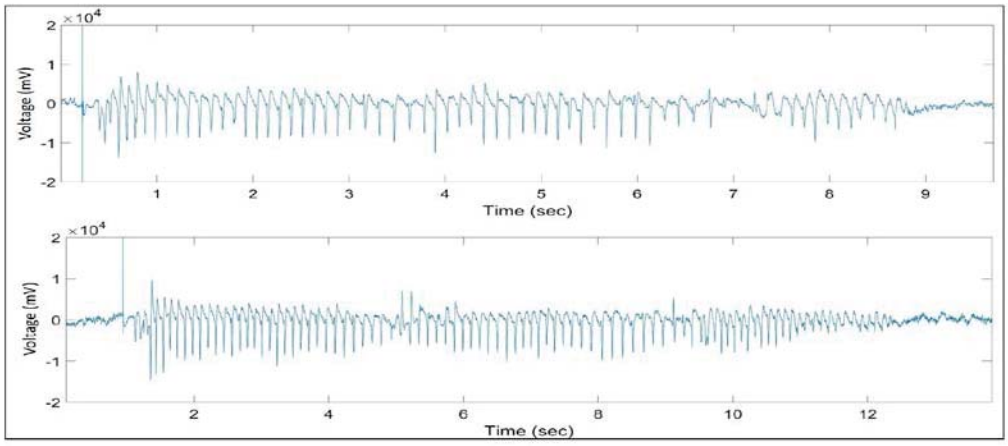
(a)



(b)



(c)



(d)

(e)

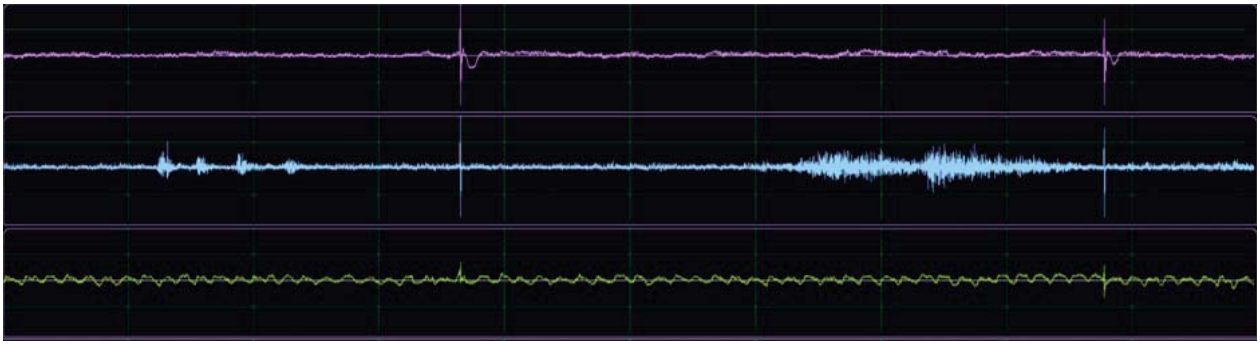


Figure S 18: Snapshots of pulse-stimulation evoked responses in the different brain states

One of the interesting observations during the conduct of the pulsatile stimulation study was that the effect of stimulation was state-dependent. Note that these observations were incidental and not thoroughly analyzed. Timing of pulse stimulation is clearly seen in each snapshot.

a. Pulse stimulation during SWS spindles did not show any evoked response: No effect during spindles, if not spindling it causes a k-complex. Snapshots of cortical LFP recording.
b. Pulse stimulation during SWS right after a k-complex evoked a second k-complex followed by a spindle. Snapshots of cortical LFP recording.
c. Pulse stimulation during transition state from wake to sleep evoked High voltage spindles. Snapshots of cortical LFP recording.
d. Pulse stimulation during REM: brief disruption of theta cycle in hippocampus and evoked k-complex in cortex without spindle. Snapshots of cortical LFP recording (top) and hippocampal LFP (bottom).
e. Pulse stimulation during awake state: brief disruption of theta cycle in hippocampus and evoked k-complex in cortex without spindle. Snapshots of cortical LFP recording. Snapshots of cortical LFP recording (top), EMG (middle) and hippocampal LFP (bottom).

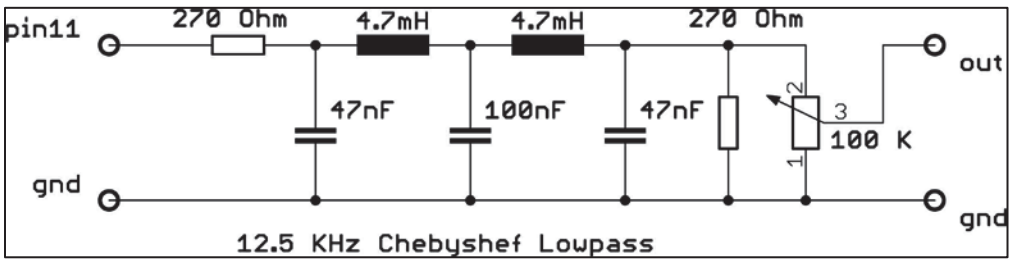


Figure S 19: Chebyshev low pass filter as a sine wave generator

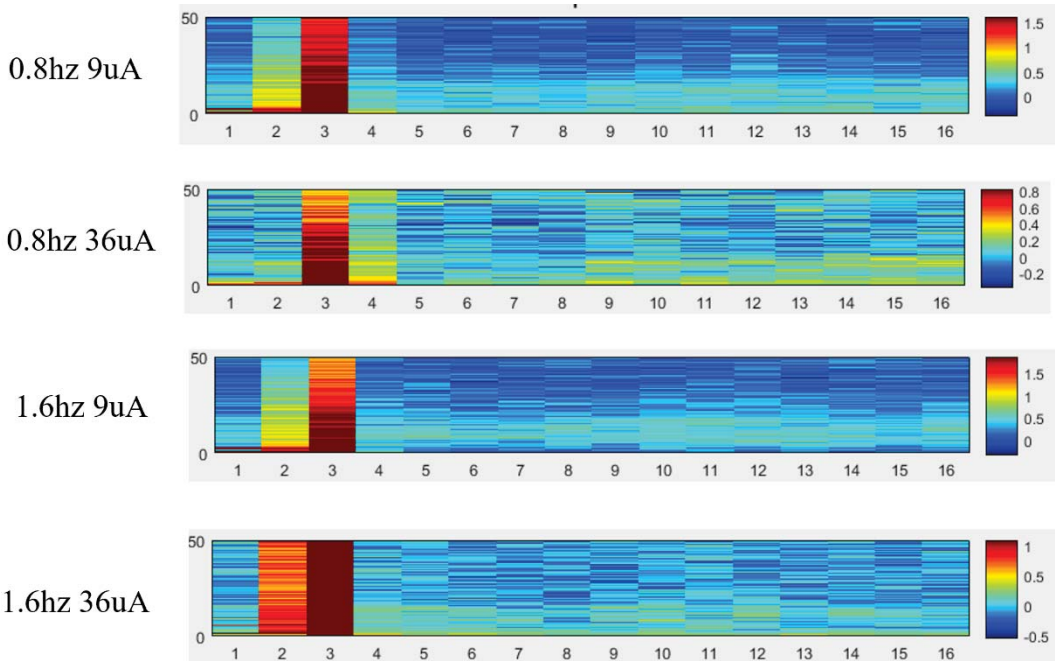


Figure S 20: Spectrogram of 2 time-bins before stim-off and 13 time-bins after stim-off

Each time-bin is 2sec; stim-off: time-bin 2/3 with the observable transient.
x-axis: Timebins
y-axis: upto 50Hz frequency
z-axis: z-scores of inter-stimulation periods compared with mean and standard deviation of comparable sham stimulation periods.