Episodic-like memory consolidation during slow-wave sleep

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Tübingen, den . . . .

Carlos Nicolás Oyanedel Salmerón

"Memory requires more than the mere dating of a fact
in the past. It must be dated in *my* past."

William James, 1890

Table of Contents

Abstract

Episodic memory as a form of declarative memory allows an individual to remember events from his or her own past; it is defined as the ability to replay in mind – with autonoetic consciousness – an event as it happened in a specific spatio-temporal context. Animal experiments on episodic memory, because they cannot address the subjectivity of autonoetic consciousness, focus on its major feature, i.e., the binding of an event into its spatio-temporal context. Two major paradigms have been used to investigate episodic memory in rodents: the 'what-where-when' (WW-When) and the 'what-where-which' (WW-Which) task. Both tasks measure exploration preferences for objects and locations to assess behavioral correlates of event-context binding underlying episodic memory. Sleep supports the consolidation of episodic memories and particularly the components dependent on hippocampal function. The *systems consolidation model* proposes that slow wave sleep (SWS) orchestrates different brain rhythms, namely the co-occurrence of neocortical slow oscillations (SOs), thalamic sleep spindles and hippocampal ripples. The latter accompany memory reactivation.

In this thesis, I investigate in rats how sleep affects the consolidation of episodic memory at behavioral and electrophysiological level. In the first set of studies (1 and 2), I demonstrated that sleep is critical for preserving an integrated episodic representation over intermediate time intervals. The first study also adds novel evidence supporting a positive relationship between the amount of slow oscillatory EEG activity during SWS and the successful performance of WW-When task. Moreover, sleep not only supports the consolidation of episodic memory measured by this task, but is also critical when the broader context in which the episode took place is considered (WW-Which task). In the third and fourth studies, I focused on brain oscillatory dynamics in neocortex and hippocampus during sleep. First, I addressed the question whether sleep and its composing sleep stages, i.e., SWS, intermediate stage and REM sleep, occur as unitary phenomena affecting the whole brain in a congruent fashion. The results show that although SWS congruently arose in signals covering the activity of both regions, REM sleep often started substantially earlier in the hippocampus than in neocortex. This not only shows a region-specific regulation of REM sleep, but also might give a unique time window for memory-related synaptic plasticity. Finally, the dialogue between neocortex and hippocampus during SWS presents a loop-like interaction of oscillatory events, where SOs can trigger spindles, and spindles can regulate the occurrence of ripples, independently from the occurrence of SOs. In turn, ripples can contribute to the emergence of SOs independently from spindles. These results shed new light on the role of

sleep for the consolidation of episodic memory by unraveling its electrophysiological underpinnings and the temporal dynamics between neocortical and hippocampal networks. Together, these findings pave the way for future studies exploring the mechanisms mediating the dissociation between hippocampal and neocortical networks and its relevance for episodic memory consolidation.

Synopsis

1. Memory formation

Each day, our brains are exposed to a huge amount of information coming from the surrounding world. However, a high percentage of this information, mainly the bits we might consider irrelevant to our survival, is actively forgotten (Poe, 2017). What happens with the information that must be retained? Certainly, the need to preserve important memories, i.e., to create new memory traces in our brains, is fundamental for adapting our behavior and our interaction with the environment more efficiently, and, therefore, enabling us to survive. Nowadays, the idea that memories are established in three stages is broadly supported. New information is acquired (encoding), then strengthened (consolidation) and finally the memory is accessed, and thus recalled (retrieval) (Feld & Diekelmann, 2015). Here, the so-called consolidation process becomes important, since it is during this period when the newly encoded information is transformed so it can persist over time.

The memory consolidation concept was first proposed by Müller and Pilzecker in 1900. After performing a series of 40 experiments, they concluded that learning does not induce instantaneous and permanent memories, but rather that memories need time to become permanent, and that, before this step, these representations remain vulnerable to disruptions (Mullner & Pilzecker, 1900; reviewed in Lechner et al., 1999). The concept of memory consolidation accounts for the idea that items or representations change their susceptibility to be forgotten or modulated over time. Lately this has led to the idea that consolidation is a time-limited mechanism by which memory representations are coded or fixed (Glickman, 1961; McGaugh, 1966). However, the notion of memories being simply fixed has given way to the reconsolidation hypotheses (Nader, 2003). During the reconsolidation process, previously consolidated memories first become labile during retrieval and then are stabilized (Tronson & Taylor, 2007). Therefore, consolidation of memories balances stability and flexibility, allowing adaptive representations (Dudai et al., 2015).

This leads to the question how exactly memories are consolidated. They could be stored independently as individual associations, or the new information could be integrated within systematic organizational structures. During the first half of the last century, Piaget (1928) and Bartlett (1932) suggested that memories are not stored in isolation but form parts of an integrated schematic organization from which we are able to extract direct and indirect associations (Eichenbaum, 2017). In the late 1940s, Hebb postulated that the basic unit for perception and memory is a cell assembly. If a locally interconnected assemble of neurons is

activated during a particular event, the efficacy of the connection will increase and, thus, also the strength of the representation (Hebb, 1949). Therefore, our current understanding of memory formation relates new memory representations to structural synaptic alterations.

However, memory consolidation is usually addressed at two different levels: the cellular/synaptic level and the brain system level (Dudai et al., 2015). The first level, 'synaptic consolidation', which is directly related to Hebb's postulate, refers to the molecular mechanisms of long-term potentiation of a synaptic connection, i.e., higher efficacy of pre-synaptic transmission and post-synaptic excitability; these processes are associated with long-term memory (Bliss & Collingridge, 1993; Jarome & Helmstetter, 2014; Mayford et al., 2012; Asok et al., 2019). They involve a stimulus-induced activation of molecular signaling cascades allowing gene expression, protein synthesis and post-translational modifications that ultimately alter synaptic efficacy (Day & Sweatt, 2010; Gräff & Tsai, 2013; Jarome & Helmstetter, 2014). The synaptic consolidation process occurs within minutes to hours after its initiation (Mayford et al., 2012; Asok et al., 2019).

The second level of consolidation is a much slower process called 'system consolidation'. This process involves a time-dependent reorganization of long-term memories to larger and more distributed brain circuits (Dudai & Morris, 2000; Dudai, 2012). It has been proposed that synaptic consolidation is a subroutine within system consolidation, i.e., that system consolidation relies on recurrent waves of synaptic consolidation in the brain areas where the new or reprocessed experience-based representations are established (Dudai, 2004, 2012; Kandel et al., 2014).

1.1 Declarative memories

The idea that memories are not a uniform mental faculty started to gain relevance in the middle of the last century with studies in the amnestic patient Henry Molaison (1922 – 2008), famously known as patient H.M. until his death (Scoville & Milner, 1957). After a bilateral medial temporal lobe resection, carried out in the patient to relieve epilepsy, Brenda Milner observed a severe effect on his memory functions. The patient exhibited profound forgetfulness without having any perceptual disorder or intellectual impairment (Scoville & Milner, 1957; Squire, 2009). He suffered from anterograde amnesia, i.e., inability of forming new memories, and also retrograde amnesia, i.e., he could not access some memories acquired before the surgery. Nevertheless, he proved capable of learning a hand-eye coordination skill within a period of three days (Milner, 1962). Remarkably, although he learned quite fast and

efficiently, he could not remember having trained on the day before. Together all these findings established the fundamental principle that forming memories is a distinct brain function separated from other abilities like perceptual and cognitive abilities, and that memory is not a single entity. Moreover, these results identified the medial temporal lobe as a key structure for memory (Squire & Wixted, 2011; Eichenbaum, 2013).

Nowadays, it is known that there are different kinds of memories, and one major distinction can be made between working memory and long-term memory. Working memory is the capacity to temporarily maintain a limited amount of information, which can support different cognitive abilities like reasoning and learning (Baddeley, 2003). Long-term memories refers to the storage of information on a prolonged time scale, such as days, weeks or even years after encoding, and can be further separated into declarative memory and non-declarative memories (Squire & Dede, 2015). While declarative memory are memories that are accessible to conscious recollection, non-declarative memory involves skills and habits, simple forms of conditioning, priming and perceptual learning, and is expressed through performance rather than conscious recollection (Squire & Wixted, 2011; Squire & Dede, 2015). Non-declarative memory uses unconscious ways to respond to our environment. Declarative memory is the tool we use to represent the world and that allows us to remember contents, i.e., scenes, words, faces, people, etc., so that they can be consciously processed, for example compared with each other (Squire & Wixted, 2011).

Declarative memory can be divided into two categories: semantic memory, which represents facts about the world, and episodic memory, which is memory for events (Squire & Dede, 2015). The encoding of declarative memories can be intentional or unintentional but is typically consciously accessible to active recall. Episodic memories are learned quickly, but can also be forgotten very fast (Wixted, 2004), whereas semantic memories are formed as a result of the repeated encoding or activation of overlapping episodic memories (Winocur et al., 2010; Inostroza & Born, 2013).

1.2 Episodic-like memory

The term episodic memory (as far as humans are concerned) was first coined by Endel Tulving and corresponds to a neurocognitive system that is differentiable from other memory systems and maintains events that create our personal experiences (Tulving, 2002). Tulving further defined episodic memory as the ability of humans to replay in mind, with autonoetic consciousness, a past event as it happened in a specific spatio-temporal context, i.e., perform a

conscious recollection and re-experience of a past event (Allen & Fortin, 2013; Lou et al., 2004; Tulving, 2002). Thus, episodic memory is explicitly located in the past and its essence lies in the conjunction of 'self, autonoetic awareness and subjectively sensed time' (Tulving, 2002). It is precisely here that Tulving makes a distinction between 'remembering' and just 'knowing'. Different from episodic memory, the knowledge we acquire is only factual, without having the flavor of past experiences associated with it (Clayton et al., 2007).

Unlike humans, animals cannot give a verbal report of a past experience. However, it cannot be excluded that animals might not only remember facts of a past event, but also be aware of those facts from a personal experience. This question can be solved to some extent by factoring out conscious recollection and using Tulving's original definition of episodic memory. In 1990, Gallistel proposed that animals record the time and place at which a certain event occurred, together with the actual features of the event, i.e., episodic recall will basically be the retrieval of information about 'what' happened and 'where' and 'when' it took place. (Gallistel, 1990; Hampton & Schwartz, 2004; Babb & Crystal, 2005). In order to consider an animal's memory as episodic-like, it must include an integrated representation of multiple aspects of a specific past experience which can be recalled in order to solve novel problems. In nature, for instance, some animals might benefit from the capacity of remembering episodic information, e.g., western scrub-jays. These birds are members of the crow family and regularly cache or hide perishable food, like insects and fruits, and non-perishable food, like nuts and seeds. Thus, they need to relay on memory to be able to retrieve these caches at a later time point and to dig for the perishable food first. In 1998, Nicola Clayton and colleagues described 'Episodic-like memory during cache recovery by scrub-jays'. They demonstrated under laboratory conditions that these birds are not only capable of knowing what they have cached on a specific day, but also where they hid it and how long ago they cached the different foods, considering that different types of food perish at a different rate (Clayton & Dickinson, 1998). They concluded that scrub-jays have memory of when and where a particular food was cached and therefore fulfill the behavioral criteria of episodic-like memory in non-human animals (Clayton & Dickinson, 1998; Clayton et al., 2003).

These results raised the question of whether other animals are also capable of remembering unique, personal past events. Using a similar design to the one employed with the scrub-jays, Babb and Crystal (2006) found that after an intensive training, laboratory rats also showed signs of episodic-like memory. Rats learned that an attractive food was only available after a certain time had elapsed since they discovered its location. They returned to

this location only after the appropriate period had passed. Considering these results, the authors concluded that rats are able to retrieve, and therefore encode, the content of a specific episodic-like memory (Babb & Crystal, 2006).

The above-mentioned evidence of episodic-like memory in birds and rats is however based on food-rewarded tests that require relatively long sessions in which the animals are trained to learn certain rules, making the encoding of the 'what-where-when' information rather semantic (Dere et al., 2006). Animals develop expectations about what has to be remembered during a subsequent – expected – test, and therefore probably rely on relatively "un-retrospective" memory retrieval (Zentall, 2005; Dere et al., 2006).

An alternative to the use of food-rewarded paradigms is the measurement of memory for unique events, including 'what-where-when' information, but in a way that is not expected or anticipated by the animal. For this purpose, one-trial paradigms that do not require previous training and measure spontaneous behavior, are suitable. These tests are based on a rodent's natural tendency to explore novel objects (Ennaceur & Delacour, 1988). The preferred exploration of novel objects indicates a memory for the familiar object (Ennaceur, 2010). The tasks do not require any previous learning of response-reward associations and no reinforcers and can be used to address simple recognition memory as well as more complex spatio-temporal episodic-like memory (Ennaceur & Delacour, 1988; Ennaceur et al., 1997; Mitchell & Laiacona, 1998; Dere et al., 2005a, 2005b; Kart-Teke et al., 2006).

In one-trial recognition tasks, animals are exposed to different objects in an open field which they have to identify as novel or familiar based on a memory of an earlier experience with the objects in the same open-field. The general procedure of these paradigms consists of three phases: 1) a sample phase (encoding), 2) a retention interval, and 3) a test phase (recall). Although the memory involved in these tasks represents an episode in the life of the animal, each test only focusses on specific aspects of that episode, e.g. spatial component (Ennaceur, 2010).

1.2.1 Components of an episode: what, where and when tasks

The 'what' component of an episode (item memory) can be investigated with the novel-object recognition (NOR) task. Here, animals are tested to determine if they are able to discriminate a familiar object from a new one. During the sample phase, two identical objects are presented. In the subsequent test phase, one of the objects is the same but the other one is a new one. More pronounced exploration of the novel object indicates a memory for the

familiar one (Ennaceur & Delacour, 1988). In a similar way, the object-place recognition (OPR) task is used to address the 'where' component of an episode (spatial memory). The sample phase for this task is exactly the same as the one used for the NOR task, i.e., animals are presented with two identical objects. However, before the test phase, one of the objects is displaced to a new position, while the other one remains at the same location as during the sample phase. In this case, a longer exploration time for the displaced object indicates a memory for the non-displaced one (Ennaceur et al., 1997).

Unlike NOR and OPR tasks, the temporal memory (TM) task, testing the 'when' component (temporal memory) of an episode, consists of two sample phases separated by a short interval. During the first sample phase two identical objects are presented (old familiar objects), and during the second sample phase two different identical objects are presented (recent familiar objects) at the same locations as the old familiar ones. In the test phase, one of the objects from each sample phase is presented, i.e., during the test phase, the animals are presented with two familiar objects that only differ in the temporal order in which they were encountered. More pronounced exploration of the object presented first is assumed to indicate memory for temporal order (Mitchell & Laiacona, 1997). However, the old familiar object might be explored more because of a decay in the memory trace representing that object, and not necessary because of a recollection-based retrieval, whereas the representation for the recent familiar object might still be available in memory (Eacott & Easton, 2010; Ennaceur, 2010). Thus, this test does not provide direct evidence that the animal actually remembers how much time has passed since it explored the object.

It is important to keep in mind that episodic memory cannot be reduced only to the sum of the 'what', 'where' and 'when' components of the episode. What sets episodic memories apart from other types of memory is the uniqueness of the episode, i.e., what is important are the bonds that keep all three components together (Gallistel, 1990, Babb & Crystal, 2005, 2006).

1.2.2 What-Where-When recognition memory in rodents

In order to evaluate 'what', 'where' and 'when' memory for a unique episode, Dere and colleagues designed an exploration task combining the above-mentioned versions of novelty-preference paradigms, i.e., NOR, OPR and TM, the so called What-Where-When (WW-When) task (Dere et al, 2005a, 2005b). The idea was to create a task that allows rodents to

simultaneously encode and remember information for ‘what’, ‘where’, and ‘when’ acquired during two sample phases and tested after 50-min delay (Dere et al., 2005a).

Similar to the TM task, in the WW-When task animals are exposed to two sample phases and one test phase. During the first sample phase, animals are exposed to four copies of an object. After a short interval, in the second sample phase, four copies of a new object are presented. Finally, during the test phase animals are exposed to four objects: two copies of the object from the first sample phase (old familiar objects) and two copies of the object from the second sample phase (recent familiar objects). Moreover, one old familiar object is spatially displaced, whereas the recent familiar objects are presented at familiar locations (Dere et al., 2005b). In this task, animals usually spend more time exploring the old familiar objects than the recent familiar ones. This result shows memory for ‘what’ and ‘when’ as previously described by Mitchell and Laiacona (1997). Additionally, animals show more exploration for the old familiar displaced object than the old familiar stationary one, thus demonstrating memory for ‘what’ and ‘where’.

Although this behavior shows that animals are able to remember ‘what’, ‘when’ and ‘where’ of a single event, it cannot clarify whether this type of multidimensional object memory is an integration of the different aspects or simply the sum of them. Therefore, the same authors proposed a slightly modified paradigm, where one of the recent familiar objects is also displaced, thus, asking whether it matters if an old or a recent familiar object is displaced (Karl-Teke et al., 2006). The results showed that animals spend more time exploring the stationary old familiar object than the stationary recent familiar one, indicating that the sequence of presentation was remembered. Moreover, animals can recognize whether the objects were displaced or not after their first appearance, thus demonstrating memory for ‘what’ and ‘where’ complementing the ‘when’ memory. However, while the rats indeed explore the displaced recent familiar object to a greater extent than the stationary recent ones, they prefer the stationary old familiar objects to the displaced old familiar object, suggesting an interaction between ‘when’ and ‘where’. This argues against the possibility that spatial and temporal object information is encoded, consolidated and retrieved independent from each other, showing that rats can establish an integrated memory from an event in a specific spatio-temporal context (Karl-Teke et al., 2006, Dere et al., 2006).

The WW-When paradigm includes several features of human episodic memory. First, animals must remember a specific episode rather than learn a task, which might take multiple trials to apply one or more rules (Zentall et al., 2001; Schwartz et al., 2005). Second, task performance demonstrates integration of information for ‘what’, ‘where’, and ‘when’

components, fulfilling the criterion of episodic-like memory (Clayton & Dickinson, 1998). Third, the inter-phase interval excludes the possibility of the animal relaying on short-term memory (Hampton & Schwartz, 2004). Finally, since the test phase represents a novel situation that cannot be anticipated by the animals, the performance might require retrospective memory retrieval (Zentall, 2005). Importantly, this paradigm is based only on recognition memory and cannot yield evidence of conscious recollection (Tulving, 2002).

1.2.3 What-Where-Which recognition memory in rodents

As mentioned above, the use of the TM task has been criticized because several studies failed to assess the temporal aspect of the episodic memory, assuming memory deficits when animals fail to spend more time on old familiar objects (Hannesson et al., 2004; Dere et al., 2005a; Hotte et al., 2005). In fact, preference for the old familiar object might reflect a decay in the memory for that object, rather than a recollection-based retrieval of temporal aspects of that episode (Bird et al., 2003; Hampton et al., 2005; Eacott & Easton, 2010; Ennaceur, 2010). For instance, Hampton et al. showed that Rhesus monkeys can remember the location of preferred and less-preferred food after 1 and 25 h, however they do not learn that the preferred food is only available at the short delay (Hampton et al., 2005). Therefore, if animals can only discriminate whether an event was more or less recent – presented first or last – but do not form a memory for the absolute occasion of the event, we might be evaluating the strength of a 'what-where' memory rather than the integration of the episodic memory in a specific spatio-temporal context (Eacott & Easton, 2010). This has led to the conclusion that measuring the temporal component of an episodic representation is prone to artifacts and some distinctions between the actual 'when' and 'how long ago' components might also relate to different memory systems (Roberts, 2008).

Considering our experiences as humans, it might not be necessary to remember the precise time of the day at which a particular event took place because we might use certain cues that set that event apart from others (Eacott & Norman, 2004; Eacott & Easton, 2010). In this context, Eacott and colleagues have proposed that the retrieval of an episode, rather than relying on absolute time, depends more on some 'occasion setters' provided by physical context, e.g., colors, objects, cues, thus replacing the 'when' component with a 'which' component (Easton & Eacott, 2008; Eacott & Easton, 2010; Ennaceur, 2010). They introduced the 'What-When-Which' (WW-Which) task, using non-temporal context to provide information about the particular episode to be remembered (Eacott & Easton, 2010).

As in the WW-When task, in the WW-Which task animals take part in two sample phases and a single test phase. During the first sample phase, animals explore two different objects in a context A. In the second sample phase, the same objects are presented, however, their location is reversed relative to the first sample phase, and they are placed in a context B. During the test phase, animals are returned to one of the previous contexts, i.e., context A or context B, and two identical copies of one of the objects are presented. In this way, one of the objects is located at the same location and the same context as in one of the sample phases, i.e., the configuration of context and location is familiar, while the second object (the copy of the object) is located in a position that is new for this particular test context, i.e., the configuration of context and location is novel (Eacott & Norman, 2004; Langston & Wood, 2010). Here, animals show increased exploration of the object that appears in the novel configuration of context and location, which reflects the episodic binding of what (object), where (location) and which (context) of that particular episode. The episodic information is not different from 'what-where' but includes an additional intervening 'which' information (Ennaceur, 2010; Eacott & Easton, 2010).

1.3 Neuroanatomy of episodic memory: Cortico-hippocampal organization of memories

The idea that the hippocampus is important for memory formation has been around for some time, and in recent decades, the hippocampus and prefrontal cortex (PFC) have been well established to play a major role in the processing of episodic memories (Eichenbaum, 2017). The findings from the studies in patient H.M. already suggested that certain types of memory rely on the integrity of the hippocampal formation (Scoville & Milner, 1957). In particular, the amnesia of patient H.M. selectively affected declarative memories (Corkin, 1984; Eichenbeum, 2013) and included impaired recall of specific personal experiences in the recent and distant past (Steivorth et al., 2005; Eichenbaum, 2013). Finally, the hippocampus was shown to be not only important for supporting memory, but to support the permanent consolidation of memories (Squire & Wixted, 2011; Eichenbaum, 2013). Studies in humans have buttressed this assumption by demonstrating that the hippocampus is engaged whenever detailed associative or contextual information is recalled, and that this is not dependent on how old that particular memory is (Wiltgen et al., 2010; Hoscheidt et al., 2010). Furthermore, the hippocampus is even involved when we imagine details and context that we have never actually experienced (Addis et al., 2007; Hassabis et al., 2007).

Since the publication by Scoville and Milner in 1957, it has been shown in both humans and animal models that the hippocampus contributes to binding different elements of an episode into cohesive units (reviewed in Dubai et al., 2015), and to organizing the different aspects of the episode in the context in which they were encoded. In rodents, studies have pointed out a very important role of the hippocampus whenever it is required to remember particular events in the spatio-temporal context in which they have been experienced (e.g., Squire, 1992; Eacott & Norman, 2004; Langston & Wood, 2010; Butterly et al., 2012; Inostroza et al., 2013a). Indeed, findings in rats show that during post-learning sleep, hippocampal place cell ensembles that were active during encoding are replayed in the same order as during the learning period (Wilson & McNaughton, 1994). Importantly, the pre-learning sleep period did not show such firing patterns. Moreover, not only during sleep, but also during the offset of a trial, hippocampal ensembles showed forward and reverse replay of the firing sequence that emerged during the trial, and this has been proposed to relate to the binding of episodic sequences (Diba & Buzsaki, 2007; Carr et al., 2011). Additionally, hippocampal firing patterns might represent specific episodic features, such as what happened within a spatial and temporal context (Komorowski et al., 2009; Moser et al., 2015; Bulkin et al., 2016).

Although evidence that the hippocampus plays a major role during the first stages of the consolidation process has been gathered in experiments which mainly studied amnesia following hippocampal lesions (Squire et al., 2001; Squire, 2004), it has also been demonstrated that neocortical areas are involved in memory formation already during encoding (Baker & Warburton, 2008; Tse et al., 2011; Brodt et al., 2018). The first hours of encoding new memories engage different neocortical areas (van Kesteren et al., 2010; Tse et al., 2011; Brodt et al., 2018). Furthermore, the neocortex has been proposed to initiate an 'encoding set' immediately before the actual encoding, i.e., a hypothetical state of predisposition or readiness to encode (reviewed in Cohen et al., 2015; Dudai et al., 2015).

2. The role of sleep role in episodic memory consolidation

2.1 The sleeping brain

A major function of sleep is the consolidation of long-term memories. This idea goes back to the experiments performed by Jenkins and Dallenbach in the 1920s. They compared the

retention of nonsense syllables after retention periods of different length (1, 2, 4, and 8 hours), filled either with sleep or wakefulness. They reported that subjects who slept immediately after learning showed a better memory recall than those who did not sleep (Jenkins & Dallenbach, 1924; Stickgold, 2005; Rasch & Born, 2013).

Sleep is a universal natural behavior present in both vertebrate and invertebrate animals (Cirelli & Tononi, 2007; Vorster & Born, 2015; Fruth et al., 2018). It is a reversible condition of reduced responsiveness, which is usually associated with immobility (Cirelli & Tononi, 2007). Compared to wakefulness, sleep is associated with a decreased ability to react to stimuli, and, unlike coma, sleep is a reversible state. Sleep is highly regulated. The brain has several mechanisms to compensate for loss of sleep, e.g., by increasing the duration and/or the depth of sleep after periods of sleep deprivation (homeostatic regulation). Furthermore, a clocklike mechanism is also present, which is independent of prior sleep and waking and triggers sleep at a certain phase of the 24-h cycle (circadian regulation) (Borbély & Achermann, 1999). Harmful consequences of sleep deprivation have been described in many studies. Sleep loss or chronic sleep alterations have an impact on metabolism and immune functions (Mullington et al., 2010; Arble et al., 2015). Most importantly, prolonged sleep deprivation can lead to death in several species, including humans with fatal familial insomnia who die after developing the syndrome (Rechtschaffen & Bergmann, 2002; Shaw et al., 2002; Stephenson et al., 2007; Baldelli & Provini, 2019).

In mammals, sleep consists of two core stages: slow-wave sleep (SWS), which represents the deepest form of non-rapid eye movement (Non-REM) sleep, and rapid eye movement (REM) sleep. In rodents, an additional transition state, termed intermediate stage (IS) or pre-REM sleep, is often discriminated (Neckelmann et al., 1994; Oyanedel et al., 2015). In general, Non-REM sleep and SWS are hallmarked by slow high-amplitude EEG oscillations – so-called slow wave activity (SWA), and REM sleep is characterized by wake-like fast and low-amplitude oscillatory brain activity. In human nocturnal sleep, SWS and REM sleep alternate in cycles of approximately 90 min (Rasch & Born, 2013; Vorster & Born, 2015). Early nocturnal sleep is dominated by SWS, while REM sleep predominates during late sleep.

During SWS, there are three characteristic brain oscillations: slow oscillations (SOs; < 1 Hz), spindles (10-16 Hz) and ripples (100-200 Hz). SOs are a global phenomenon that preferentially originates in the prefrontal cortex but also involves subcortical structures like the thalamus (Steriade, 2003; Crunelli & Huges, 2010), and travels towards posterior cortex, reaching also the hippocampus (Massimini et al., 2004). During SOs, the entire neocortex

oscillates between vigorous synaptic activity (up-states) and relative silence (down-states) (Crunelli & Huges, 2010; Neske, 2016). While the SO down-state is relatively short and associated with a generalized hyperpolarization and reduced neuronal firing, the SO up-state is longer and associated with synchronized membrane depolarization and an increase in the neuronal firing rate, driving also the generation of thalamic spindles (Steriade et al., 1993a, 1993b; Neske, 2016; Niethard et al., 2018). Sleep spindles (10-16 Hz) are waxing and waning EEG rhythms generated in intrathalamic circuits involving GABAergic networks of the thalamic reticular nucleus, which spread through thalamo-cortical fibers to the entire cortex (Steriade et al., 1993a; 1993b; Kim et al., 2015). Spindles also reach the hippocampus, where they are in synchrony with ripples (Clemens et al., 2007, 2011; Staresina et al., 2015). Hippocampal ripples are brief, fast oscillatory patterns of hippocampal local field potential (LFP) signals (100-200 Hz) (Buzsáki, 2015). They originate in the CA1 region of the hippocampus and are usually associated with large amplitude deflections observed also in the CA1 region, so-called sharp waves (SPW). SPWs results from strong depolarization of CA3 collaterals due to synchronous bursting of CA1 pyramidal cells (Buzsáki, 2006). Hippocampal ripples typically accompany the reactivation of neural ensembles that were active during prior wake phases (Wilson & McNaughton, 1994; Diba and Buzsáki, 2007; Khodagholy et al., 2017).

REM sleep is characterized by wake-like, low amplitude, mixed fast frequency activity. Additionally, REM sleep is hallmarked by phasic rapid movements of the eyes and muscle atonia. Particularly in rats, the hippocampal LFP shows high theta (4.0-8.0 Hz) activity during REM sleep (Inostroza & Born, 2013; Rasch & Born, 2013).

2.2 Active system consolidation during sleep

Although the contribution of sleep to memory formation has been recognized and studied for a long time, initial studies described its role as a passive protection of newly encoded memories from interference (Jenkins & Dallenbach, 1924). More recently, many studies have shown that sleep ‘actively’ facilitates the consolidation of memories, in addition to the solely protective effect, leading to the hypothesis of an active system consolidation of memories during sleep (Diekelmann & Born, 2010; Payne & Kensinger, 2010; Lewis & Durrant, 2011; Inostroza & Born, 2013).

The theory of active system consolidation of memory highlights the role of sleep in the memory formation process (Diekelmnan & Born, 2010; Lewis & Durrant, 2011; Born &

Wilhelm, 2012). It proposes a dialogue between hippocampal and neocortical networks that coordinates memory formation (Diekelmann & Born, 2010; Inostroza & Born, 2013). In this context, events experienced during wakefulness are rapidly encoded by the hippocampus and extra-hippocampal structures. The hippocampus serves as a fast learner that binds different memory components, which are also stored in neocortical areas, into a unique episodic representation, i.e., places the experienced event into a spatio-temporal context. At this stage, memory retrieval is highly dependent on the hippocampus. During subsequent periods of sleep, the newly acquired memory representations are repeatedly reactivated. Thus, cortical ensembles that were active during encoding are reactivated by the hippocampus. These reactivations feed the memory information from hippocampus into neocortical networks via efferent CA1 entorhinal pathways, i.e., memory representations are gradually redistributed and integrated into pre-existing long-term memories (Gais et al., 2007; Brodt et al., 2018). After the strengthening of neocortical representations, the memory becomes less hippocampus-dependent (Born & Wilhelm, 2012; Inostroza & Born, 2013). These reactivations occur during SWS, where different EEG oscillations regulate this process. SOs are thought to temporally group neuronal activity into hyperpolarizing down-states – in which neurons are silent – and succeeding depolarizing up-states – in which neuronal firing increases (see section 2.1; Steriade et al., 1993a). These oscillations not only synchronize neural activity of neocortical networks, but also of other brain regions relevant for memory formation, e.g., the thalamus, where spindles are generated, and the hippocampus, where memory reactivations are co-occurring with SPW-ripples (Clemens et al., 2007, 2011; Bergmann et al., 2012). On the one hand, spindles nest in the depolarizing SO up-state (Staresina et al., 2015), and on the other hand, hippocampal ripples tend to nest in excitable spindle troughs, and this coupling has been proposed as a mechanism promoting hippocampus-to-neorcortex information flow, i.e., spindles might have a crucial role in enhancing the memory representation in cortical areas (Sirota et al., 2003; Clemens et al., 2011, Klinzing et al., 2019). As for spindles, hippocampal ripple activity is also suppressed during the hyperpolarizing SO down-state and enhanced during the following depolarizing up-state (Mölle et al., 2006, 2009; Clemens et al., 2007, 2011). Overall, the temporal pattern is consistent with a loop-like scenario during SWS where SOs can trigger thalamic spindles in a top-down fashion. Spindles can also regulate hippocampal networks independently from the occurrence of SOs (Klinzing et al., 2019). Moreover, hippocampal ripples, in a bottom-up and thalamus-independent fashion, can directly contribute to the emergence of a neocortical SOs (Maingret et al., 2016).

Synaptic consolidation processes assumed to take place during REM sleep following the above-mentioned reactivation during SWS, may help stabilize the newly transformed representations (Diekelmann & Born, 2010; Inostroza & Born, 2013). Moreover, as mentioned above, synaptic consolidation mechanisms act as local subroutines to support the eventual system consolidation (Dudai, 2012; Dudai et al., 2015; Inostroza & Born, 2013).

2.3 Episodic memory consolidation during sleep

Several studies in both humans and animals have shown that sleep after encoding episodic information, as well as other forms of declarative memory contents, improves the retention of these memories (Marshall & Born, 2007; Diekelmann & Born, 2010; Binder et al., 2012; Inostroza et al., 2013a; Sawangjit et al., 2018). Post-learning sleep compared to wake induced a slower trajectory of forgetting over time and made the memory representation resistant to interference (Ellenbogen et al., 2006a, 2006b). Additionally, experiments in rats and humans have shown that sleep is particularly critical for maintaining episodic features and contributes to the associative integration and binding of the spatio-temporal memory components (Ellenbogen et al., 2007, Lau et al., 2010; Inostroza et al., 2013a; Weber et al., 2014).

Hippocampus-dependence is a hallmark of episodic-like memory as assessed in both WW-When and WW-Which tasks (Kart-Teke et al., 2006; Langston et al., 2010; DeVito & Eichenbaum, 2010; Chao et al., 2017). Animals with hippocampal lesions are able to perform on tasks that separately test 'what', 'where', 'when' and 'which' aspects, but fail on a task requiring the binding of those aspects into an integrated episodic-like representation (Li & Chao, 2008; Langston & Wood, 2010; Inostroza et al., 2013b). Since all of these tasks are based on object recognition paradigms, these results may be due to alterations in the coherent hippocampal processing of item and contextual information transferred from lateral and medial entorhinal inputs, respectively (Inostroza et al., 2013b). As mentioned before, sleep is also critical for forming a persistent integrated episodic memory in the WW-When task, with this effect particularly linked to slow oscillatory activity during SWS (Inostroza et al., 2013a; Oyanedel et al., 2014, 2019). In contrast, memory aspects that are not essentially relying on hippocampal function, like 'what' (item) memory, motor skills and emotional aspects in episodic memory, might benefit particularly from REM sleep (Gais & Born, 2004; Rauchs et al., 2005; Walker & Stickgold, 2006; Rasch & Born, 2013). Post-encoding sleep also supports independent spatial and temporal components in an episodic memory that are hippocampus-dependent, whereas object recognition memory (item memory) that is independent of

hippocampal function does not profit from sleep, at least when it comes to short to intermediate-long term memories (Binder et al., 2012; Inostroza et al., 2013a; Kelemen et al., 2014, Oyanedel et al., 2014).

Neuroimaging and lesion studies have demonstrated that contextual information of episodic memory rely on hippocampal function, however, item memory is supported by extra-hippocampal structures (Davachi, 2006; Eichenbaum et al., 2007). Moreover, van der Helm and colleagues showed that napping selectively enhances signs of context memory, leaving item memory unaffected (van der Helm et al., 2011). Notably, a recent study in rats has shown that hippocampal activity during post-encoding sleep but not during retrieval is critical for forming long-term non-hippocampal memory (Sawangjit et al., 2018). In this study, sleep effects were tested using either an NOR task, which does not require hippocampal function but activity of the perirhinal cortex, or a hippocampus-dependent OPR task (Brown & Aggleton, 2001; Winters et al., 2008; Langston & Wood, 2010; Inostroza et al., 2013b). Post-learning sleep enhanced the memory for the OPR task at immediate and remote (1 week) recall. Surprisingly, sleep distinctly enhanced memory for the NOR task only during remote recall: sleep compared to sleep deprivation after encoding preserved memory for items tested three, but not one week after encoding. Additionally, selective silencing of the hippocampus during post-learning sleep completely abolished the sleep-dependent maintenance of NOR memory. However, hippocampal silencing during remote recall did not disturb recognition performance, indicating that although the hippocampus was required for sleep-dependent consolidation, these memories did not depend on the hippocampus for subsequent retrieval (Sawangjit et al., 2018).

It has been proposed that there are at least two processes contributing to episodic memory retrieval: recognition and familiarity (Yonelinas, 2001). While familiarity accounts for a classical single-detection process, recollection reflects a process whereby qualitative information about the memory is retrieved. A specific influence of sleep on episodic recognition can be also detected by the remember/know paradigm (Yonelinas, 2001; Yonelinas and Levy, 2002). One of the main features of episodic memory is the conscious recollection of the encoded event, which is associated with the remembering process. On the other hand, knowing something or knowing to have seen something but not being able to recall any episodic features of the event when this certain something was learned or encountered triggers the feeling of familiarity, which is not considered episodic in nature. The first, explicit recollection critically relies on hippocampal function, while familiarity-based recognition processes involve only extra-hippocampal regions (Yonelinas, 2001). Post-

learning sleep consistently enhances explicit recollection of episodic memories, whereas familiarity-based judgments remain unaffected (Rauchs et al., 2004; Drosopoulos et al., 2005, Daurat et al., 2007; Atienza & Cantero, 2008). Moreover, SWS during post-learning sleep is directly associated with the enhanced recall of this aspect of episodic memories (Rauchs et al., 2004; Daurat et al., 2007).

Consolidation of declarative memories during sleep has been linked to distinct oscillatory activity hallmarking the EEG during SWS, such as spindles, sharp-wave ripples, and in particular SOs (Eschenko et al., 2006, Marshall et al., 2006; Eschenko et al., 2008; van der Helm et al., 2011; Ngo et al., 2013; Binder et al., 2014a, 2014b). However, while different functions may not be attributed to the two major sleep stages in isolation, i.e., SWS and REM sleep, evidence suggests that the succession of the two stages is critical for memory consolidation (e.g., Giuditta & Ambrosini, 1995; Stickgold et al., 2000; Rasch & Born, 2013). The co-occurrence of reactivations of hippocampal memories and spindles during the SO up-state is thought to support the distribution of memory representations across hippocampal and extra-hippocampal networks. The extra-hippocampal representations, which are considered to be linked to the context-independent semantic aspects of the memory, might be subsequently stabilized during ensuing REM sleep (Diekelmann & Born, 2010).

Altogether, sleep's effect on memory formation has been mainly conceptualized for the hippocampus-dependent declarative memory system; however, memory processing may be similar for non-hippocampus-dependent memories (Dudai et al., 2015; Sawangjit et al., 2018, Schapiro et al., 2019). There is a strengthening effect of sleep, in particular of SWS, on contextual and binding aspects of episodic and episodic-like memory. Moreover, these benefits are mostly linked to SWS, although REM sleep seems to contribute to other aspects of the memory, e.g., emotional aspects.

3. Summary of the conducted research

The goal of my research over the last seven years has been to deepen our understanding of the role of sleep, especially SWS, for the consolidation of episodic memories. Certainly, the beneficial aspects of sleep on long-term memory formation have been well investigated; nevertheless, there are several open questions. For instance, while previous experiments demonstrated the importance of sleep for the consolidation of episodic-like memory, they

lacked the EEG-based assessment of the underlying electrophysiological sleep features. This dissertation aims to answer some of these open questions related to the consolidation of episodic-like memories during sleep, focusing in particular on episodic representations and analyzing sleep and its memory-improving features from a behavioral and electrophysiological perspective.

3.1 Summary of Study 1: Episodic-like memory consolidation during sleep is particularly associated with SWS and slow oscillatory activity

The first study, Oyanedel et al., 2014, had two aims: first, to replicate the results of Inostroza et al. (2013a) who showed that sleep after encoding is critical for maintaining episodic features of hippocampal function, i.e., the binding of an event into spatio-temporal context (Inostroza et al., 2013a); and second, we asked whether the enhancing effect of sleep on episodic-like memory is particularly linked to SWS and the occurrence of slow oscillatory and spindle EEG activity during the post-encoding retention interval.

Rats were tested on four non-stressful object recognition tasks, based on the animal's natural preference for novelty (see section 1.2): on the WW-When task as a measure of episodic memory, and also on three tasks separately covering the 'what' (NOR task), 'where' (OPR task) and 'when' (TM task) components of the episodic memory. Each session contained one or two sample phases (encoding), followed by an 80-min retention interval (consolidation) and a subsequent test phase (retrieval). The sample phase of each task allowed the animals to explore two (or four) objects placed inside an open field arena until the objects were explored at least 15 s within an interval of 2 to 5 minutes. During the retention interval, each animal was allowed to sleep and, respectively, was sleep-deprived. The order of the retention conditions was balanced across rats and between each condition and an inter-test interval of three days was used to minimize the impact of the first manipulation. During the retention interval, animals were placed in their own recording box and EEG/EMG signals were recorded. Finally, during the test phase, the rats were placed back into the open field arena and allowed to explore the set-up for three minutes. Memory during this phase was assessed using the rat's natural preference for novelty versus familiarity, i.e., its tendency to explore to a greater extent a new object, or a new location of a familiar object, or objects that were encountered earlier than later during the sample phase.

We confirmed the previously published results by demonstrating that post-encoding sleep is critical for maintaining significant aspects of episodic memory. Moreover, consistent

with previous findings, rats that slept showed significantly better spatial memory and a trend towards improved temporal memory; however, NOR memory did not benefit from post-encoding sleep. Additionally, we showed that the supporting influence of sleep on episodic memory is associated with increased slow oscillatory EEG activity. We also found that NOR performance positively correlates with the percentage of REM sleep, as well as with the number of spindles, and that OPR performance correlates with the percentage of SWS. There was no association of WW-When performance with spindle activity during SWS. These findings emphasize the importance of SWS and associated slow oscillatory activity for episodic-like memory consolidation during sleep (Inostroza & Born, 2013). Contrary to our expectation, we did not observe an association of spindle activity with memory performance on the WW-When task, which diverges from previous studies in humans and rats that rather consistently indicated that increased spindle activity during post-learning sleep is linked to an enhanced retention performance (e.g., Binder et al., 2012; Eschenko et al., 2006; Fogel et al., 2009; Rasch & Born, 2013; Schabus et al., 2004; Schmidt et al., 2006).

These results indicate that SWS, and slow oscillatory EEG activity in particular, plays a key role in consolidating hippocampus-dependent episodic memory, whereas the role of spindles in this process needs to be further examined.

3.2 Summary of Study 2: Sleep affects episodic-like consolidation in dependence of the episodic paradigm

The second study, Oyanedel et al., 2019, compared two different episodic memory paradigms in rats: the WW-When task – also used in the first study (see above) – and the WW-Which task (see section 1.2.3). Although both tasks assess episodic memory, the WW-When task focusses on the temporal aspects of an episode, whereas the WW-Which task focusses on the occasion setters or contextual components. Here, we asked two main questions: first, whether sleep supports episodic memory consolidation assessed by means of the WW-Which task, and second, given the difference in memory assessment between the WW-When and WW-Which tasks, whether the consolidation would differ between the tasks.

As described previously, the WW-When task aims to measure the memory as an event bound into spatio-temporal context, while the WW-Which task lacks the temporal component, but introduces an 'occasion setter' which represents the contextual configuration in which the event took place. The rats used to evaluate the WW-When task were the same that were used in the first study (Oyanedel et al., 2014). An additional set of rats was used to evaluate the

WW-Which task, which was performed as previously described (Langston & Wood, 2010), with minor modifications. It consisted of two sample phases. During the first sample phase, two different objects were presented in a context A. During the second sample phase, the same objects were presented, however their locations were swapped, and the context was changed to context B. Afterwards, as after the WW-When task, animals were placed in their home cage for a 90-min retention interval, filled with either natural morning sleep or sleep deprivation. For the test phase, the open field was configured as either context A or context B, and two identical copies of one of the objects were presented. Thus, one of the objects was located at the same location and context as in one of the sample phases (familiar configuration of location and context), whereas the second object was placed in a new location. This new location was new for this particular test context (novel configuration of location and context) (see section 1.2.3; Eacott & Norman, 2004; Langston & Wood, 2010). The memory was tested using the rat's natural preference for novelty versus familiarity, i.e., its enhanced exploration of the object that appears in a novel configuration of location and context.

In both tasks, sleep was found to be crucial for the consolidation of the episodic memory content. This again confirms that post-encoding sleep is critical for maintaining aspects of episodic memory measured in both temporal and contextual paradigms. More interestingly, we found that consolidating effects of sleep were stronger for the WW-Which than WW-When task. While the sleep effect on WW-When memory gradually emerged during the three minutes of the test phase, reaching significance only in the last minute, the sleep effect on WW-Which memory was already present from the first minute onwards. Furthermore, additional analyses of the spatial and temporal components of the WW-When task showed that the WW-When memory delay originated from the temporal component that did not emerge until the last minute of test, unlike the spatial component that was already present from the very first minute.

This study is the first to show that sleep is crucial for episodic-like memory consolidation tested with two different tasks (WW-When and WW-Which), with the delayed emergence of the sleep effect on the WW-When memory resulting from the temporal coverage of the task.

3.3 Summary of Study 3: Different sleep stage dynamics in neocortex and hippocampus

In the third study, Durán, Oyanedel et al., 2018, we focused on sleep stage dynamics in different brain areas. Our question was whether the different sleep stages, i.e., SWS, IS and REM sleep, are expressed in a coherent way throughout the whole brain, i.e., as unitary phenomena. The main aim was to characterize sleep stages and their transitions between neocortex and hippocampus, supposedly the two most relevant structures involved in memory consolidation.

We simultaneously recorded surface EEG from the frontal and parietal cortex and LFP from medial prefrontal cortex (mPFC) and dorsal hippocampus (dHC) of rats during the resting phase of their sleep/wake cycle. Our main finding was that sleep is not organized synchronously across the different brain regions. Although sleep, as a whole, and particularly SWS showed high congruence between the different recording sites, representing thus a global phenomenon, the congruence was lower for REM sleep and lowest for IS. For REM sleep, this incongruence was more pronounced during sleep stage transitions, where in more than 35% of the epochs, REM sleep started systematically earlier at the dHC LFP recording sites than in neocortical networks. The earlier onset of REM sleep in hippocampus was associated with a REM sleep-typical decrease in muscle tone.

These findings indicate that there is a region-specific regulation of REM sleep, which has important implications not only for our understanding of how sleep is organized, but also for its functions in memory consolidation.

3.4 Summary of Study 4: Oscillatory dynamics during slow-wave sleep

This study, Oyanedel, Durán et al., was performed in order to achieve a more fine-grained picture of the temporal relationship between the relevant oscillations in the hippocampal-neocortical memory system during spontaneous SWS, i.e., the oscillations thought to orchestrate memory processing during sleep. The respective analyses were performed on the recordings obtained in study three.

Consistent with the previously described top-down influence of cortex on hippocampus, we found that there is a decrease in spindles and hippocampal ripples during the neocortical SO hyperpolarizing down-state. This decrease is followed by an increase in both spindle and hippocampal ripple activity during the subsequent SO up-state. Spindle onsets were followed by an increase in hippocampal ripple activity, and, in turn, the ripple

maximum troughs were always preceded by increased spindle activity. Moreover, when comparing ripple activity during co-occurring SO-spindle events with that during isolated SOs or spindles, we found that ripple dynamics are mainly determined by spindles rather than SOs. With regard to bottom-up influences, we found an increase in the activity of hippocampal ripples to precede the SO down-states, especially in the mPFC. However, no similar dynamic was seen for spindles, suggesting that ripples might directly contribute to the occurrence of neocortical SOs.

Taken together, this temporal pattern is consistent with the assumption of a loop-like interaction of oscillatory events in which, top-down, SOs can trigger thalamic spindles, and spindles can regulate the occurrence of ripples in hippocampal networks independently from the occurrence of SOs. Finally, hippocampal ripples, in a bottom-up manner, can directly and independently from thalamic spindles contribute to the emergence of neocortical SOs.

4. Discussion

4.1 The role of sleep in episodic-like memory consolidation

Episodic memory comprises several components, i.e., the ability to recognize particular events or objects ('what' memory) and the ability to associate these events with their spatial location ('where' memory), as well as the particular occasional setters or context ('which' memory) and the time ('when' memory) they have been encountered. Episodic memory crucially depends on the hippocampus. Whereas the strengthening effect of sleep on long-term memory is well established, it has been only recently proposed that sleep particularly supports associate integration and binding in memory (Ellenbogen et al., 2007; Lewis & Durrant, 2011; Inostroza & Born, 2013).

Our first two studies confirm that sleep is critical for preserving an integrated episodic memory. The first study adds novel evidence supporting a relationship between specific sleep parameters and memory performance: the supportive effect of sleep on episodic-like memory, measured by the integration of an event into a spatio-temporal context (using the WW-When task), is associated with increased slow oscillatory EEG activity during the post-encoding sleep interval. In analyses of the memory tasks addressing the different components of an episode, we found that performance on the NOR task, i.e., memory for the object, positively correlated with the percentage of REM sleep and the number of spindles during SWS. The latter finding led us to further investigate the relation between SWS and the remote retrieval of object memory (Sawangjit et al., 2018). Moreover, we found that performance on the OPR task, i.e., spatial memory, positively correlated with the percentage of SWS. Contrary to our expectation, we did not find any association of WW-When task performance with spindle activity during SWS, which diverges from previous studies in humans and rats showing that increased spindle activity during post-learning sleep is linked to enhanced retention performance (e.g., Schabus et al., 2004; Eschenko et al., Schmidt et al., 2006; Fogel et al., 2009; Binder et al., 2012; Rasch & Born, 2013). I can only speculate about the reasons for this discrepancy between our and previously published studies. One possible reason could be that in the rat compared to the human EEG, spindle activity is a less prominent phenomenon and more difficult to determine because often it does not express itself in a distinct spectral peak (Mölle et al., 2009; Fogel et al., 2010). However, this was not the case in the first study where spindle peaks could be readily identified in the power spectra of all animals. Therefore, a more plausible explanation for the missing link between spindle activity and WW-When performance relates to the relatively short retention intervals (of 80 min) used in this study.

Considering that spindles represent direct communication between thalamus and cortex and contribute to memory consolidation by supporting the redistribution (and strengthening) of reactivated information from hippocampus to extra-hippocampal sites, and assuming that this process is a gradual process, a short retention interval might only allow sleep-related reactivations of the hippocampal representations *per se* rather than the redistribution of memory representations (Bergmann et al., 2012; Inostroza & Born, 2013). The assumption that spindles generally favor the formation of extra-hippocampal representations would also account for the unexpectedly high correlation we observed between spindle counts and NOR memory, which is considered to primarily involve perirhinal rather than hippocampal networks (e.g., Aggleton et al., 2010; Barker et al., 2007; Brown & Aggleton, 2001). Yet, this post-hoc explanation is obviously tentative and therefore needs to be tested in future studies.

Sleep not only supports the consolidation of episodic memory measured as the integration of spatial and temporal information, but is also critical when the broader context, or 'occasion setter', in which the episode took place, is considered (WW-Which task). In combination, these results corroborate the fact that sleep is important for preserving an integrated episodic representation over intermediate time intervals (Kesner & Hunsaker, 2010; Inostroza & Born, 2013). The comparison of both tasks, however, showed that sleep's effect on memory consolidation was in general weaker for the WW-When than for the WW-Which task. We also found that memory for WW-When emerged gradually across the 3-min test, whereas in the WW-Which task above-chance performance was present already in the first minute. In a more fine-grained analysis, it was possible to elucidate that this delayed emergence of significant episodic memory performance in the WW-When task originated from the 'when' component of the task that gained relevance at the end of the test phase. The 'where' component in contrast was readily expressed from the beginning of the test phase. Taken together, these results show that temporal and spatial components of episodic information differentially emerge during the test phase.

The behavioral assessment of memory performance after sleep compared to a period of sleep deprivation replicated performance patterns observed in our previous studies (Binder et al., 2012; Inostroza et al., 2013a; Kelemen et al., 2014), indicating not only sleep-dependency of intermediate-term episodic-like memory but also distinct beneficial effects of sleep on hippocampus-dependent spatial and temporal aspects of memory, whereas the maintenance of recognition memory ('what' memory), which does not critically depend on hippocampal function, did not depend on sleep (Graves et al., 2003; Cai et al., 2009). While we did not observe an effect of sleep versus sleep deprivation on NOR task performance, in

several previous publications such an effect was reported. Object recognition in mice was shown to be sensitive to six-hour sleep deprivation immediately following learning (Palchykova et al., 2006a; 2006b) and also to four-hour fragmentation of sleep following learning (Rolls et al., 2011). Obviously, the effect of sleep on NOR varies substantially depending on the exact experimental procedures. Here, we used rats instead of mice and employed a distinctly shorter (80 min) retention interval than in the cited previous studies. However, the finding that sleep deprivation does not affect NOR performance was consistent with our previous results relying on the same paradigm (Inostroza et al., 2013a; Kelemen et al., 2014; Sawangjit et al., 2018). Yet, we cannot exclude that longer retention intervals would yield a different pattern. In any case, the NOR task was the only task that did not benefit from sleep in this and our previous studies. Considering that this task is also the only one of the task we used that does not require hippocampal function (Aggleton et al., 2010; Barker et al., 2007; Brown & Aggleton, 2001; Eichenbaum et al., 2007; Forwood et al., 2005), our findings corroborate the view that sleep preferentially benefits hippocampus-dependent memory (Albouy et al., 2013; Cai et al., 2009; Graves et al., 2003; Inostroza & Born, 2013). Importantly, a recent study of our group tested the long-term effect of post-encoding sleep on performance on this task. Compared with wakefulness, NOR performance only benefited from 2-h post-encoding sleep when tested three weeks after encoding. Spindle activity was associated with NOR memory performance. Interestingly, selectively inactivating both hippocampi during post-encoding sleep completely abolished the sleep-dependent maintenance of object recognition, demonstrating that the hippocampus plays an important role for the long-term consolidation of memories that have been traditionally considered to be hippocampus-independent (Sawangjit et al., 2018).

Of the four tasks we used in the first study, only memory for the OPR task correlated with the percentage of SWS. This is consistent with our hypothesis that consolidation of hippocampus-dependent tasks mainly benefits from SWS (Plihal & Born, 1997). On the other hand, performance on the NOR task correlated positively with the percentage of REM sleep, which is consistent with previous findings of a positive correlation between REM sleep and object recognition in rats (Chen et al., 2014) and humans (McDevitt et al., 2014). Indeed, it has been suggested that performance on familiarity-based item recognition tasks does not essentially require hippocampal function and can benefit from synaptic consolidation processes occurring during REM sleep because this sleep stage provides a neurochemical milieu, i.e., high cholinergic and theta activity, that specifically favors these processes (Diekelmann & Born, 2010; Groch et al., 2013). However, synaptic consolidation likewise

occurs in the wake state, albeit under conditions more prone to bias by interfering external stimulation. This might explain why REM sleep-related facilitation of item memory has not been consistently observed (Inostroza et al, 2013a). In the present experiments, temporal memory did not correlate with any sleep EEG features, and this observation was against our expectation. Moreover, in contrast to previous experiments (Inostroza et al., 2013a), we did not observe a beneficial effect of sleep on performance in the TM task. These findings indicate that respective effects of sleep are less robust. This negative result may also be related to the general difficulty to sensitively measure purely temporal memory at the behavioral level in animals (Ennaceur, 2010; Marshall et al., 2013). Performance on the WW-When task did not correlate with any sleep stage, which may not surprise given the fact that this measure of episodic memory performance combines recognition of object position ('where') and recognition of temporal order ('when'), and these two measures, as discussed above, showed an inconsistent pattern of correlations with the sleep stages of interest. However, WW-When task performance did correlate with power in the SO frequency band, in support of our hypothesis that episodic memory consolidation particularly benefits from SWS and SOs (Ngo et al., 2013; Binder et al., 2014a, 2014b). As SOs hallmark SWS, the correlation of WW-When performance with slow oscillatory EEG power in the absence of a similarly significant correlation with the percentage of time spent in SWS might surprise; nevertheless, previous studies have shown that SO amplitude can change independently of the time spent in SWS (e.g., Van Der Werf et al., 2009; Ngo et al., 2013). This also illustrates that measures like time spent in a specific sleep stage may not provide more than a rough approximation to the processes relevant for sleep-dependent memory consolidation. On the other hand, the correlation of this measure of episodic memory task performance with slow oscillatory activity suggests a particular functional specificity of these oscillations for the integration of spatio-temporal context into an episodic memory. This is certainly a highly speculative assumption because, although not significant, correlations between spatial and temporal memory and SO power pointed in the same direction. Indeed, the observed correlation between episodic memory formation and slow oscillatory activity during retention sleep converges with studies performed mostly in humans that indicate that SOs generally contribute to the consolidation of memory that depends on hippocampal function (e.g., Marshall et al., 2006; Binder et al., 2012; Ngo et al., 2013; Wilhelm et al., 2013).

4.2 Neocortical and hippocampal oscillatory dynamics during sleep

Sleep and its stages have been usually assumed to be homogeneous states involving the whole organism. Researchers have traditionally characterized sleep via polysomnography, which includes simultaneous EEG and EMG recordings, and, in humans, electrooculographic recordings (Rechtschaffen & Kales, 1968; Neckelmann et al., 1994; Oyanedel et al., 2015). In mammals, sleep comprises two core sleep stages: SWS, the deepest stage of Non-REM sleep, and REM sleep. In the third study, we addressed the question whether the occurrence of the different sleep stages is congruent between different brain areas. This is of special interest because different sleep stages are thought to fulfil specific functions. For instance, the dual process theory of memory consolidation during sleep proposes that SWS supports declarative memory consolidation, whereas REM sleep supports procedural memory consolidation (Maquet, 2001; Rasch & Born, 2013). Furthermore, the functions assigned to the different sleep stages typically are not established merely within a single structure such as the neocortex but rather rely on interactions between cortical and subcortical brain areas. Thus, episodic memory consolidation during sleep has been linked to specific oscillatory features during SWS and is assumed to involve a coordinated dialogue between neocortex and hippocampus (Diekelmann & Born, 2010; Watson & Buzsáki, 2015).

We compared the expression of sleep stages in frontal and parietal EEG recordings and in LFP recordings from mPFC and dHC of rats. Our results show that there are distinct differences between cortical and hippocampal signals that are mainly related to the onset of REM sleep. REM sleep epochs often started tens of seconds earlier at hippocampal than other recording sites, confirming recent findings by Emrick and colleagues (Emrick et al., 2016). Moreover, the earlier REM sleep onset in dHC LFP recordings was associated with a REM sleep-typical decrease in muscle tone. The differences in temporal REM sleep dynamics between neocortex and hippocampus might reflect distinct regulation of this sleep stage in these areas. The most relevant differences were found in analyses of IS, the transition state between SWS and REM sleep that displays as its main characteristic the co-occurrence of spindle-like and theta activity. We observed IS mainly in EEG signals recorded from frontal cortical areas. Moreover, when the hippocampus was already in REM sleep, mPFC was still in IS, suggesting that spreading of hippocampal theta activity – via volume-conductance – might contribute to the emergence of IS in the cortex. In fact, due to the difficulties to determine this sleep stage, many studies do not consider IS as a separate sleep stage from SWS (e.g., Niethard et al. 2016; Latchoumane et al., 2017)

Our findings also showed differences in the occurrence of SWS at different recording sites; nevertheless, compared to the differences in REM sleep onset they were overall negligible. Indeed, there was a very high congruence of SWS between neocortical and hippocampal recordings, suggesting that SWS represents a rather unitary phenomenon covering large brain areas. SWS is mainly hallmarked by the occurrence of slow oscillatory activity including SOs (< 1.0 Hz). These oscillations not only synchronize activity in the thalamo-cortical networks, where they are generated, but also synchronize activity between other brain regions, like the hippocampus and the cortex, allowing a precisely timed interaction between these regions (Steriade et al., 1993a; 2006; Siapas & Wilson, 1998; Mölle et al., 2006; Wierzynski et al., 2009; Crunelli & Hughes, 2010; Maingret et al., 2016). However, in more fine-grained analyses, the mPFC signal compared to that in other recording sites showed a shorter mean duration of SWS epochs. This is particularly surprising because the prefrontal cortex is thought to be a major source of slow waves (Riedner et al., 2011). However, deep-layer LFP recordings are supposed to be more sensitive to locally generated slow potential changes, and thus, in comparison with the other recording sites, the respective slow wave potentials are higher. Skull EEG electrodes detect diminished wave potentials because they cover slow wave signals from rather broad cortical areas. Consistently, our data show that SWS was present first in the frontal EEG signal. It is essential to highlight that these differences were rather marginal and might only reflect differences in sensitivity of LFP and EEG recordings to slow-wave activity.

It is important to note that our findings do not suggest independent regulation of REM sleep in the hippocampus. REM sleep is characterized by the presence of theta activity, which also occurs during active wakefulness (Peever & Fuller, 2017). This rhythm in generated in the medial septum with the diagonal band of Broca that projects to the hippocampus; in conjunction these areas are the major generators of the theta rhythm (Buzsáki, 2002; Pignatelli et al., 2012; Peever & Fuller, 2017). This suggests that the early appearance of REM sleep in the hippocampus might be due to the direct innervation of the hippocampus by theta-generating structures. Furthermore, the advanced emergence of REM sleep in the hippocampus was also associated with a decrease in muscle tone, which is a major feature of REM sleep. This coupling shows that the brain stem seems to be directly involved in the distinct hippocampal regulation of REM sleep. It has been proposed that the meso-pontine area acts as a switch between REM sleep and SWS, including REM-off and REM-on networks (Lu et al., 2006; Fraigne et al., 2015). Different populations of the REM-on network also project to areas in the basal forebrain including structures associated with theta

generation, i.e., medial septum and the diagonal band of Broca, and the ventral medial medulla, which carries inhibitory projections to skeletal motor neurons, thereby contributing to the establishment of muscle atonia (Fraigne et al., 2015). Finally, when REM sleep appeared first in the hippocampus, the cortical EEG signal showed enhanced power in the slow-wave (0.5-4.0 Hz) and sigma band (10-16 Hz) – both features of SWS – co-occurring with high theta activity, which is synchronized to hippocampal theta. This pattern suggests that neocortical networks show SWS-related activity in the presence of concurrent REM-related hippocampal theta activity representing volume-conducted activity (Scheffzük et al., 2011, 2013).

In sum, these findings show that sleep in general, and SWS in particular, are mostly present as global unified phenomena. Nevertheless, there is a region-specific mechanism modulating the occurrence of REM sleep so that in many cases it emerges earlier in the hippocampus than in neocortical networks. Ultimately, these results underline that differences in the regional expression of sleep stages need to be considered when characterizing the function of sleep stages, especially of REM sleep.

In the fourth study, we further examined the sleep-, and in particular SWS-related communication between neocortex and hippocampus via SOs, spindles and hippocampal ripples. With regard to top-down modulation, i.e., cortex-to-hippocampus modulation, we found that SO down-states in the EEG are related to a decrease in spindle and hippocampal activity, and that the following SO up-states are associated with an increase in both spindles and ripples, which are associated with the reactivation of hippocampal memory representations during SWS. Interestingly, we did not observe this SO-spindle dynamic in mPFC recordings. Spindles in turn were accompanied by an increase in hippocampal ripple activity. The spindle-related increase in ripple activity was not dependent on whether or not the spindle was co-occurring with a SO. As to bottom-up influences, we found an increase in hippocampal activity preceding the occurrence of SO down-states. No correspondent dynamics were found for spindle-SO interactions. These temporal dynamics suggest a dialogue between neocortex and hippocampus that unfolds in a loop-like manner that includes top-down as well as bottom-up influences.

We found a rather low proportion of SOs co-occurring with spindles, mainly due to the fact that the number of detected SOs was 10 to 14 times higher than the number of spindles. This finding may challenge the assumption of a strong influence of SOs on the generation of spindles in the thalamus. Nevertheless, spindle-generating mechanisms display fast refractoriness preventing SOs from triggering excessive amounts of spindles (Destexhe et

al., 1998; Ngo et al., 2015). Our findings in EEG recordings are in line with previous studies showing a robust increase in spindle activity accompanying the early SO up-state (Mölle et al., 2009, 2011; Nir et al., 2011), and support the view that the generation of spindles in the thalamus is driven by membrane depolarization of cortico-thalamic projections during the up-state of SOs (Steriade et al., 1993a, 1993b; Crunelli and Hughes, 2010). Surprisingly, spindles and SOs detected in mPFC LFP recordings turned out to appear completely uncoupled. Although this finding might be unexpected at a first glance because most SOs arise from prefrontal networks (Massimini et al., 2004), it is in line with weak prefrontal SO-spindle co-occurrence found in human intracranial recordings (Andrillion et al., 2011) and may reflect weaker cortico-thalamic projections transmitting the frontal depolarization to thalamic generators of spindles (Carman et al., 1964). Thus, SOs coming from the prefrontal cortex may primarily propagate intracortically toward posterior areas. This view is consistent with our observation that up-states of SOs in mPFC recordings are associated with an increase in the activity of spindles in parietal EEG recordings.

We found hippocampal ripples to be nested in SO up-states. For frontal and parietal SOs, there was a significant decrease in hippocampal ripple activity during the SO down-state, which followed a SO-up-state increase of activity. Hippocampal ripple activity also showed an increase after the onset of spindles in parietal EEG recordings. Additionally, ripple activity was preceded by an increase in spindles in all recorded areas. Time-frequency analyses revealed that this spindle-related increase in ripple power occurred independent of whether the spindles co-occurred with a SO or not. However, ripple power was significantly elevated when a SO co-occurred with a spindle but not during the occurrence of an isolated SO. Considering all these findings, it is possible that spindles, independent of cortical SOs, are the primary modulator of hippocampal ripple activity. In this view, the main role of SOs might be the suppression of ripples during their down-state, i.e., the down-state might effectively inactivate the hippocampal circuitry (Behrens et al., 2005). Furthermore, in a previous study, optogenetically induced spindles, independent of whether or not they were induced during an SO up-state, synchronized hippocampal ripple activity (Latchoumane et al., 2017). It is still unclear how exactly spindles affect hippocampal ripples; a candidate mediator between the two rhythms is the nucleus reuniens of the thalamus (Cassel et al., 2013; Varela et al., 2014).

Although previous studies have shown that the stimulation of thalamic spindles consistently induces neocortical SOs (Lewis et al., 2015; Latchoumane et al., 2017), we did not find any increase in SOs following spindles. This suggests that thalamic spindles can

contribute to the generation of SOs but that this rarely happens under natural conditions as investigated here. Previous evidence that spindle-generating networks undergo refractoriness faster than SO-generating networks supports this conclusion (Ngo et al., 2015; Antony et al., 2018).

Memory reactivation during sleep has been proposed to be mediated by repeated reactivations of hippocampal memory representations, which in turn are supposed to be under top-down control of cortical SOs (Born et al., 2006; Marshall et al., 2006). The co-occurrence of spindle-ripple events is thought to be a mechanism of the hippocampal-to-neocortical pathway during the excitable up-state phase of the SO. Our data hint at a possible bottom-up contribution of hippocampal ripple to neocortical SOs because we found that hippocampal ripples were followed by increased SO activity in both EEG channels. Moreover, when ripples were locked to SO down-states, there was an increase in hippocampal ripple activity preceding the SO down-state in both mPFC and dHC. Furthermore, cortical SO down-states were suppressed during ripples, suggesting a rebound mechanism that produces the later (~200 ms) increase of SOs. Such a mechanism might also be related to the fact during the SO down-state, cortical interneurons are inactive (Niethard et al., 2018).

In sum, our findings show a loop-like interaction of oscillatory events regulating the information flow between hippocampus and neocortex during SWS. On the one hand, there is top-down global inactivation of the loop during hyperpolarizing SO down-states, followed by spindle-mediated ripple activity in hippocampal networks during the SO up-state. On the other hand, there is a bottom-up mechanism whereby hippocampal ripples can trigger SOs that appears to bypass spindle-generating thalamic circuits.

5. References

Addis, D.R., Wong, A.T. & Schacter, D.L. (2007). Remembering the past and imagining the future: common and distinct neural substrates during event construction and elaboration. *Neuropsychologia*, 45, 1363–1377.

Aggleton, J.P., Albasser, M.M., Aggleton, D.J., Poirer, G.L. & Pearce, J.M. (2010). Lesions of the rat perirhinal cortex sparethe acquisition of a complex configural visual discrimination yet impair object recognition. *Behavioral Neuroscience*, 124, 55–68.

Antony, J.W., Piloto, L., Wang, M., Pacheco, P., Norman, K.A. & Paller, K.A. (2018). Sleep spindle refractoriness segregates periods of memory reactivation. *Current Biology*, 28, 1736–1743.

Albouy, G., Sterpenich, V., Vandewalle, G., Darsaud, A., Gais, S., Rauchs, G.,… Maquel, P. (2013). Interaction between hippocampal and striatal systems predicts subsequent consolidation of motor sequence memory. *PLoS ONE*, 8, e59490.

Allen, T.A. & Fortin, N.J. (2013). The evolution of episodic memory. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 10379–10386.

Arble, D.M., Bass, J., Behn, C.D., Butler, M.P., Challet, E., Czeisler, C.,… Wright, K.P. (2015). Impact of sleep and circadian disruption on energy balance and diabetes: a summary of workshop discussions. *Sleep*, 38, 1849–1860.

Asok, A, Leroy, F., Rayman, J.B. & Kandel, E.R. (2019). Molecular mechanisms of the memory trace. *Trends in Neuroscience*, 42, 14–22.

Atienza, M. & Cantero, J. (2008). Modulatory effects of emotion and sleep on recollection and familiarity. *Journal of Sleep Research*, 17, 285–294.

Babb, D.J. & Crystal, J.D. (2005). Discrimination of what, when, and where: Implication for episodic-like memory in rats. *Learning and Motivation*, 36, 177–189.

Babb, S.J. & Crystal, J.D. (2006). Episodic-like memory in the rat. *Current Biology*, 16, 1317–1321.

Baddeley, A. (2003). Working memory: looking back and forward. *Nature Reviews: Neuroscience*, 4, 829–839.

Baldelli, L. & Provini, F. (2019). Fatal familial insomnia and Agrypnia Excitata: Autonomic dysfunctions and pathophysiological implications. *Autonomic Neuroscience: Basic and Clinical*, 218, 68–86.

Barker, G.R.I., Bird, F., Alexander, V. & Warburton, E.C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *The Journal of Neuroscience*, 27, 2948–2957.

Bartlett, F.C. (1932). Remembering: A study in experimental and social psychology. London: Cambridge University Press.

Behrens, C.J., Van den Boom, L.P., De Hoz, L., Friedman, A. & Heinemann, U. (2005). Induction of sharp wave-ripple complexes *in vitro* and reorganization of hippocampal networks. *Nature Neuroscience*, 8, 1560–1567.

Bergmann, T.O., Mölle, M., Diedrichs, J., Born, J. & Siebner, H.R. (2012). Sleep spindle-related reactivation of category specific cortical regions after learning face-scene associations. *NeuroImage*, 59, 2733–2742.

Binder, S., Baier, P.C., Mölle, M., Inostroza, M., Born, J. & Marshall, L. (2012). Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats. *Neurobiology of Learning and Memory*, 97, 213–219.

Binder, S., Berg, K., Gasca, F., Lafon, B., Parra, L.C., Born, J. & Marshall, L. (2014b). Transcranial slow oscillation stimulation during sleep enhances memory consolidation in rats. *Brain Stimulation*, 7, 508–515.

Binder, S., Rawohl, J., Born, J. & Marshall, L. (2014a). Transcranial slow oscillation stimulation during NREM sleep enhances acquisition of the radial maze task and modulates cortical network activity in rats. *Frontiers in Behavioral Neuroscience*, 7, 220.

Bliss, T.V. & Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, 361, 31–39.

Borbély, A.A. & Achermann, P. (1999). Sleep homeostasis and models of sleep regulation. *Journal of Biological Rhythms*, 14. 559–568.

Born, J., Rasch, B. & Gais, S. (2006). Sleep to remember. *The Neuroscientist*, 12, 410–424.

Born, J. & Wilhelm, I. (2012). System consolidation of memory during sleep. *Psychological Research*, 76, 192–203.

Brodt, S., Gais, S., Beck, J., Erb, M., Scheffler, K. & Schönauer, M. (2018). Fast track to the neocortex: A memory engram in the posterior parietal cortex. *Science*, 362, 1045–1048.

Brown, M.W. & Aggleton J.P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nature Reviews: Neuroscience*, 2, 51–61.

Bulkin, D.A., Law, L.M. & Smith, D.M. (2016). Placing memory in context: hippocampal representations promote retrieval of appropriate memories. *Hippocampus*, 26, 958–971.

Butterly, D.A., Petroccione, M.A. & Smith, D.M. (2012). Hippocampal context processing is critical for interference free recall of odor memories in rats. *Hippocampus*, 22, 906–913.

Buzsáki, G. (2002). Theta oscillation in the hippocampus. *Neuron*, 33, 325–340.

Buzsáki, G. (2006). Rhythms of the brain. New York: Oxford University Press.

Buzsáki, G. (2015). Hippocampal sharp wave-ripple: a cognitive biomarker for episodic memory and planning. *Hippocampus*, 25, 1073–1188.

Cai, D.J., Shuman, T., Gorman, M.R., Sage, J.R. & Anagnostaras, S.G. (2009). Sleep selectively enhances hippocampus-dependent memory in mice. *Behavioral Neuroscience*, 123, 713–719.

Carman, J.B., Cowan, W.M. & Powell, T.P.S. (1964). Cortical connexions of the thalamic reticular nucleus. *Journal of Anatomy*, 98, 587598.

Carr, M.F., Jadhav, S.P. & Frank, L.M. (2011). Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nature Neuroscience*, 14, 147–153.

Cassel, J.C., Pereira de Vasconcelos, A., Loureiro, M., Cholvin, T., Dalrymple-Alford, J.C., Vertes, R.P. (2013). The reuniens and rhomboid nuclei: neuroanatomy, electrophysiological characteristics and behavioral implications. *Progress in Neurobiology*, 111, 34–52.

Chao, O.Y., Nikolaus, S., Lira Brandão, M., Huston, J.P. & De Souza Silva, M.A. (2017). Interaction between the medial prefrontal cortex and hippocampal CA1 area is essential for episodic-like memory in rats. *Neurobiology of Learning and Memory*, 141, 72–77.

Chen, L., Tian, S. & Ke, J. (2014). Rapid eye movement sleep deprivation disrupts consolidation but not reconsolidation of novel object recognition memory in rats. *Neuroscience Letters*, 563, 12–16.

Cirelli, C. & Tononi, G. (2007). Is sleep essential? *PLoS Biology*, 6, e216.

Clayton, N. S., Bussey, T. J. & Dickinson, A. (2003). Can animals recall the past and plan for the future? Nature Reviews Neuroscience, 4, 685–691.

Clayton, N.S. & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature*, 395, 272–274.

Clayton, N.S., Salwiczek, L.H. & Dickinson, A. (2007). Episodic Memory. *Current Biology*, 17, 189–191.

Clemens, Z., Mölle, M., Eross, L., Barsi, P., Halász, P. & Born, J. (2007). Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans. *Brain*, 130, 2868–2878.

Clemens, Z., Mölle, M., Eross, L., Jakus, R., Rásonyi, G., Halász, P. & Born, J. (2011). Fine-tuned coupling between human parahippocampal ripples and sleep spindles. *European Journal of Neuroscience*, 33, 511–520.

Cohen, N., Pell, L., Edelson, M.G., Ben-Yakov, A., Pine, A. & Dudai, Y. (2015). Peri-encoding predictors of memory encoding and consolidation. *Neuroscience and Biobihavioral Reviews*, 50, 128–142.

Corkin, S. (1984). Lasting consequences of bilateral medial temporal lobectomy: clinical course and experimental findings in H.M. *Seminars in Neurology*, 4, 249–259.

Crunelli, V. & Hughes, S.W. (2010). The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nature Neuroscience*, 13, 9–17.

Daurat, A., Terrier, P., Foret, J. & Tiberge, M. (2007). Slow wave sleep and recollection in recognition memory. *Consciousness and Cognition*, 16, 445–455.

Davachi, L. (2006). Item, context and relational episodic encoding in humans. *Current Opinion in Neurobiology*, 16, 693–700.

Day, J.J. & Sweatt, J.D. (2010). DNA methylation and memory formation. *Nature Neuroscience*, 13, 1319–1323.

Dere, E., Huston, J.P. & De Souza Silva, M.A. (2005a). Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. *Neurobiology of Learning and Memory*, 84, 214–221.

Dere, E., Huston, J.P. & De Souza Silva, M.A. (2005b). Episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. *Brain Research Protocols*, 16, 10–19.

Dere, E., Kart-Teke, E., Huston, J.P. & De Souza Silva, M.A. (2006). The case for episodic memory in animals. *Neuroscience and Biobehavioral Reviews*, 30, 1206–1224.

Destexhe, A., Contreras, D. & Steriade, M. (1998). Mechanisms underlying the synchronizing action of corticothalamic feedback through inhibition of thalamic relay cells. *Journal of Neurophysiology*, 79, 999–1016.

DeVito, L.M. & Eichenbaum, H. (2010). Distinct contributions of the hippocampus and medial prefrontal cortex to the "what–where–when" components of episodic-like memory in mice. *Behavioural Brain Research*, 215, 318–325.

Diba, K. & Buzsáki, G. (2007). Forward and reverse hippocampal place-cell sequences during ripples. *Nature Neuroscience*, 10, 1241–1242.

Diekelmann, S. & Born, J. (2010). The memory function of sleep. *Nature Reviews: Neuroscience*, 11, 114-126.

Drosopoulos, S., Wagner, U. & Born, J. (2005). Sleep enhances explicit recollection in recognition memory. *Learning and Memory*, 12, 44–51.

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annual Review of Psychology*, 55, 51–86.

Dudai, Y. (2012). The restless engram: consolidations never end. *Annual Review of Neuroscience*, 35, 227–247.

Dudai, Y., Karni, A. & Born, J. (2015). The consolidation and transformation of memory. *Neuron*, 88, 20–32.

Dudai, Y. & Morris, R.G. (2000). To consolidate or not to consolidate: what are the questions? In J.J. Bulhuis (Ed.), *Brain, Perception, Memory. Advances in Cognitive Sciences* (pp. 149–162). Oxford: Oxford University Press.

Durán, E., Oyanedel, C.N., Niethard, N., Inostroza, M. & Born, J. (2018) Sleep stage dynamics in neocortex and hippocampus. *Sleep*, 41, zsy060.

Eacott, M.J. & Easton, A. (2010). Episodic memory in animals: Remembering which occasion. *Neuropsychologia*, 48, 2273–2280.

Eacott, M.J. & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? *The Journal of Neuroscience*, 24, 1948–1953.

Easton, A. & Eacott, M.J. (2008). A new working definition of episodic memory: replacing "when" with "which". In E. Dere, A. Easton, L. Nadel, J.P. Huston (Eds.), *Handbook of Episodic Memory* (pp. 185–196). Amsterdam: Elsevier.

Eichenbaum, H. (2013). What H.M. taught us. *Journal of Cognitive Neuroscience*, 25, 14–21.

Eichenbaum, H. (2017). Memory: Organization and control. *Annual Review of Psychology*, 68, 19–45.

Eichenbaum, H., Yonelinas, A.P. & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annual Review of Neuroscience*, 30, 123–152.

Ellenbogen, J.M., Hu, P.T., Payne, J.D., Titone, D. & Walker, M.P. (2007). Human relational memory requires time and sleep. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 7723–7728.

Ellenbogen, J.M., Hulbert, J.C., Stickgold, R., Dinges, D.F. & Thompson-Schill, S.L. (2006a). Interfering with theories of sleep and memory: sleep, declarative memory, and associative interference. *Current Biology*, 16, 1290 – 1294.

Ellenbogen, J.M., Payne, J.D. & Stickgold, R. (2006b). The role of sleep in declarative memory consolidation: passive, permissive, active or none? *Current Opinion in Neurobiology*, 16, 716–722.

Emrick, J., Gross, B.A., Riley, B.T. & Poe, G.R. (2016) Different simultaneous sleep states in the hippocampus and neocortex. *Sleep*, 39, 2201–2209.

Ennaceur, A. & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*, 31, 47–59.

Ennaceur, A., Neave, N. & Aggleton, J.P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research*, 113, 509–519.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research*, 215, 244–254.

Eschenko, O., Mölle, M., Born, J. & Sara, S.J. (2006). Elevated sleep spindles density after learning or after retrievals in rats. *The Journal of Neuroscience*, 26, 12914–12920.

Eschenko, O., Ramadan, W., Mölle, M., Born, J. & Sara, S.J. (2008). Sustained increase in hippocampal sharp-wave ripple activity during slow-wave sleep after learning. *Learning and Memory*, 15, 222–228.

Feld, G.B. & Diekelmann, S. (2015). Sleep smart–optimizing sleep for declarative learning and memory. *Frontiers in Psychology*, 6, 622.

Fogel, S.M., Smith, C.T. & Beninger, R.J. (2009). Evidence for 2-stage models of sleep and memory: Learning-dependent changes in spindles and theta in rats. *Brain Research Bulletin*, 79, 445–451.

Fogel, S.M., Smith, C.T. & Beninger, R.J. (2010). Too much of a good thing? Elevated baseline sleep spindles predict poor avoidance performance in rats. *Brain Research*, 1319, 112–117.

Fraigne, J.J., Torontali, Z.A., Snow, M.B. & Peever, J.H. (2015). REM sleep at its core – circuits, neurotransmitters, and pathophysiology. *Frontiers in Neurology*, 6, 123.

Fruth, B., Tagg, N. & Stewart, F. (2018). Sleep and nesting behavior in primates: A review. *American Journal of Physical Anthropology*, 166, 499–509.

Gais, S., Albouy. G., Boly, M., Dang-Vu, T.T., Darsaud, A., Desseilles, M.,… Peigneux, P. (2007). Sleep transforms the cerebral trace of declarative memories. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 18778–18783.

Gais, S. & Born, J. (2004). Declarative memory consolidation: Mechanisms acting during human sleep. *Learning and Memory*, 11, 679–685.

Gallistel, C.R. (1990). The organization of learning. Cambridge, MA: MIT Press.

Glickman, S.E. (1961). Perseverative neural processes and consolidation of the memory trace. *Psychological Bulletin*, 58, 218–233.

Gräff, J. & Tsai, L.-H. (2013). Histone acetylation: molecular mnemonics on the chromatin. *Nature Reviews: Neuroscience*, 14, 97–111.

Graves, L.A., Heller, E.A., Pack, A.I. & Abel, T. (2003). Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learning and Memory*, 10, 168–176.

Groch, S., Wilhelm, I., Diekelmann, S. & Born, J. (2013). The role of REM sleep in the processing of emotional memories: evidence from behavior and event-related potentials. *Neurobiology of Learning and Memory*, 99, 1–9.

Hampton, R.R., Hampstead, B.M. & Murray, E.A. (2005). Rhesus monkeys (*Macaca mulatt*a) demonstrate robust memory for what and where, but not when, in an open-field test of memory. *Learning and Motivation*, 36, 245–259.

Hampton, R.R. & Schwartz, B.L. (2004). Episodic memory in nonhumans: what, and where, is when? *Current Opinion in Neurobiology*, 14, 192–197.

Hassabis, D., Kumaran, D., Vann, S.D. & Maguire, E.A. (2007). Patients with hippocampal amnesia cannot imagine new experiences. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 1726–1731.

Hebb, D.O. (1949). The organization of the behavior. New York: Wiley.

Hoscheidt, S.M., Nadel, L., Payne, J. & Ryan, L. (2010). Hippocampal activation during retrieval of spatial context from episodic and semantic memory. *Behavioural Brain Research*, 12, 121–132.

Hotte, M., Naudon, L. & Jay, T.M. (2005). Modulation of recognition and temporal order memory retrieval by Dopamine D_1 receptor in rats. *Neurobiology of Learning and Memory*, 84, 85–92.

Inostroza, M., Binder, S. & Born, J. (2013a). Sleep-dependency of episodic-like memory consolidation in rats. *Behavioural Brain Research*, 237, 15-22.

Inostroza, M. & Born, J. (2013). Sleep for preserving and transforming episodic memory. *Annual Review of Neuroscience*, 36, 79–102.

Inostroza, M., Brotons-Mas, J., Laurent, F., Cid, E. & Mendez de la Prida, L. (2013b). Specific impairment of "what-where-when" episodic-like memory in experimental models of temporal lobe epilepsy. *The Journal of Neuroscience*, 33, 17749–17762.

James, W. (1890). The principles of psychology. New York: Henry Holt & Co.

Jarome, T.J. & Helmstetter, F.J. (2014). Protein degradation and protein synthesis in long-term memory formation. *Frontiers in Molecular Neuroscience*, 7, 61.

Jenkins, J.G. & Dallenbach, K.M. (1924). Obliviscence during sleep and waking. *American Journal of Psychology*, 35, 605–612.

Kandel, E.R., Dudai, Y. & Mayford, M.R. (2014). The molecular and systems biology of memory. *Cell*, 157, 163–186.

Kart-Teke, E., De Souza Silva, M.A., Huston, J.P. & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory*, 85, 173–182.

Kelemen, E., Bahrendt, M., Born, J. & Inostroza, M. (2014). Hippocampal corticosterone impairs memory consolidation during sleep but improves consolidation in the wake state. *Hippocampus*, 24, 510–515.

Kesner, R.P. & Hunsaker, M.R. (2010). The temporal attributes of episodic memory. *Behavioural Brain Research*, 215, 299–309.

Khodagholy, D., Gelinas, J.N. & Buzsáki, G. (2017). Learning-enhanced coupling between ripple oscillations in association cortices and hippocampus. *Science*, 358, 369–372.

Kim, D., Hwang, E., Lee, M., Sung, H. & Choi, J.H. (2015). Characterization of topographically specific sleep spindles in mice. *Sleep*, 38, 85–96.

Klinzing, J., Niethard, N. & Born, J. (2019). Mechanisms of system memory consolidation during sleep. *Nature Neuroscience*.

Komorowski, R.W., Manns, J.R. & Eichenbaum, H. (2009). Robust conjunctive item–place coding by hippocampal neurons parallels learning what happens where. *The Journal of Neuroscience*, 29, 9918–9929.

Langston, R.F., Stevenson, C.H., Wilson, C.L., Saunders, I. & Wood, E.R. (2010). The role of hippocampal subregions in memory for stimulus associations. *Behavioural Brain Research*, 215, 275–291.

Langston, R.F. & Wood, E.R. (2010). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus*, 20, 1139–1153.

Latchoumane, C.F., Ngo, H.-V.V., Born, J. & Shin, H.S. (2017). Thalamic spindles promote memory formation during sleep through triple phase-locking of cortical, thalamic and hippocampal rhythms. *Neuron*, 95, 424–435.

Lau, H., Tucker, M.A. & Fishbein, W. (2010). Daytime napping: Effects on human direct associative and relational memory. *Neurobiology of Learning and Memory*, 93, 554–560.

Lechner, H.A., Squire, L.R. & Byrne, J.H. (1999). 100 years of consolidation – Remembering Müller and Pilzecker. *Learning and Memory*, 6, 77–87.

Lewis, P.A. & Durrant, S.J. (2011). Overlapping memory replay during sleep builds cognitive schemata. *Trends in Coginitive Sciences*, 15, 343–351.

Lewis, L.D., Voigts, J., Flores, F.J., Schmitt, L.I., Wilson, M.A., Halassa, M.M. & Brown, E.N. (2015). Thalamic reticular nucleus induces fast and local modulation of arousal state. *Elife*, 4, e08760.

Li, J.-S. & Chao, Y.-S. (2008). Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. *Neurobiology of Learning and Memory*, 89, 192–198.

Lou, H.C., Luber, B., Crupain, M., Keenan, J.P., Nowak, M., Kjaer, T.W.,… Lisanby, S.H. (2004). Parietal cortex and representation of the mental Self. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 6827–6832.

Lu, J., Sherman, D., Devor, M. & Saper, C.B. (2006). A putative flip-flop switch for control of REM sleep. *Nature*, 441, 589–594.

Maingret, N., Girardeau, G., Todorova, R., Goutierre, M. & Zugaro, M. (2016). Hippocampo-cortical coupling mediates memory consolidation during sleep. *Nature Neuroscience*, 19, 959–964.

Maquet, P. (2001). The role of sleep in learning and memory. *Science*, 294, 1048–1052.

Marshall, L. & Born, J. (2007). The contribution of sleep to hippocampus-dependent memory consolidation. *Trend in Cognitive Sciences*, 11, 442–450.

Marshall, L., Helgadóttir, H., Mölle, M. & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature*, 444, 610–613.

Marshall, R.E., Hurly, T.A., Sturgeon, J., Shuker, D.M. & Healy, S.D. (2013). What, where and when: deconstructing memory. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20132194.

Massimini, M., Huber, R., Ferrarelli, F., Hill, S. & Tononi, G. (2004). The sleep slow oscillation as a traveling wave. *The Journal of Neuroscience*, 24, 6862–6870.

Mayford, M., Siegelbaum, S.A. & Kandel, E.R. (2012). Synapses and memory storage. *Cold Spring Harbor. Perspectives in Biology*, 4, a005751.

McGaugh, J.L. (1966). Time-dependent processes in memory storage. *Science*, 153, 1351–1358.

McDevitt, E.A., Rowe, K.M., Brady, M., Duggan, K.A. & Mednick, S.C. (2014). The benefit of offline sleep and wake for novel object recognition. *Experimental Brain Research*, 232, 1487–1496.

Milner, B. (1962). Les troubles de la memoire accompagnant des lesions hippocampiques bilaterales. In P. Passquant (Ed.) *Physiologie de hippocampe* (pp. 257–272). Paris: C.N.R.S. Paris.

Mitchell, J.B. & Laiacona, J. (1998). The medial frontal cortex and temporal memory: tests using spontaneous exploratory behaviour in the rat. *Behavioural Brain Research*, 97, 107–113.

Mölle, M., Bergmann, T.O., Marshall, L. & Born, J. (2011). Fast and slow spindles during the sleep slow oscillation: disparate coalescence and engagement in memory processing. *Sleep*, 34, 1411–1421.

Mölle, M., Eschenko, O., Gais, S., Sara, S.J. & Born, J. (2009). The influence of learning on sleep slow oscillatons and associated spindles and ripples in humans and rats. *European Journal of Neuroscience*, 29, 1071–1081.

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

Moser, M.-B., Rowland, D.C. & Moser, E.I. (2015). Place cells, grid cells, and memory. *Cold Spring Harbor. Perspectives in Biology*, 7, a021808.

Mullington, J.M., Simpson, N.S., Meier-Ewert, H.K. & Haack, M. (2010). Sleep loss and inflammation. *Best Practices & Research Clinical Endocrinology & Metabolism*, 24, 775–784.

Nader, K. (2003). Memory traces unbound. *Trends in Neuroscience*, 26, 65–72.

Neckelmann, D., Olsen, Ø.E., Fagerland, S. & Ursin, R. (1994). The reliability and functional validity of visual and semiautomatic sleep/wake scoring in the Møl1-Wistar rat. *Sleep*, 17, 120–131.

Neske, G.T. (2016). The slow oscillation in cortical and thalamic networks: mechanisms and functions. *Frontiers in Neural Circuits*, 9, 88.

Ngo, H.-V.V., Martinetz, T., Born, J. & Mölle, M. (2013). Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron*, 78, 545–553.

Ngo, H.-H.V., Miedema, A., Faude, I., Martinetz, T., Mölle, M. & Born, J. (2015). Driving sleep slow oscillations by auditory closed-loop stimulation–a self-limiting process. *The Journal of Neuroscience*, 35, 6630–6638.

Niethard, N., Hasegawa, M., Itokazu, T., Oyanedel, C.N., Born, J. & Sato, T.R. (2016). Sleep-stage-specific regulation of cortical excitation and inhibition. *Current Biology*, 26, 2739–2749.

Niethard, N., Ngo, H.-V.V., Ehrlich, I. & Born, J. (2018). Cortical circuit activity underlying sleep slow oscillations and spindles. *Proceedings of the National Academy of Sciences of the United States of America*, 115, E9220–E9229.

Nir, Y., Staba, R.J., Andrillon, T., Vyazovskiy, V.V., Cirelli, C., Fried, I. & Tononi, G. (2011). Regional slow waves and spindles in human sleep. *Neuron*, 70, 153–169.

Oyanedel, C.N., Binder, S., Kelemen, E., Petersen, K., Born, J. & Inostroza, M. (2014). Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats. *Behavioural Brain Research*, 275, 126–130.

Oyanedel, C.N., Kelemen, E., Scheller, J., Born, J. & Rose-John, S. (2015). Distinct sleep effects of interleukin-6 trans-signaling in brain and periphery. *Behavior, Brain, and Immunity*, 50, 178–185.

Oyanedel, C.N., Sawangjit, A., Born, J. & Inostroza, M. (2019). Sleep-dependent consolidation patterns reveal insights into episodic memory structure. *Neurobiology of Learning and Memory*, 160, 67–72.

Palchykova, S., Crestani, F., Meerlo, P. & Tobler, I. (2006a). Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters. *Physiology & Behavior*, 87, 144 – 153.

Palchykova, S., Winsky-Sommerer, R., Meerlo, P., Dürr, R. & Tobler, I. (2006b). Sleep deprivation impairs object recognition in mice. *Neurobiology of Learning and Memory*, 85, 263–271.

Payne, J.D. & Kensinger, E.A. (2010). Sleep's role in the consolidation of emotional episodic memories. *Current Directions in Psychological Science*, 19, 290–295.

Peever, J & Fuller, P.M. (2017). The biology of REM sleep. *Current Biology*, 27, R1237–R1248.

Piaget, J. (1928). Judgment and reasoning in the child. London: K. Paul, Trench, Trubner & Co.

Pignatelli, M., Beyeler, A. & Leinekugel, X. (2012) Neural circuits underlying the generation of theta oscillations. *Journal of Physiology-Paris*, 106, 81–92.

Plihal, W. & Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience*, 9, 534–547.

Poe, G.R. (2017). Sleep is for forgetting. *The Journal of Neuroscience*, 37, 464–473.

Rasch, B. & Born, J. (2013). About sleep's role in memory. *Physiological Reviews*, 93, 681–766.

Rauchs, G., Bertran, F., Guillery-Girard, B., Desgranges, B., Kerrouche, N., Denise, P., Foret., J. & Eustache, F. (2004). Consolidation of strictly episodic memories mainly requires rapid eye movement sleep. *Sleep*, 27, 395–401.

Rauchs, G., Desgranges, B., Foret, J. & Eustache, F. (2005). The relationship between memory systems and sleep stages. *Journal of Sleep Research*, 14, 123–140.

Rechtschaffen, A. & Bergmann, B.M. (2002). Sleep deprivation in the rat: an update of the 1989 paper. *Sleep*, 25, 18–24.

Rechtschaffen, A. & Kales, A. (1968). A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Bethesda, MD: U.S. National Institute of Neurological Diseases and Blindness, Neurological Information Network.

Riedner, B.A., Hulse, B.K., Murphy, M.J., Ferrarelli, F. & Tononi, G. (2011). Temporal dynamics of cortical sources underlying spontaneous and peripherally evoked slow waves. *Progress in Brain Research*, 193, 201–218.

Roberts, W.A. (2008). The current status of cognitive time travel research in animals. In E. Dere, A. Easton, L. Nadel, & J.P. Huston (Eds.), Handbook of episodic memory (pp. 135–153). Amsterdam: Elsevier.

Rolls, A., Colas, D., Adamantidis, A., Carter, M., Lanre-Amos, T., Heller, H.C. & de Lecea, L. (2011). Optogenetic disruption of sleep continuity impairs memory consolidation. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 13305–13310.

Sawangjit, A., Oyanedel, C.N., Niethard, N., Salazar, C., Born, J. & Inostroza, M. (2018). The hippocampus is crucial for forming non-hippocampal long-term memory during sleep. *Nature*, 564, 109–113.

Schabus, M., Gruber, G., Parapatics, S., Sauter, C., Klösch, G., Anderer, P.,… Zeitlhofer, J. (2004). Sleep spindles and their significance for declarative memory consolidation. *Sleep*, 27, 1479–1485.

Schapiro, A.C., Reid, A.G., Morgan, A., Manoach, D.S., Verfaellie, M. & Stickgold, R. (2019). The hippocampus is necessary for the consolidation of a task that does not require the hippocampus for initial learning. *Hippocampus*, doi: 10.1002/hipo.23101 Epub ahead of print.

Scheffzük, C., Kukushka, V.I., Vyssotski, A.L., Draguhn, A., Tort, A.B. & Brankačk, J. (2011). Selective coupling between theta phase and neocortical fast gamma oscillations during REM-sleep in mice. *PLoS ONE*, 6, e28489.

Scheffzük, C., Kukushka, V.I., Vyssotski, A.L., Draguhn, A., Tort, A.B. & Brankačk, J. (2013). Global slowing of network oscillations in mouse neocortex by diazepam. *Neuropharmacology*, 65, 123–133.

Schmidt, C., Peigneux, P., Muto, V., Schenkel, M., Knoblauch, V., Münch, M.,… Cajochen, C. (2006). Encoding difficulty promotes postlearning changes in sleep spindle activity during napping. *The Journal of Neuroscience*, 26, 8976–8982.

Schwartz, B.L., Hoffman, M.L. & Evans, S. (2005). Episodic-like memory: A review and new findings. *Learning and Motivation*, 36, 226–244.

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

Shaw, P.J., Tononi, G., Greenspan, R.J. & Robinson, D.F. (2002). Stress response genes protect against lethal effect of sleep deprivation in *Drosophila*. *Nature*, 417, 287–291.

Siapas, A.G. & Wilson, M.A. (1998). Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron*, 21, 1123–1128.

Squire, L.R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological Reviews*, 99, 195–231.

Squire, L.R. (2004). Memory systems of the brain: a brief history and current perspective. *Neurobiology of Learning and Memory*, 82, 171–177.

Squire, L.R. (2009). The legacy of patient H.M. for neuroscience. *Neuron*, 61, 6–9.

Squire, L.R., Stark, C.E.L. & Clark, R.E. (2001). The medial temporal lobe. *Annual Review of Neuroscience*, 27, 279–306.

Squire, L.R. & Dede, A.J.O. (2015). Conscious and unconscious memory systems. *Cold Spring Harbor. Perspectives in Biology*, 7, a021667.

Squire, L.R. & Wixted, J.T. (2011). The cognitive neuroscience of human memory since H.M. *Annual Review of Neuroscience*, 34, 259–288.

Staresina, B.P., Bergmann, T.O., Bonnefond, M., van der Meij, R., Jensen, O., Deuker, L.,… Fell, J. (2015). Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nature Neuroscience*, 18, 1679–1686.

Stephenson, R., Chu, K.M. & Lee, J. (2007). Prolonged deprivation of sleep-like rest raises metabolic rate in the Pacific beetle cockroach, *Diploptera punctata* (Eschscholtz). *The Journal of Experimental Biology*, 210, 2540 – 2547.

Steriade, M. (2003). The corticothalamic system in sleep. *Frontiers in Bioscience*, 8, 878–899.

Steriade, M., Contreras, D., Curró Dossi, R. & Nuñez, A. (1993a). The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *The Journal of Neuroscience*, 13, 3284–3299.

Steriade, M., McCormick, D.A. & Sejnowski, T.J. (1993b). Thalamocortical oscillations in the sleeping and aroused brain. *Science*, 262, 679–685.

Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature*, 437, 1272–1278.

Stickgold, R., Whidbee, D., Schirmer, B., Patel, V & Hobson, J.A. (2000). Visual discrimination task improvement: a multi-step process occurring during sleep. *Journal of Cognitive Neuroscience*, 12, 246–254.

Tronson, N.C. & Taylor, J.R. (2007). Molecular mechanisms of memory reconsolidation. *Nature Reviews: Neuroscience*, 8, 262–275.

Tse, D., Takeuchi, T., Kakeyama, M., Kajii, Y., Okuno, H., Tohyama, C.,… Morris, R.G.M. (2011). Schema-dependent gene activation and memory encoding in neocortex. *Science*, 333, 891–895.

Tulving, E. (2002). Episodic memory: From mind to brain. *Annual Review of Psychology*, 53, 1–25.

Van der Helm, E., Gujar, N., Nishida, M. & Walker, M.P. (2011). Sleep-dependent facilitation of episodic memory details. *PLoS ONE*, 6, e27421.

Van Der Werf, Y.D., Altena, E., Schoonheim, M.M., Sanz-Arigita, E., Vis, J.C., De Rijke, W. & Someren, E.J. (2009). Sleep benefits subsequent hippocampal functioning. *Nature Neuroscience*, 12, 122–123.

Van Kesteren, M.T.R., Fernández, G., Norris, D.G. & Hermans, E.J. (2010). Persistent schema-dependent hippocampal-neocortical connectivity during memory encoding and post-encoding rest in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 7550–7555.

Varela, C., Kumar, S., Yang, J.Y. & Wilson, M.A. (2014). Anatomical substrates for direct interactions between hippocampus, medial prefrontal cortex, and the thalamic nucleus reuniens, *Brain Structure & Function*, 219, 911–929.

Vorster, A.P. & Born, J. (2016). Sleep and memory in mammals, birds and invertebrates. *Neuroscience and Biobehavioral Reviews*, 50, 103–119.

Walker, M.P. & Stickgold, R. (2006). Sleep, memory, and plasticity. *Annual Review of Psychology*, 57, 139–166.

Watson, B.O. & Buzsáki, G. (2015). Sleep, memory & brain rhythms. *Daedalus*, 144, 67–82.

Weber, F.D., Wang, J.Y., Born, J. & Inostroza, M. (2014). Sleep benefits in parallel implicit and explicit measures of episodic memory. *Learning and Memory*, 21, 190–198.

Wierzynski, C.M., Lubenov, E.V., Gu, M. & Siapas, A.G. (2009). State-dependent spike-timing relationships between hippocampal and prefrontal circuits during sleep. *Neuron*, 61, 587–596.

Wilhelm, I., Rose, M., Imhof, K.I., Rasch, B., Büchel, C. & Born, J. (2013). The sleeping child outplays the adult's capacity to convert implicit into explicit knowledge. *Nature Neuroscience*, 16, 391–393.

Wilson, M.A. & McNaughton, B.L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science*, 265, 676–679.

Wiltgen, B.J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M.G., Parivash, S.N.,… Silva, A.J. (2010). The hippocampus plays a selective role in the retrieval of detailed contextual memories. *Current Biology*, 20, 1336–1344.

Winocur, G., Moscovitch, M. & Bontempi, B. (2010). Memory formation and long-term retention in humans and animals: convergence towards a transformation account of hippocampal-neocortical interactions. *Neuropsychologia*, 48, 2339–2356.

Winters, B.D., Saksida, L.M. & Bussey, T.J. (2008). Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neuroscience and Biobehavioral Reviews*, 32, 1055–1070.

Wixted, J.T. (2004). The psychology and neuroscience of forgetting. *Annual Review of Psychology*, 55, 235–269.

Yonelinas, A.P. (2001). Components of episodic memory: the contribution of recollection and familiarity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 356, 1363–1374.

Yonelinas, A.P. & Levy, B.J. (2002). Dissociating familiarity from recollection in human recognition memory: Different rates of forgetting over short retention intervals. *Psychonomic Bulletin & Review*, 9, 575–582.

Zentall, T.R., Clement, T.S., Bhatt, R.S. & Allen, J. (2001). Episodic-like memory in pigeons. *Psychonomic Bulletin & Review*, 8, 685–690.

Zentall, T.R. (2005). Animals may not be stuck in time. *Learning and Motivation*, 36, 208–225.

List of papers and statement of contributions

Study 1: Oyanedel, C.N., Binder, S., Kelemen, E., Petersen, K., Born, J. & Inostroza, M. (2014). Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats. *Behavioural Brain Research*, 275, 126–130.

I developed the research question and analysis plan, created the figures, and wrote the first draft of the manuscript. The detection algorithms were written by EK. The manuscript was edited by JB and MI. The data was collected by SB and KP.

Study 2: Oyanedel, C.N., Sawangjit, A., Born, J. & Inostroza, M. (2019). Sleep-dependent consolidation patterns reveals insights into episodic memory structure. *Neurobiology of Learning and Memory*, 160, 67–72.

I designed the experiment together with MI and JB. I collected the data, analyzed the data and wrote the first draft of the manuscript. MI, JB and I edited the manuscript. AS performed the visual sleep scoring.

Study 3: Durán, E., Oyanedel, C.N., Niethard, N., Inostroza, M. & Born, J. (2018) Sleep stage dynamics in neocortex and hippocampus. *Sleep*, 41, zsy060.

I designed the study together with MI, JB and ED. I collected the data together with ED. I analyzed the data and wrote the first draft of the manuscript together with ED. Together with ED and JB I edited the manuscript. NN and I performed the time-frequency analysis.

Study 4: Oyanedel, C.N., Durán, E., Niethard, N., Inostroza, M. & Born, J. Temporal association between sleep slow oscillations, spindles and ripples. *Submitted*.

I designed the study together with MI, JB and ED. I collected the data together with ED. I analyzed the data and wrote the first draft of the manuscript. NN and I performed the signal processing and event detections. ED performed the sleep scoring. JB, ED, NN and I edited the manuscript.

Appended Papers

Study 1 – Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats

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Short Communication

Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats

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HIGHLIGHTS

- We studied sleep effects on consolidation of episodic-like memory and its components in rats.
- We confirmed that sleep following learning enhances episodic-like memory and object-place memory.
- Episodic-like memory correlated with power of slow oscillations in EEG during slow wave sleep following learning.
- Object-place memory correlated with percentage of slow wave sleep during consolidation period.

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ABSTRACT

Our previous experiments showed that sleep in rats enhances consolidation of hippocampus dependent episodic-like memory, i.e. the ability to remember an event bound into specific spatio-temporal context. Here we tested the hypothesis that this enhancing effect of sleep is linked to the occurrence of slow oscillatory and spindle activity during slow wave sleep (SWS). Rats were tested on an episodic-like memory task and on three additional tasks covering separately the *where* (object place recognition), *when* (temporal memory), and *what* (novel object recognition) components of episodic memory. In each task, the sample phase (encoding) was followed by an 80-min retention interval that covered either a period of regular morning sleep or sleep deprivation. Memory during retrieval was tested using preferential exploration of novelty vs. familiarity. Consistent with previous findings, the rats which had slept during the retention interval showed significantly stronger episodic-like memory and spatial memory, and a trend of improved temporal memory (although not significant). Object recognition memory was similarly retained across sleep and sleep deprivation retention intervals. Recall of episodic-like memory was associated with increased slow oscillatory activity (0.85–2.0 Hz) during SWS in the retention interval. Spatial memory was associated with increased proportions of SWS. Against our hypothesis, a relationship between spindle activity and episodic-like memory performance was not detected, but spindle activity was associated with object recognition memory. The results provide support for the role of SWS and slow oscillatory activity in consolidating hippocampus-dependent memory, the role of spindles in this process needs to be further examined.

Episodic memory is defined by the ability to replay in mind a past event as it happened in a specific spatio-temporal context [1,2]. Accumulating evidence suggests that sleep supports the consolidation of hippocampus-dependent memory [3–5]. This benefitting effect is primarily conveyed by slow wave sleep (SWS) and the slow oscillations [6]. It has been proposed that the slow oscillations enhance hippocampal memories by synchronizing the reactivation of respective neuronal representations to the excitable up-state [7]. The depolarizing up-state of the slow oscillation drives spindle activity originating in thalamo-cortical networks [8]. The co-occurrence of reactivations of hippocampal memories and spindles during the up-state is thought to support the formation of a

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distributed memory representations spanning across hippocampal and extra-hippocampal networks [4].

In animals, the investigation of episodic-like memory concentrates on its major feature, i.e. the binding of an event into its spatio-temporal context, as the subjective component of autonoetic consciousness cannot be addressed. Our previous studies in rats showed that a short period of sleep after encoding is critical for maintaining episodic-like memory, spatial memory and temporal memory, which are linked to hippocampal function, whereas object recognition memory did not profit from sleep [9,10]. However, while those experiments demonstrated the importance of sleep for memory consolidation, they lacked an assessment of the underlying sleep EEG. Here we set out to test the hypothesis that the enhancing effect of sleep on episodic-like memory in rats is linked to SWS and the occurrence of slow oscillatory and spindle activity during the post-encoding retention interval.

Twelve adult male Long Evans rats were kept in regulated light/dark (12 h/12 h) conditions with light onset at 6 a.m., with water and food available *ad libitum*. The experiments were performed between 8:00 a.m. and 1:00 p.m. (i.e., in the first half of the rest phase when sleep pressure is typically high). All experimental procedures were performed in accordance with the European animal protection laws and policies (Directive 86/609, 1986, European Community) and were approved by the Schleswig–Holstein state authority.

In order to characterize sleep pattern and slow oscillation activity, four screw EEG electrodes were chronically implanted under isofluorane anesthesia (two frontal electrodes AP: +2.6 mm, L: ±1.5 mm relative to Bregma and two occipital reference electrodes AP: −10.0 mm, L: ±1.5 mm). Two stainless steel wire electrodes were implanted bilaterally in the neck muscles for EMG recordings. The electrodes were fixed to the skull with cold polymerizing dental resin. At the time of recordings the electrodes were connected through a swiveling commutator to an amplifier (Model 15A54, Grass Technologies, USA). EEG and EMG signals were amplified, filtered (EEG: 0.01–300 Hz; EMG: 30–300 Hz), and sampled at the rate of 1000 Hz. After at least seven days for recovery, rats were habituated for three days to the empty open field arena (10 min per day) and immediately afterwards to the recording box (80 min per day). To test retention of episodic-like memory and its components, we used a non-stressful, one-trial based episodic-like memory (EM) task, and three additional tasks assessing spatial (object place recognition – OPR), temporal (temporal memory – TM) and item memory (novel object recognition – NOR). The behavioral procedures were described in [10]. The tasks were executed in the following order: NOR, OPR, TM, EM, with at least two days between subsequent tests. This sequence of tasks was repeated twice, with the sleep and sleep deprivation conditions alternating across tasks in a within subject design. All tasks comprised a sample phase, an 80-min retention interval, and a test phase. The sample phase for each task allowed the rat to explore two (or four) objects in the open field until it had accumulated at least 15 s of exploration for each object within an interval of 2–5 min. The retention interval was filled either with normal morning sleep, or sleep deprivation in the recording box. In the sleep condition the rats were left undisturbed. Sleep deprivation was achieved by gentle handling; if the animal displayed a sleeping posture it was aroused by tapping on the box, gently shaking the box or if necessary disturbing the sleeping nest. For the test phase, the rats were placed in the open field arena to allow exploration for 3 min.

The EM task (Fig. 1A) included two sample sub-phases which were separated by an interval of 20 min. In the first sample sub-phase four identical objects were presented (old-familiar objects). In the second sub-phase a set of four identical objects (different from those used in the first sub-phase) was presented (recent-familiar objects). In the test phase, animals were exposed to two old-familiar and two recent-familiar objects. One of the old-familiar objects and one of the recent-familiar objects was placed at the same location as in the corresponding sample phase (old-familiar stationary and recent-familiar stationary) while the other two objects were placed in new locations (old-familiar displaced and recent-familiar displaced). This arrangement allows testing directly the binding of spatial and temporal component. The interaction between spatial and temporal component effects is basically assessed by comparing exploration time for the object that is both old and displaced (i.e., the old-familiar displaced object) with exploration times for the objects for which either only the temporal component (i.e., the old-familiar stationary object) or only the spatial component (i.e., the recent-familiar displaced) is manipulated [11]. In the OPR task, two identical objects were presented in the open field during the sample phase. In the test phase the same two objects were presented with one of the objects being displaced from its original position. Relatively enhanced exploration of the displaced object indicates memory for the location of the non-displaced object. The TM task consisted of two sample sub-phases, separated by a 20-min interval. During the first sub-phase, two identical objects were presented and in the second sub-phase, two different identical objects were presented in the same locations. For the test phase one object from each sample sub-phase was presented (at the original location). Relatively enhanced exploration of the earlier presented object indicates temporal order memory. In the NOR task the sampling phase was the same as in the OPR task. In the test phase, one of the objects was replaced by a different novel object. Relatively enhanced exploration time for the novel object indicates memory for the familiar object.

Exploration behavior was analyzed offline using the ANY-maze tracking system (Stoelting Europe, Ireland). For the EM task, the time an individual rat spent exploring each object during the test phase (retrieval) was converted into an discrimination ratio binding temporal and spatial context components: [(old-familiar stationary object − recent-familiar stationary object) + (recent-familiar displaced object − recent-familiar stationary object)]/(old-familiar stationary object + old-familiar displaced object + recent-familiar stationary object + recent-familiar displaced object) [10]. For the OPR, TM, and NOR tasks discrimination ratios were based on the formula: [(novel object − familiar object)/(novel object + familiar object)], where "novel" refers to the displaced object on the OPR task, the old-familiar object on the TM task, and novel object on the NOR task; "familiar" refers to the respective other object. Sleep was scored using 10-s epochs according to standard criteria [12] (SleepSign for Animal, Kissei Comtec, Japan). Periods of waking, SWS, REM sleep and pre-REM sleep were identified. Furthermore, Fast Fourier Transformation was performed and average power during SWS was then calculated for the 0.85–2.0 Hz slow oscillation (SO) range. Sleep spindles were detected based on the algorithm used by [13]. For the TM and EM tasks, data from one rat were discarded due to the loss of the implant, and for the EM task, an additional rat was discarded due to technical failure, thus resulting in a final $n = 12$ for the NOR and OPR tasks, $n = 11$ for the TM task and $n = 10$ for the EM task. For statistical analyses SPSS 21.0 (IBM, Armonk, USA) software was used.

In the EM task, sleep during the 80-min post-encoding retention interval distinctly improved performance in the test phase, as compared with the sleep deprivation condition (Student's *t*-test: $t(9) = 2.67$, $p = 0.026$, Fig. 1B). In fact, only when rats had slept during the retention interval did they achieve discrimination ratio above chance level (One-sample t-tests: sleep: $t(9) = 2.74$, $p < 0.05$, sleep deprivation: $t(9) = -0.38$, $p = 0.71$). In addition, exploration time was analyzed separately for the four objects of the EM task using a $2 \times 2 \times 2$ repeated measures ANOVA with the factors "retention condition" (sleep vs. sleep deprivation), "temporal component" (old-familiar vs. recent-familiar) and "spatial

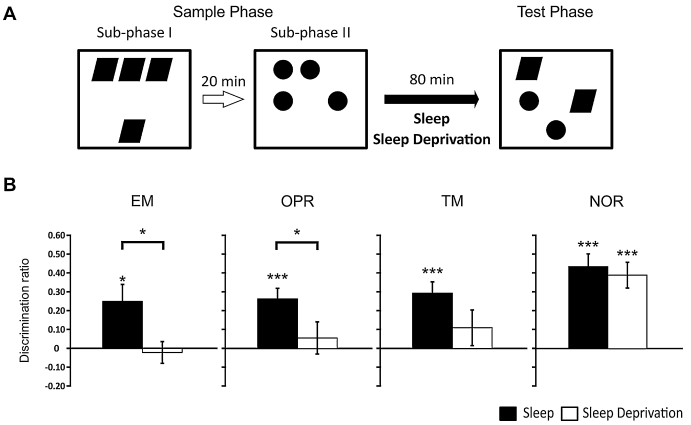

Fig. 1. (A) Schematic of EM task showing example arrangements of objects during sample and test phases. (B) Discrimination ratios indicating memory performance during the test phase in EM task, OPR task, TM task and NOR task. Means (±SEM) discrimination ratios are indicated for the sleep (black bars) and sleep deprivation (empty bars) conditions. Asterisks on top of the bars indicate significance in comparison with chance level, asterisks above horizontal lines indicate significance for pairwise comparisons between conditions (*$p < 0.05$, ***$p < 0.001$).

component" (displaced vs. stationary). Consistent with our hypothesis of a sleep effect on EM performance, this analysis revealed a significant 3-way interaction between retention condition x spatial component x temporal component ($F(1,9) = 20.4$, $p = 0.001$). Sub-ANOVAs run separately for the sleep and sleep deprivation conditions confirmed the presence of episodic-like memory in the sleep condition as reflected by high significance for the spatial component × temporal component interaction ($F(1,9) = 18,7$, $p = 0.002$). The same interaction was also significant in the sleep deprivation condition ($F(1,9) = 7.7$, $p = 0.022$). However, this interaction mainly originated from a distinctly enhanced exploration time for the old-displaced object, i.e., a pattern diverging from that of normal episodic memory binding (see [10]). In the OPR task, the memory was also better after the sleep retention interval than after sleep deprivation ($t(11) = 2.27$, $p = 0.044$, Fig. 1B). Only after the sleep retention interval did discrimination ratio significantly differ from chance (sleep: $t(11) = 4.63$, $p < 0.001$; sleep deprivation: $t(11) = 0.66$, $p = 0.53$). In the TM task, the memory improvement in the sleep condition compared to the sleep deprivation condition did not reach significance ($t(10) = 1.30$, $p = 0.22$). The discrimination ratio after the sleep retention interval was distinctly above chance ($t(10) = 4.91$, $p < 0.001$), whereas after sleep deprivation was not ($t(10) = 1.17$, $p = 0.27$). Performance in the NOR task did not profit from sleep during the retention interval ($t(11) = 0.49$, $p = 0.64$, Fig. 1B). Discrimination ratios in both the sleep and sleep deprivation conditions were significantly above chance level (sleep: $t(11) = 6.38$, $p < 0.001$; sleep deprivation: $t(11) = 5.74$, $p < 0.001$).

Total object exploration times during the sample phase did not differ significantly between the sleep and sleep deprivation condition for any of the tasks. Total exploration did also not differ between the retention conditions for the test phases of the NOR and TM tasks. On the OPR and EM tasks, total exploration time during the test phase was longer after sleep than after sleep deprivation ($t(11) = 2.75$, $p = 0.019$ and $t(9) = 3.05$, $p = 0.014$). However, we did not find significant correlation between total exploration time during the test phase and memory performance on any one of the tasks ($p > 0.05$). Furthermore, the notion that sleep effects on memory do not result from an unspecific facilitation of exploration is supported by our previous findings showing an enhancing effect of sleep on memory in the absence of any change in exploration times [14].

Total sleep time (35.45 ± 1.33 min) and sleep architecture during the sleep retention interval did not differ between the tasks ($p > 0.5$, Supplementary Table 1). EM task performance was associated with increased slow oscillatory EEG activity (0.85–2.0 Hz; Pearson $r = 0.64$, $p = 0.047$, Fig. 2A). Contrary to our expectation, spindle counts or density were not correlated with retention on the EM task ($p = 0.63$). OPR performance correlated positively with percentage of SWS within total sleep ($r = 0.750$, $p < 0.01$, Fig. 2B). NOR performance correlated negatively with the percentage of SWS ($r = -0.629$, $p < 0.05$) and showed a significant association of spindle counts during SWS ($r = 0.63$, $p = 0.027$, Fig. 2C). Supplementary Table 2 provides correlations between sleep parameters and performance in the four tasks.

We further examined whether the sample phase experience affected subsequent retention sleep, compared with baseline sleep (i.e., sleep after the last of the three habituation sessions). Except for a trend toward increased 2.0–4.0 Hz delta power during SWS ($F(3, 24) = 3.07$, $p = 0.07$), these analyses did not reveal any systematic change in sleep following the sample phase.

Behavioral effects of sleep largely replicated observation of our previous studies, indicating a critical sleep-dependency of intermediate-term episodic (EM task) and spatial (OPR task) memory [10,14,15]. NOR performance was not affected by sleep, again consistent with our previous findings. The observed enhancement of TM performance by sleep was not significant, in contrast to our previous report, suggesting less robust effects of sleep in this task. We suspect this negative outcome is owed partly to the general difficulty to sensitively measure purely temporal memory at the behavioral level in animals.

While we did not observe an effect of sleep vs. sleep deprivation in the NOR task, in several previous papers such effect was reported [16,17]. In contrast to these studies, we used rats rather than mice and employed a distinctly shorter retention interval. In addition, the high performance of the sleep deprived condition in our study

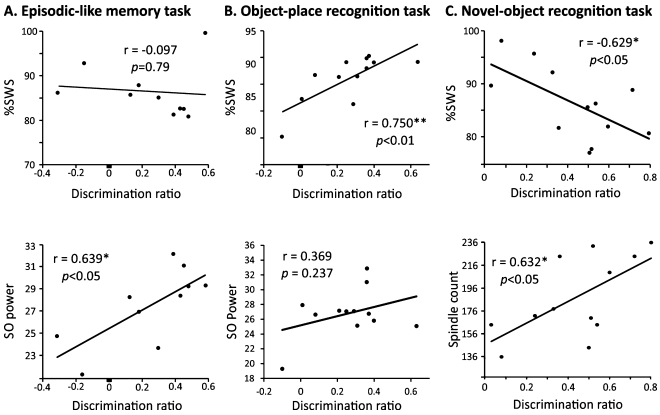

Fig. 2. (A) Correlations (Pearson) between EM performance and the percentage of SWS and EEG power in the slow oscillation frequency band (0.85–2.0 Hz). (B) Correlations between OPR performance and percentage of SWS and EEG power in the slow oscillation frequency band. (C) Correlations between NOR performance and the percentage of SWS, and spindle counts during SWS (*$p < 0.05$, **$p < 0.01$).

could have made it harder to detect a sleep induced improvement. Nevertheless, our data show unambiguously that the consolidation of this task did not require sleep. Considering the notion that unlike the other tasks, which are hippocampus dependent, the role of hippocampus in NOR appears limited [18, but see 19], our findings may corroborate the view that sleep preferentially benefits hippocampus-dependent memory [20,21].

In support for our main hypothesis that slow oscillatory activity associated with SWS is important for consolidation of hippocampus-dependent memories, we observed that (i) performance on the episodic-like memory task was positively correlated with slow oscillation power and (ii) performance on the object-place recognition task was positively correlated with percentage of SWS during retention sleep. Although correlation analyses strictly speaking cannot provide information on causality, these findings add novel evidence in support of a relationship between slow wave activity and memory performance.

As the slow oscillations hallmark SWS, the correlation of EM performance with slow oscillatory EEG power in the absence of a similarly significant correlation with the percentage of time spent in SWS might surprise. However, previous studies have shown that slow oscillation amplitude can change independently of the time spent in SWS (e.g. [22]). The correlation with slow oscillation power was revealed only for performance on the EM task, which might suggest a particular functional specificity of this oscillation for integrating spatio-temporal context into an episodic memory. The observed correlation between episodic memory formation and slow oscillatory activity during retention sleep converges with studies mostly in humans (e.g. [6]).

Contrary to our expectation, we did not observe an association of spindle activity with memory performance on the EM task, which diverges from previous studies in humans and rats (e.g. [13,23,24]). The lacking link between spindle activity and episodic-like memory performance may relate to the rather short retention interval. Assuming that spindles benefit consolidation primarily by supporting the transfer of reactivated hippocampal memory information to extra-hippocampal sites, and assuming that this redistribution of hippocampal representations is a more gradual process, retrieval testing after a retention interval of only 80 min might reflect primarily strengthening effects of sleep-associated reactivations on the hippocampal representation per se, rather than effects originating from the redistribution of the memory representation.

We believe that stress and tiredness in our protocol did not impair memory performance after sleep deprivation. The period of sleep deprivation was relatively short, and gentle handling procedures applied during such short periods do not produce substantial increases in corticosterone concentrations [25,26]. Furthermore, in our previous experiments which directly controlled for possible stress effects by testing the rats after retention periods of spontaneous wakefulness in the late evening hours (i.e., during their natural active phase), we observed an identical pattern of memory performance [9,10].

An 80-min retention interval was chosen in this and our previous studies because spatial memory in the OPR task may degrade across longer intervals and cannot be reliably assessed (e.g. [16]). However, with the short duration of the retention period we might have only probed effects of sleep on intermediate-term memory ranging between minutes and hours. Therefore, future studies have to scrutinize how these effects of sleep translate into the formation of long-term memories.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2014.09.008.

References

[1] Allen TA, Fortin NJ. The evolution of episodic memory. Proc Natl Acad Sci USA 2013;110(Suppl.):10379–86.

[2] Pause BM, Zlomuzica A, Kinugawa K, Mariani J, Pietrowsky R, Dere E. Perspectives on episodic-like and episodic memory. Fron Behav Neurosci 2013, http://dx.doi.org/10.3389/fnbeh.2013.00033.
[3] Giuditta A, Ambrosini MV, Montagnese P, Mandile P, Cotugno M, Grassi Zucconi G, et al. The sequential hypothesis of the function of sleep. Behav Brain Res 1995;69:157–66.
[4] Inostroza M, Born J. Sleep for preserving and transforming episodic memory. Ann Rev Neurosci 2013;36:79–102.
[5] Rasch B, Born J. About sleep's role in memory. Physiol Rev 2013;93:681–766.
[6] Marshall L, Helgadóttir H, Mölle M, Born J. Boosting slow oscillations during sleep potentiates memory. Nature 2006;444:610–3.
[7] Mölle M, Born J. Slow oscillations orchesting fast oscillations and memory consolidation. Prog Brain Res 2011;193:93–110.
[8] Steriade M. The corticothalamic system in sleep. Front Biosci 2003;8:878–99.
[9] Binder S, Baier PC, Mölle M, Inostroza M, Born J, Marshall L. Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats. Neurobiol Learn Mem 2012;97:213–9.
[10] Inostroza M, Binder S, Born J. Sleep-dependency of episodic-like memory consolidation in rats. Behav Brain Res 2013;237:15–22.
[11] Kart-Teke E, De Souza Silva MA, Huston JP, Dere E. Wistar rats show episodic-like memory for unique experiences. Neurobiol Learn Mem 2006;85:173–82.
[12] Neckelmann D, Olsen OE, Fagerland S, Ursin R. The reliability and functional validity of visual and semiautomatic sleep/wake scoring in the Møll–Wistar rat. Sleep 1994;17:120–31.
[13] Eschenko O, Mölle M, Born J, Sara SJ. Elevated sleep spindle density after learning or after retrieval in rats. J Neurosci 2006;26:12914–20.
[14] Kelemen E, Bahrendt M, Born J, Inostroza M. Hippocampal corticosterone impairs memory consolidation during sleep but improves consolidation in the wake state. Hippocampus 2014;24:510–5.
[15] Ishikawa H, Yamada K, Pavlides C, Ichitani Y. Sleep deprivation impairs spontaneous object-place but not novel-object recognition in rats. Neurosci Lett 2014;580:114–8.
[16] Palchykova S, Winsky-Sommerer R, Meerlo P, Dürr R, Tobler I. Sleep deprivation impairs object recognition in mice. Neurobiol Learn Mem 2006;85:263–71.
[17] Rolls A, Colas D, Adamantidis A, Carter M, Lanre-Amos T, Heller HC, et al. Optogenetic disruption of sleep continuity impairs memory consolidation. Proc Natl Acad Sci USA 2011;108:13305–10.
[18] Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J Neurosci 2004;24:5901–8.
[19] Cohen SJ, Munchow AH, Rios LM, Zhang G, Asgeirsdóttir HN, Stackman Jr RW. The rodent hippocampus is essential for nonspatial object memory. Curr Biol 2013;23:1685–90.
[20] Cai DJ, Shuman T, Gorman MR, Sage JR, Anagnostaras SG. Sleep selectively enhances hippocampus-dependent memory in mice. Behav Neurosci 2009;123:713–9.
[21] Graves LA, Heller EA, Pack AI, Abel T. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem 2003;10:168–76.
[22] Van Der Werf YD, Altena E, Schoonheim MM, Sanz-Arigita EJ, Vis JC, De Rijke W, et al. Sleep benefits subsequent hippocampal functioning. Nat Neurosci 2009;12:122–3.
[23] Fogel SM, Smith CT, Beninger RJ. Evidence for 2-stage models of sleep and memory: learning-dependent changes in spindles and theta in rats. Brain Res Bull 2009;79:445–51.
[24] Schabus M, Gruber G, Parapatics S, Sauter C, Klösch G, Anderer P, et al. Sleep spindles and their significance for declarative memory consolidation. Sleep 2004;27:1479–85.
[25] Meerlo P, Koehl M, van der Borght K, Turek FW. Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. J Neuroendocrinol 2002;14:397–402.
[26] Zant JC, Leenaars CHC, Kostin A, Van Someren EJW, Porkka-Heiskanen T. Increases in extracellular serotonin and dopamine metabolite levels in the basal forebrain during sleep deprivation. Brain Res 2011;1399:40–8.

Supplementary Data

Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats

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	EM (N=10)	**OPR** (N=12)	**TM** (N=11)	**NOR** (N=12)
TST (min)	33.78 ± 3.32	33.28 ± 2.29	37.65 ± 2.04	38.17 ± 3.00
%SWS	86.49 ± 1.88	86.14 ± 1.28	86.38 ± 1.16	86.41 ± 1.99
%PreREM sleep	6.15 ± 1.26	6.85 ± 1.03	6.48 ± 0.76	5.15 ± 0.84
%REM sleep	7.36 ± 1.59	7.02 ± 0.89	7.14 ± 0.94	8.43 ± 1.48
Latency SWS (min)	19.18 ± 3.74	23.44 ± 3.29	18.79 ± 1.79	17.64 ± 2.85
REM sleep (min)	53.47 ± 5.82	56.51 ± 3.73	54.77 ± 2.87	54.17 ± 4.77

Supplementary Table 1: Sleep parameters during the sleep retention intervals for the EM, OPR, TM and NOR tasks. Sample size is indicated in parenthesis. Means (±SEM) are shown for the total sleep time (TST, in min), the percentages (with reference to TST) of slow wave sleep (%SWS), preREM sleep (%PreREM sleep) and REM sleep (%REM sleep), as well as for latencies of SWS and REM sleep (in min, with reference to the time animals were put into the recording box). There were no significant differences between tasks in any of the sleep parameters.

	EM (N=10)	**OPR** (N=12)	**TM** (N=11)	**NOR** (N=12)
%SWS	r = -0.097	r = 0.750**	r = -0.090	r = -0.629*
SO power	r = 0.639*	r = 0.369	r = 0.387	r = 0.071
Spindle count	r = 0.075	r = -0.380	r = -0.317	r = 0.632*

Supplementary Table 2: Relationship of slow wave sleep (SWS) parameters and performance on each of the four tasks (EM, OPR, TM and NOR tasks). SWS parameters are: percentage of SWS (%SWS), slow oscillation power and spindle count. (*$p < 0.05$, **$p < 0.01$)

Study 2 – Sleep-dependent consolidation patterns reveals insights into episodic memory structure

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Sleep-dependent consolidation patterns reveal insights into episodic memory structure

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ABSTRACT

Episodic memory formation is considered a genuinely hippocampal function. Its study in rodents has relied on two different task paradigms, *i.e.* the so called "what-where-when" (WW-When) task and "what-where-which" (WW-Which) task. The WW-When task aims to assess the memory for an episode as an event bound into its context defined by spatial and distinct temporal information, the WW-Which task lacks the temporal component and introduces, instead, an "occasion setter" marking the broader contextual configuration in which the event occurred. Whether both tasks measure episodic memory in an equivalent manner in terms of recollection has been controversially discussed. Here, we compared in two groups of rats the consolidating effects of sleep on episodic-like memory between both task paradigms. Sampling and test phases were separated by a 90-min morning retention interval which did or did not allow for spontaneous sleep. Results show that sleep is crucial for the consolidation of the memory on both tasks. However, consolidating effects of sleep were stronger for the WW-Which than WW-When task. Comparing performance during the post-sleep test phase revealed that WW-When memory only gradually emerged during the 3-min test period whereas WW-Which memory was readily expressed already from the first minute onward. Separate analysis of the temporal and spatial components of WW-When performance showed that the delayed episodic memory on this task originated from the temporal component which also did not emerge until the third minute of the test phase, whereas the spatial component already showed up in the first minute. In conclusion, sleep differentially affects consolidation on the two episodic-like memory tasks, with the delayed expression of WW-When memory after sleep resulting from preferential coverage of temporal aspects by this task.

1. Introduction

Episodic memory in humans has been defined as the conscious recollection of past events, *i.e.* as replaying in mind a past event as it happened (Tulving, 2002). However, animals are likewise capable of forming integrated memories of events that occur in a specific spatial-temporal context (Clayton, Bussey, & Dickinson, 2003; Clayton, Griffiths, Emery, & Dickinson, 2001). Research mainly in rodents led to the development of different behavioral task paradigms to assess memory of event-context binding which rely on preferential object exploration. A frequently used task is the so-called "what-where-when" (WW-When) task which aims to measure spatial and temporal context features in a dissociable manner (Dere, Huston, & De Souza Silva, 2005a; Kart-Teke, de Souza Silva, Huston, & Dere, 2006). However, it has been argued that rodents have a poor memory for the time and temporal order of events *per se*, which in addition relied more on familiarity-based judgement strength decay rather than representing a recollection-based retrieval of the time aspects of an episode (Bird, Roberts, Abroms, Kit, & Crupi, 2003; Hampton, Hampstead, & Murray, 2005). Based on such reasoning Eacott and coworkers (Eacott and Easton, 2010; Eacott and Norman, 2004) proposed that remembering episodes, instead, relied more on "occasion setters", as provided by the broader physical context (including objects, colors, etc.) in which an event occurred. They, accordingly, introduced the "what-where-which" (WW-Which) task in which the memory for a unique episode relies on remembering the particular occasion of an event, independent of its temporal order. Memory performance on the task was moreover shown to be based on recollection rather than familiarity based mechanisms (Panoz-Brown et al., 2016).

Sleep supports consolidation of memory, in particular in the hippocampus-dependent episodic memory system (Inostroza and Born, 2013). The hippocampal dependency represents a hallmark of episodic-

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like memory as assessed in both WW-When and WW-Which tasks (Chao, Nikolaus, Lira Brandão, Huston, & de Souza Silva, 2017; DeVito and Eichenbaum, 2010; Kart-Teke et al., 2006; Langston, Stevenson, Wilson, Saunders, & Wood, 2010). Sleep has also been shown to be critical for forming a persistent episodic memory in the WW-When task, with this effect linked to the slow oscillatory activity (Inostroza, Binder, & Born, 2013; Oyanedel et al., 2014). Here, we asked whether sleep likewise supports consolidation on the WW-Which task and, given the difference in covering temporal aspects of episodic memory between the WW-When and WW-Which tasks, whether sleep-dependent consolidation would differ between the tasks.

2. Material and methods

2.1. Animals

Twenty adult male Long Evans rats (Janvier, France) were used, ten for the evaluation of each task. Four animals were discarded from the analysis of the WW-Which task because they did not sleep during the sleep retention condition (final N = 6). The ten animals for evaluating the WW-When task were used in a previous study (Oyanedel et al., 2014). Animals were kept at a 12 h/12 h dark/light cycle with light onset at 06:00 a.m. Water and food were available *ad libitum* throughout the experiments. All experimental procedures were performed in accordance with the European animal protection laws (Directive 86/609, 1986, European Community) and were approved by the respective state authorities of Baden-Württemberg and Schleswig–Holstein, Germany.

2.2. Procedures and experimental task

Before performance on the experimental tasks, the animals were handled for at least four consecutive days. Then, they were habituated to the empty open fields (10 min per day) and immediately afterwards, to the resting box (90 min per day) during 5 (WW-Which task) or 3 (WW-When task) consecutive days. Both tasks contained two sample phases (encoding) separated by a 20-min interval, followed by a 90-min retention period, and a subsequent test phase (retrieval). Each rat participated in two different retention conditions in which the retention interval was filled either with normal sleep (Sleep) or sleep deprivation (S-Deprivation). During the Sleep condition, rats were left undisturbed in the resting box. In the S-Deprivation condition, they were deprived from sleep by gently handling (tapping on the resting box or, if necessary shaking the cage). No intense stimulation was used to minimize stress. The order of retention conditions was balanced across rats, with conditions separated by three days. All experiments were performed between 7:00 and 14:00 h.

2.3. Tasks

The WW-When task was performed as previously described (Inostroza et al., 2013, Fig. 1A). During the sample phases the animals were exposed to two different sets each comprising 4 identical objects, designated as “old-familiar” (those presented in the first sample phase) and as “recent-familiar” objects (those presented in the second sample phase). During the test phase, animals were exposed to a mixed set of objects, consisting of two old-familiar and two recent-familiar objects. One of the two old-familiar objects and one of the two recent-familiar objects were placed at the same location as in the corresponding sample phase (old-familiar stationary and recent-familiar stationary) while the other two objects were placed at new locations (old-familiar displaced and recent-familiar displaced). Relatively enhanced exploration of the recent-familiar displaced object in comparison with the recent-familiar stationary object is considered to reflect memory for the spatial context (Where component); relatively enhanced exploration of the old-familiar stationary object compared to the recent-familiar stationary object is considered to reflect memory for the temporal context (When component). The arrangement allows directly testing the binding of an event into spatiotemporal context, as a hallmark of episodic memory which is basically indicated by a statistical interaction between spatial and temporal context effects (Oyanedel et al., 2014).

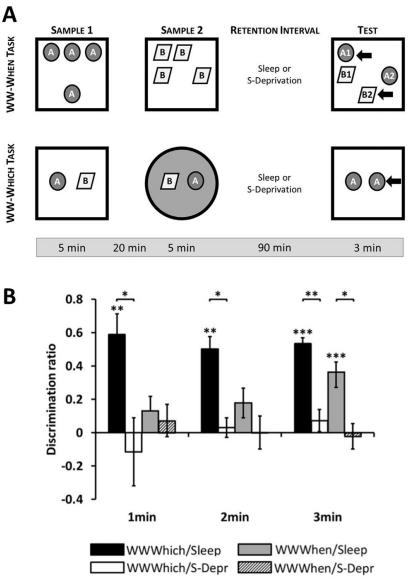

Fig. 1. Episodic-like memory tasks. (A) Schema of What-Where-When (WW-When, top) and What-Where-Which (WW-Which, bottom) tasks showing example arrangement of objects during the sample and test phases. Arrows during the test phase indicate which objects, in case of a significant episodic memory, are expected to be preferentially explored, according to the rat's natural tendency to explore novelty. For the WW-When task, object B2 (recent-familiar displaced) is expected to be more explored than object B1 (recent-familiar stationary) also as a reflection of the Where component, and object A1 (old-familiar stationary) is expected to be explored more than object B1 (recent-familiar stationary), also as a reflection of the When component. See methods for formula to calculate episodic memory DI ratios (“displaced” vs “stationary“ denotes whether at test the object is placed at a different or the same location as during the sample phase; old-familiar” vs. “recent-familiar” denotes whether the object is presented in the first or second sample phase). For the WW-Which task, in case of a significant episodic memory the object with a novel configuration of place and context (right) is expected to be more explored than the object that is presented in a familiar location and context (left). (B) Discrimination ratios indicating episodic memory across the 3-min test phase for the WW-Which (black) and WW-When (grey) tasks. Mean (± SEM) discrimination ratios are indicated for both Sleep (filled) and S-Deprivation (hatched) conditions. Asterisks on top of the bars indicate “significant above chance level”, asterisks above horizontal lines indicate significance for respective pairwise comparison between conditions (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

The WW-Which task was performed as previously described by Langston and Wood (2010) with minor modifications (Fig. 1A). During the first sample phase, the open field was configured as context A where two different objects were presented. During the second sample phase, the open field was configured as context B, and the same two different

objects were presented but their location was swapped relative to the first sample phase. For the test phase, the open field was configured as either context A or context B, and two identical copies of one of the objects from the sample phases were presented. One of the objects was presented at the same location and context as in the respective sampling phase (familiar configuration of location and context), and the other was at a position that was new for this particular context (novel configuration of location and context). Here, binding in episodic memory is basically expressed by an enhanced exploration time for the object that appears in the novel configuration of place and context in comparison with the object that is presented at a familiar location and context.

2.4. Apparatus and data analysis

The WW-When task and the WW-Which task for context A took place in a quadratic dark grey open field (80 × 80 × 40 cm, PVC). The context B for the WW-Which task was a circular white open field (diameter 80 cm). To measure the rat's behavior, a camera was mounted above the open field. Objects were made of glass and had sufficient weight to ensure the rats could not displace them. They differed in height (10–15 cm), base diameter (8–10 cm), color and shape. Pilot studies ensured that the rats could discriminate the different objects and did not show particular preferences for any objects. To further prevent any confounds by possible object preferences the use of objects as well as the locations were randomized across animals, tasks and the Sleep vs. S-Deprivation conditions. After each phase, the apparatus and objects were cleaned with water containing 70% ethanol.

Exploration behavior was analyzed offline by an experienced observer using the ANYmaze tracking system (Stoelting Europe, Dublin, Ireland). Exploration was defined by the rat being within 2 cm of an object, directing its nose towards the object and engaging in active exploration behavior, such as sniffing. For both tasks, the time an individual rat spent exploring each object during the test phase was converted into a discrimination ratio reflecting the binding of an event into spatial and temporal components (WW-When task) and the binding of the event into its contextual component (WW-Which task), respectively, as a measures of episodic memory.

The formulas were for the $DI_{What\text{-}Where\text{-}When}$:

[(OLD-FAMILIAR STATIONARY OBJECT − RECENT-FAMILIAR STATIONARY OBJECT) + (RECENT-FAMILIAR DISPLACED OBJECT − RECENT-FAMILIAR STATIONARY OBJECT)]/(OLD-FAMILIAR STATIONARY OBJECT + OLD-FAMILIAR DISPLACED OBJECT + RECENT-FAMILIAR STATIONARY OBJECT + RECENT FAMILIAR DISPLACED OBJECT);

and for the $DI_{What\text{-}Where\text{-}Which}$:

(NOVEL-LOCATION-CONTEXT OBJECT− FAMILIAR-LOCATION-CONTEXT OBJECT)/(NOVEL-LOCATION-CONTEXT OBJECT + FAMILIAR-LOCATION-CONTEXT OBJECT).

For the WW-When task the Where and When components were also analyzed according to the following formulas:

$DI_{Where\ component}$: (RECENT-FAMILIAR DISPLACED OBJECT − RECENT-FAMILIAR STATIONARY OBJECT)/(RECENT-FAMILIAR STATIONARY OBJECT + RECENT FAMILIAR DISPLACED OBJECT);

$DI_{When\ component}$: (OLD-FAMILIAR STATIONARY OBJECT − RECENT-FAMILIAR STATIONARY OBJECT)/(OLD-FAMILIAR STATIONARY OBJECT + RECENT-FAMILIAR STATIONARY OBJECT)

Statistical comparisons concentrated on cumulative DI scores for 1-min intervals across the initial 3-min interval of the test phase.

Sleep during the retention interval was assessed using standard visual procedures (Kelemen, Behrendt, Born, & Inostroza, 2014; Pack et al., 2007; Van Twyver, Webb, Dube, & Zackheim, 1973). Sleep was scored whenever the rat showed a typical sleep posture and stayed immobile for at least 10 s. If brief, movements interrupted sleep epochs by < 5 s, continuous sleep was scored. In a validation study (in 5 separate animals) we compared visual sleep scoring with EEG/EMG-based scoring. Confirming previous reports (Pack et al., 2007; Van Twyver et al., 1973), the data indicated an average agreement of visually scored sleep time with EEG/EMG-based scoring of 94.8% (with the rat's EEG/EMG-based sleep time set to 100%). The mean ± SEM total sleep time in the 5 animals was 68.81 ± 8.05 min for EEG/EMG-based scoring, and 65.92 ± 6.80 min for visual scoring.

Results are reported as the mean ± SEM. Statistical analyses were performed using SPSS 18.0 for Windows. To analyze discrimination ratios, we used analyses of variance (ANOVA) with the Tasks (WW-When, WW-Which) as group factor and Sleep/S-Deprivation and Time during the test phase (1st, 2nd, 3rd minute) as repeated measures factors. Only if an ANOVA indicated significance for main or interaction effects of interest, it was followed by *post hoc* t-tests. Discrimination ratios for each group were also compared with chance level performance (zero) using one-sample t-tests. A $p < 0.05$ was considered significant.

3. Results

Both tasks (WW-When and WW-Which), indicated that a significant episodic memory was preserved only after Sleep, but not when the rats had stayed awake during the 90-min retention interval (see Fig. 1B, for respective comparisons with chance level, $F(1, 14) = 14.685$, $p < 0.01$, for main effect of Sleep/S-Deprivation). Separate ANOVA on both the WW-When and WW-Which task confirmed a main effect for the Sleep/S-Deprivation factor ($F(1, 9) = 10.543$, $p < 0.01$ and $F(1, 5) = 21.223$, $p < 0.01$, respectively). However, the consolidating effect of sleep was generally weaker in the WW-When than WW-Which task ($F(1, 14) = 5.311$, $p < 0.05$, for main effect of Task) and, in addition showed a differential pattern across the test phase between the tasks ($F(2, 28) = 3.816$, $p < 0.05$, for the Sleep/S-Deprivation x Time x Task interaction). Discrimination ratios for WW-When task differed between the Sleep and S-Deprivation condition not until the third minute of the test phase (1st min: $t(9) = 0.415$, $p = 0.688$; 2nd min: $t(9) = 1.091$, $p = 0.304$; 3rd min: $t(9) = 3.247$, $p < 0.05$), whereas for the WW-Which task, this difference expressed itself from the first minute onward (1st min: $t(5) = 2.564$, $p < 0.05$; 2nd min $t(5) = 3.714$, $p < 0.05$; 3rd min $t(5) = 4.607$, $p < 0.01$; Fig. 1B). Similarly, on the WW-When task, discrimination ratios after sleep were above chance only at the 3rd min (1st min: $t(9) = 1.414$, $p = 0.191$; 2nd min: $t(9) = 1.942$, $p = 0.084$; 3rd min: $t(9) = 5.675$, $p < 0.001$) whereas on the WW-Which task post-sleep discrimination ratios were significant throughout the 3-min test phase (1st min: $t(5) = 4.550$, $p < 0.01$; 2nd min: $t(5) = 6.588$, $p < 0.01$; 3rd min: $t(5) = 14.552$, $p < 0.001$; Fig. 1B).

Analyses of the Where and When components of the WW-When task revealed that the slower emergence of episodic memory on this task during the post-sleep test phase was linked to the When component (ANOVA; $F(2, 18) = 8.319$, $p < 0.01$; for When/Where x Time interaction; Fig. 2). For the Where component, post-sleep discrimination ratios reached above chance levels throughout the 3-min test phase (1st min: $t(9) = 4.687$, $p < 0.01$; 2nd min: $t(9) = 3.840$, $p < 0.01$; 3rd min: $t(9) = 3.112$, $p < 0.05$), whereas for the When component these scores only differed from chance level at the 3rd minute (1st min: $t(9) = -1.315$, $p = 0.221$; 2nd min: $t(9) = -0.479$, $p = 0.643$; 3rd min: $t(9) = 6.443$, $p < 0.001$). Confirmed the differential dynamics of Where and When components across the post-sleep test phase, direct comparisons between these components indicated significant differences for the 1st ($t(9) = 3.499$, $p < 0.01$) and 2nd minute ($t(9) = 3.153$, $p < 0.05$) but, not at the 3rd minute ($p > 0.31$). The total time spent exploring objects at the sample or test phase did not differ between conditions (all $p > 0.28$).

Time spent asleep during the sleep retention intervals was

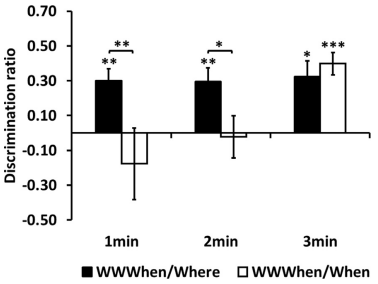

Fig. 2. Separate analysis of the Where (black bars) and When components on the WW-When task. Mean (± SEM) discrimination ratios are shown for the 3 min of test phase. Asterisks on top of the bars indicate "significant above chance level", asterisks above horizontal lines indicate significance for respective pairwise comparison between the components of the WW-When task (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

comparable between the task conditions (WW-When and WW-Which, respectively: sleep onset 19.82 ± 3.63 vs. 22.66 ± 8.03 min; $t(14) = 0.370$, $p = 0.72$; sleep duration. 24.67 ± 3.70 vs. 33.25 ± 3.08 min; $t(14) = -1.747$, $p = 0.103$).

4. Discussion

Whereas previous studies typically relied on just one specific task to assess episodic memory, the present experiments compared episodic memory assessment in two different but widely used tasks, using effects on sleep-dependent consolidation to characterize differences in the task's coverage of episodic memory function. The tasks were the WW-When task emphasizing the spatiotemporal feature in episodic memory, and the WW-Which task which, instead, emphasizes the broader context (occasional setter) in determining the memory for a specific episode. We found that on both tasks, sleep during the 90-min retention interval was necessary for the formation of a significant episodic memory in a retrieval test afterwards. Whereas for the WW-When task, this finding confirms previous work (Inostroza et al., 2013) it is novel for the WW-Which task. In combination, the findings across both tasks corroborate the notion that sleep is critical for preserving an integrated episodic memory over intermediate time intervals (Kesner and Hunsaker, 2010).

Importantly, however, the comparison of both tasks revealed that the consolidating effects of sleep were not only generally weaker on the WW-When than WW-Which task, but also emerged more gradually across the 3-min test period, whereas on the WW-Which task these effects of sleep were present already in the first minute of the test phase. The delayed emergence of significant episodic memory on the WW-When task originated from the When component of this task, as in separate analysis of the Where and When components, only the emergence of the When component was delayed but not the Where component, which was readily expressed from the beginning of the test phase onwards. This finding shows that temporal and spatial components of episodic memory differentially express over the test phase.

The hippocampus is thought not to be necessary for discriminating individual items on the basis of familiarity but, instead, to be crucial when the memory judgment requires the integration of distinct features (Jenkins, Amin, Pearce, Brown, & Aggleton, 2004) which is the case for both task paradigms used here to assess episodic memory. Rats on the WW-When task are able to remember single episodes of what happened, where and when, and this ability is based on a highly integrated "what-where-when" representation known to be supported by the hippocampus (DeVito and Eichenbaum, 2010; Barbosa, Pontes, Ribeiro, Ribeiro, & Silva, 2012; Drieskens et al., 2017). Similarly, the WW-Which task tests the rat's ability to associate an object (what), its location (where), and the broader visuospatial context in which the event takes place to form an integrated memory (Eacott and Norman, 2004). Here, the visuospatial context serves as an occasion setter, providing information to define a distinct experience that is retrieved at test. Whereas components of the task, such as object and context recognition memory, are supported by perirhinal and postrhinal cortices, respectively (Eacott and Gaffan, 2005; Gaffan, Healey, & Eacott, 2004; Norman and Eacott, 2005), the ability to integrate "what", "where" and "which" is dependent on an intact hippocampus (Eacott and Norman, 2004; Langston and Wood, 2010; Langston et al., 2010).

Our findings indicate that sleep strengthened episodic memory on both task, which agree with numerous previous studies in rodents and in humans (*e.g.* Aly and Moscovitch, 2010; Inostroza et al., 2013; Oyanedel et al., 2014; Weber, Wang, Born, & Inostroza, 2014). Considering episodic memory as a hallmark of hippocampal function our findings also tie in with the notion that sleep in particular supports the consolidation of hippocampus-dependent aspects in memory (Albouy et al., 2008; Cai, Shuman, Gorman, Sage, & Anagnostaras, 2009; Graves, Heller, Pack, & Abel, 2003; Marshall and Born, 2007; Rauchs et al., 2011), leaving unaffected the aspects which do not rely on hippocampus, such as object recognition memory (Inostroza et al., 2013; Oyanedel et al., 2014). According to the "active systems consolidation" concept, consolidation of episodic memory during sleep relies on the reactivation of the newly encoded neural representations (Inostroza and Born, 2013; Rasch and Born, 2013). These reactivations occurring during slow wave sleep, originate from hippocampal representations and spread to extrahippocampal. Neural reactivations in hippocampal networks during sleep probably exert an immediate strengthening effect on episodic memory features (Hanert, Weber, Pedersen, Born, & Bartsch, 2017; Inostroza and Born, 2013) and might secondarily, and with some delay, also support formation of extrahippocampal representations (*e.g.* Gais et al., 2007).

The WW-When and WW-Which tasks are methodologically highly comparable, inasmuch both tasks are based on the same spontaneous object exploration approach. This approach has been widely used in the evaluation of multiple aspects of memory (Dix and Aggleton, 1999; Kart-Teke et al., 2006; Mitchell and Laiacona, 1998) and possesses several advantages, mainly because it relies on an unconditioned preference thereby preventing the induction of any rule learning or semantic memory (Ennaceur and Delacour, 1988). These methodological features allow for a straight forward comparison between the tasks differing in their emphasis on spatiotemporal and broader contextual components, respectively, in the assessment of episodic memory.

A limitation of our study is that we cannot discriminate to what extent the differences in performance on both tasks at the test phase were due to differences in retrieval per se rather than to a differential consolidation of the two tasks. Differences already at encoding of the different task configurations might lead to differences in retrieval performance regardless of whether retrieval is tested immediately after encoding or following an intervening consolidation period of sleep. One might test this by comparing the raís performance on both tasks immediately after encoding. Whereas such tests have been frequently performed for the WW-Which task, the WW-When task, to our knowledge, has only been used in two studies with retention intervals shorter than 50 min (Davis, Eacott, Easton, & Gigg, 2013; Davis, Easton, Eacott, & Gigg, 2013). Interestingly, an inspection of these studies reveals that the raís pattern of exploratory performance at the test phase (after a short delay) did not coincide with that expected with longer retention intervals. It has been suggested that for the WW-When task retention intervals shorter than 50 min might produce distorted patterns of exploration at the test phase due to pro- and retroactive interference from information gathered during the first or second sample trials (Dere, Huston, & De Souza Silva, 2005b; Kart-Teke et al., 2006). In light of the

present results, we are tempted to speculate that rats can form and adequately express an integrated what-where-when episodic memory only after a period which allows them to sleep for some time, despite the fact that memory for its components (*i.e.* a separate what, where and when memory) is accessible right after encoding.

The main finding of this comparison indicated that episodic memory on the WW-When task at test expressed itself less strongly and only gradually over the 3-min period of the test phase, compared to WW-Which memory which was strongly expressed already in the first minute. In fact, for the WW-Which task, the early onset of memory expression was statistically remarkably robust, despite the smaller number of animals tested (N = 6). Separate analyses of the components revealed that this more gradual emergence of episodic memory originated from the When, rather the Where component. The When component of the episodic memory is indeed considered the most challenging feature to assess (Clayton and Russell, 2009). Several studies using different versions of the WW-When in rats and rhesus monkeys, did not find significant memory for the When component of past events, *i.e.* the animals remembered what was hidden where but not when (Bird et al., 2003; Hampton et al., 2005), and there is an ongoing discussion about whether rodents have a recollection-type of temporal memory, or whether they solved the task using, for example, decaying trace strength, *i.e.* recency as a non-hippocampal, familiarity-based strategy (Davis, Eacott, et al., 2013). Interestingly, transgenic 3xTgAD mice, a model of Alzheimer's disease were still able to form WW-When memory whereas the formation of WW-Which memory was impaired (Davis, Easton, et al., 2013) which led those authors to conclude that unlike the WW-Which task the WW-When task does not necessary require an intact hippocampus and is open to non-hippocampal solutions. However, it is also to note that in those experiments, the presence of an episodic memory was determined based only on an increased exploration time for the "old familiar-displaced" object relative to the other 3 objects. This differs from the present approach adopted from Kart-Teke et al. (2006) assuming that an integrated episodic memory for the what, where, and when components necessarily expresses itself in a much more elaborated pattern of exploration which comprises an increased exploration time for the "old familiar-stationary" object relative to the "recent familiar-stationary" object together with an increased exploration time for the "recent familiar-displaced" object relative to the "recent familiar-stationary" object. Rats can remember the when of an episodic-like memory trace in terms of the relative time elapsed (how long ago) or relative to the time of the day at which they encountered a distinctive event (Roberts, 2008; Zhou and Crystal, 2009) which argues against an absence of temporal memory, but does not exclude the use of non-hippocampal strategies for retrieving it, *i.e.* a familiarity-based strategy. This was likewise found in a human study comparing WW-When and WW-Which tasks (Easton, Webster, & Eacott, 2012). In that study, the participants successfully performed on the WW-When task with both a recollection-based strategy ("remember" judgments) and a familiarity-based strategy ("know" judgments), whereas performance on the WW-Which task was only successful with the recollection-based strategy (Ameen-Ali, Norman, Eacott, & Easton, 2017; Easton et al., 2012). Yet ultimately, we cannot determine which strategy the rats used for performing the WW-When task in our experiments. It seems, hence, appropriate to consider both WW-When and WW-Which tasks as covering the binding of differential features, *i.e.* either the spatio-temporal component or the visuospatial context, into an episodic representation, with the differences in the temporal course of object exploration during the test phases originating from difference in the components integrated into the representation.

In fact, the late expression of the When component of the WW-When task might partially explain the difficulties in its assessment. In our task arrangement, When memory was expressed at the end of the 3-min test phase. However, depending on the specific task conditions (*e.g.* retention interval, samples duration, etc.) the When memory expression might take even longer and test phases might not be long enough to catch this effect. On the other side, it has also to be noted that the WW-Which task is not devoid of any temporal component. Although per definition WW-Which performance emphasizes the binding of an object with its location and into the visuospatial context, the task involved two sample phases which also occurred at two different times (they were 20 min apart), which implicates a possible confound which is not controlled for in the present version of the WW-Which task.

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References

Albouy, G., Sterpenich, V., Balteau, E., Vandewalle, G., Desseilles, M., Dang-Vu, T., ... Peigneux, P. (2008). Both the hippocampus and striatum are involved in consolidation of motor sequence memory. *Neuron, 58*, 261–272.

Aly, M., & Moscovitch, M. (2010). The effects of sleep on episodic memory in older and younger adults. *Memory, 18*, 327–334 Learning and Memory, 24, 136–139.

Ameen-Ali, K. E., Norman, L. J., Eacott, M. J., & Easton, A. (2017). Incidental context information increases recollection. *Learning and Memory, 24*, 136–139.

Barbosa, F. F., Pontes, I. M., Ribeiro, S., Ribeiro, A. M., & Silva, R. H. (2012). Differential roles of the dorsal hippocampal regions in the acquisition of spatial and temporal aspects of episodic-like memory. *Behavioural Brain Research, 232*, 269–277.

Bird, L. R., Roberts, W. A., Abroms, B., Kit, K. A., & Crupi, C. (2003). Spatial memory for food hidden by rats (*Rattus norvegicus*) on the radial maze: Studies of memory for where, what, and when. *Journal of Comparative Psychology, 117*, 176–187.

Cai, D. J., Shuman, T., Gorman, M. R., Sage, J. R., & Anagnostaras, S. G. (2009). Sleep selectively enhances hippocampus-dependent memory in mice. *Behavioral Neuroscience, 123*, 713–719.

Chao, O. Y., Nikolaus, S., Lira Brandão, M., Huston, J. P., & de Souza Silva, M. A. (2017). Interaction between the medial prefrontal cortex and hippocampal CA1 area is essential for episodic-like memory in rats. *Neurobiology of Learning and Memory, 141*, 72–77.

Clayton, N. S., Bussey, T. J., & Dickinson, A. (2003). Can animals recall the past and plan for the future? *Nature Reviews Neuroscience, 4*, 685–691.

Clayton, N. S., Griffiths, D. P., Emery, N. J., & Dickinson, A. (2001). Elements of episodic-like memory in animals. *Philosophical Transactions of the Royal Society of London, B. 356*, 1483–1491.

Clayton, N. S., & Russell, J. (2009). Looking for episodic memory in animals and young children: Prospects for a new minimalism. *Neuropsychologia, 47*, 2330–2340.

Davis, K. E., Eacott, M. J., Easton, A., & Gigg, J. (2013). Episodic-like memory is sensitive to both Alzheimer's-like pathological accumulation and normal ageing processes in mice. *Behavioral Brain Research, 254*, 73–82.

Davis, K. E., Easton, A., Eacott, M. J., & Gigg, J. (2013). Episodic-like memory for what-where-which occasion is selectively impaired in the 3xTgAD mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease, 33*, 681–698.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005b). Episodic-like memory in mice: Simultaneous assessment of object, place and temporal order memory. *Brain Research Protocols, 16*, 10–19.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005a). Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. *Neurobiology of Learning and Memory, 84*, 214–221.

DeVito, L. M., & Eichenbaum, H. (2010). Distinct contributions of the hippocampus and medial prefrontal cortex to the "what–where–when" components of episodic-like memory in mice. *Behavioural Brain Research, 215*, 318–325.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: Evidence of object-location and object-context recognition. *Behavioural Brain Research, 99*, 191–200.

Drieskens, D. C., Neves, L. R., Pugliane, K. C., de Souza, I. B. M. B., Lima, Á. D. C., Salvadori, M. G. D. S. S., ... Barbosa, F. F. (2017). CA1 inactivation impairs episodic-like memory in rats. *Neurobiology of Learning and Memory, 24*, 28–33.

Eacott, M. J., & Easton, A. (2010). Episodic memory in animals: Remembering which occasion. *Neuropsychologia, 48*, 2273–2280.

Eacott, M. J., & Gaffan, E. A. (2005). The roles of perirhinal cortex, postrhinal cortex, and the fornix in memory for objects, contexts, and events in the rat. *The Quarterly Journal of Experimental Psychology, 58*, 202–217.

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: A possible model of episodic-like memory? *The Journal of Neuroscience, 24*, 1948–1953.

Easton, A., Webster, L. A., & Eacott, M. J. (2012). The episodic nature of episodic-like memories. *Learning and Memory, 19*, 146–150.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research, 31*, 47–59.

Gaffan, E. A., Healey, A. N., & Eacott, M. J. (2004). Objects and positions in visual scenes: Effects of perirhinal and postrhinal cortex lesions in the rat. *Behavioral Neuroscience, 118*, 992–1010.

Gais, S., Albouy, G., Boly, M., Dang-Vu, T. T., Darsaud, A., Desseilles, M., ... Maquet, P. (2007). Sleep transforms the cerebral trace of declarative memories. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 18778–18783.

Graves, L. A., Heller, E. A., Pack, A. I., & Abel, T. (2003). Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learning and Memory, 10*, 168–176.

Hampton, R. R., Hampstead, B. M., & Murray, E. A. (2005). Rhesus monkeys (*Macaca mulatta*) demonstrate robust memory for what and where, but not when, in an open-field test of memory. *Learning and Motivation, 36*, 245–259.

Hanert, A., Weber, F. D., Pedersen, A., Born, J., & Bartsch, T. (2017). Sleep in humans stabilizes pattern separation performance. *The Journal of Neuroscience, 37*, 12238–12246.

Inostroza, M., Binder, S., & Born, J. (2013). Sleep-dependency of episodic-like memory consolidation in rats. *Behavioural Brain Research, 237*, 15–22.

Inostroza, M., & Born, J. (2013). Sleep for preserving and transforming episodic memory. *Annual Review of Neuroscience, 36*, 79–102.

Jenkins, T., Amin, E., Pearce, J., Brown, M., & Aggleton, J. (2004). Novel spatial arrangements of familiar visual stimuli promote activity in the rat hippocampal formation but not the parahippocampal cortices: A c-fos expression study. *Neuroscience, 124*, 43–52.

Kart-Teke, E., de Souza Silva, M. A., Huston, J. P., & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory, 85*, 173–182.

Kelemen, E., Behrendt, M., Born, J., & Inostroza, M. (2014). Hippocampal corticosterone impairs memory consolidation during sleep but improves consolidation in the wake state. *Hippocampus, 24*, 510–515.

Kesner, R. P., & Hunsaker, M. R. (2010). The temporal attributes of episodic memory. *Behavioural Brain Research, 215*, 299–309.

Langston, R. F., Stevenson, C. H., Wilson, C. L., Saunders, I., & Wood, E. R. (2010). The role of hippocampal subregions in memory for stimulus associations. *Behavioural Brain Research, 215*, 275–291.

Langston, R. F., & Wood, E. R. (2010). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus, 20*, 1139–1153.

Marshall, L., & Born, J. (2007). The contribution of sleep to hippocampus-dependent memory consolidation. *Trend in Cognitive Sciences, 11*, 442–450.

Mitchell, J. B., & Laiacona, J. (1998). The medial frontal cortex and temporal memory: Tests using spontaneous exploratory behaviour in the rat. *Behavioural Brain Research, 97*, 107–113.

Norman, G., & Eacott, M. J. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral Neuroscience, 119*, 557–566.

Oyanedel, C. N., Binder, S., Kelemen, E., Petersen, K., Born, J., & Inostroza, M. (2014). Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats. *Behavioural Brain Research, 275*, 126–130.

Pack, A. I., Galante, R. J., Maislin, G., Cater, J., Metaxas, D., Lu, S., ... Svenson, K. (2007). Novel method for high-throughput phenotyping of sleep in mice. *Physiological Genomics, 28*, 232–238.

Panoz-Brown, D., Corbin, H. E., Dalecki, S. J., Gentry, M., Brotheridge, S., Sluka, C. M., ... Crystal, J. D. (2016). Rats remember items in context using episodic memory. *Current Biology, 26*, 2821–2826.

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological Reviews, 93*, 681–766.

Rauchs, G., Feyers, D., Landeau, B., Bastin, C., Luxen, A., Maquet, P., & Collette, F. (2011). Sleep contributes to the strengthening of some memories over others, depending on hippocampal activity at learning. *Journal of Neurosciences, 16*, 2563–2568.

Roberts, W. A. (2008). The current status of cognitive time travel research in animals. In E. Dere, A. Easton, L. Nadel, & J. P. Huston (Eds.). *Handbook of episodic memory* (pp. 135–153). Amsterdam: Elsevier.

Tulving, E. (2002). Episodic memory: From mind to brain. *Annual Review of Psychology, 53*, 1–25.

Van Twyver, H., Webb, W. B., Dube, M., & Zackheim, M. (1973). Effects of environmental and strain differences on EEG and behavioral measurement of sleep. *Behavioral Biology, 9*, 105–110.

Weber, F. D., Wang, J. Y., Born, J., & Inostroza, M. (2014). Sleep benefits in parallel implicit and explicit measures of episodic memory. *Learning and Memory, 21*, 190–198.

Zhou, W., & Crystal, J. D. (2009). Evidence for remembering when events occurred in a rodent model of episodic memory. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 9525–9529.

Study 3 – Sleep stage dynamics in neocortex and hippocampus

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ORIGINAL ARTICLE

Sleep stage dynamics in neocortex and hippocampus

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Abstract

Mammalian sleep comprises the stages of slow-wave sleep (SWS) and rapid eye movement (REM) sleep. Additionally, a transition state is often discriminated which in rodents is termed intermediate stage (IS). Although these sleep stages are thought of as unitary phenomena affecting the whole brain in a congruent fashion, recent findings have suggested that sleep stages can also appear locally restricted to specific networks and regions. Here, we compared in rats sleep stages and their transitions between neocortex and hippocampus. We simultaneously recorded the electroencephalogram (EEG) from skull electrodes over frontal and parietal cortex and the local field potential (LFP) from the medial prefrontal cortex and dorsal hippocampus. Results indicate a high congruence in the occurrence of sleep and SWS (>96.5%) at the different recording sites. Congruence was lower for REM sleep (>87%) and lowest for IS (<36.5%). Incongruences occurring at sleep stage transitions were most pronounced for REM sleep which in 36.6 per cent of all epochs started earlier in hippocampal LFP recordings than in the other recordings, with an average interval of 17.2 ± 1.1 s between REM onset in the hippocampal LFP and the parietal EEG ($p < 0.001$). Earlier REM onset in the hippocampus was paralleled by a decrease in muscle tone, another hallmark of REM sleep. These findings indicate a region-specific regulation of REM sleep which has clear implications not only for our understanding of the organization of sleep, but possibly also for the functions, e.g. in memory formation, that have been associated with REM sleep.

Statement of Significance

Sleep in mammals comprises the core sleep stages of slow-wave sleep (SWS) and rapid eye movement (REM) sleep which are thought of as unitary phenomena expressing themselves in a coherent way throughout the brain. We compared the occurrence of sleep and sleep stages in electroencephalogram recordings of cortical activity and local field potential recordings from prefrontal cortex and hippocampus. Although SWS congruently occurred in signals covering neocortical and hippocampal activity, REM sleep often started substantially earlier in the hippocampus than in neocortical networks. The findings indicate a region-specific regulation of REM sleep with implications for the functions commonly attributed to this stage.

Key words: slow-wave sleep; intermediate stage; REM sleep; prefrontal cortex; hippocampus; theta activity; muscle atonia

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Introduction

Classically, sleep and its composing sleep stages have been thought of as homogenous states that capture the whole organism, or at least the whole brain. Based on such concept, sleep was defined using behavioral criteria, such as physical quiescence and increased arousal thresholds. The most widely accepted approach to characterize sleep is polysomnography which includes the simultaneous recording of electroencephalographic (EEG) and electromyographic (EMG) recordings and, additionally in humans, electrooculographic recordings [1, 2]. These signals allow us to differentiate in mammals the two principal sleep stages of slow-wave sleep (SWS) and rapid eye movement (REM) sleep [3, 4]. Additionally, in rodents and cats, a transition state between SWS and REM sleep can be discriminated which is called intermediate stage (IS) [5–7]. Although all these sleep stages are considered as global phenomena, in recent years evidence has accumulated suggesting that sleep and sleep stages might not congruently take place in the whole brain, but can also locally occur restricted to specific networks and regions [8]. For example, in human neocortex, local activations were recorded while SWS was simultaneously present in other regions [9]. In mice, intrusions of sleep-like activity patterns were observed in local neocortical networks during prolonged wake periods and immediately after spontaneous awakening [10, 11]. In simultaneous scalp and intracranial recordings in human patients, most slow waves and spindles hallmarking the EEG during SWS were found to occur only in local neocortical networks [12].

The findings of these studies mostly examine activity within neocortical networks, underlining the local nature of phenomena defining sleep stages like spindles and slow waves. However, much less is known about the congruence in the occurrence of entire sleep stages between different brain structures. This is important, on the one hand, because the different sleep stages are often thought to fulfill specific functions. For example, "dual process theories" of memory formation during sleep assume that SWS supports consolidation of declarative memory, whereas REM sleep supports consolidation of procedural memory [13, 14]. On the other hand, the functions allocated to the different sleep stages are typically not established within only a single structure such as the neocortex, but rely on interactions between cortical and subcortical interactions. Thus, memory formation during SWS is assumed to involve the co-ordinate dialogue between neocortex and hippocampus [15, 16]. Indeed, consistent with a region-specific organization of sleep stages, intracranial recordings in human patients revealed that spindles occur in the hippocampus several minutes before sleep onset [17]. In a recent first systematic examination of sleep stages in the rat neocortex and hippocampus, both regions were found to concurrently be in different sleep stages nearly as often as they were in the same [18]. In light of the strong implications of these findings for the understanding of sleep and its functions, we here sought to confirm and extend those previous experiments. In rats, we recorded the EEG via skull electrodes over the frontal and parietal cortex and, additionally, local field potentials (LFPs) from medial prefrontal cortex (mPFC) and dorsal hippocampus (dHC). Our results reveal a high congruence in the occurrence of SWS at the different recording sites, which was decreased with regard to REM sleep. In many cases, the hippocampus appeared to enter REM sleep, together with a decrease in muscle tone, substantially earlier compared with the other recording sites.

Materials and Methods

Animals

The recordings were performed in five male Long Evans rats (Janvier, Le Genest-Saint-Isle, France, 280–340 g, 14–18 weeks old). Animals were kept on a 12 hr/12 hr light/dark cycle with lights off at 19:00 hr. Water and food were available ad libitum. All experimental procedures were approved by the University of Tübingen and the local institutions in charge of animal welfare (Regierungspräsidium Tübingen).

Surgery

Standard surgical procedures were followed as described previously [19]. Animals were anesthetized with an intraperitoneal injection of fentanyl (0.005 mg/kg of body weight), midazolam (2.0 mg/kg), and medetomidin (0.15 mg/kg). They were placed into a stereotaxic frame and were supplemented with isoflurane (0.5%) if necessary. The scalp was exposed and five holes were drilled into the skull. Three EEG screw electrodes were implanted: one frontal electrode (AP: +2.6 mm, ML: −1.5 mm, with reference to Bregma), one parietal electrode (AP: −2.0 mm, ML: −2.5 mm), and one occipital reference electrode (AP: −10.0 mm, ML: 0.0 mm). Additionally, two platinum electrodes were implanted to record LFP signals: one into the right mPFC (AP: +3.0 mm, ML: +0.5 mm, DV: −3.6 mm) and one into the right dHC (AP: −3.1 mm, ML: +3.0 mm, DV: −3.6 mm). Electrode positions were confirmed by histological analysis. One stainless steel wire electrode was implanted in the neck muscle for EMG recordings. Electrodes were connected to a six-channel electrode pedestal (PlasticsOne, USA) and fixed with cold polymerizing dental resin and the wound was sutured. Rats had at least 5 days for recovery.

Electrophysiological recordings

Rats were habituated to the recording box (dark grey PVC, 30 × 30 cm, 40 cm high) for 2 days, 12 hr per day, before actual recordings started. Experimental recordings were performed for 12 hr during the light phase, starting at 7:00 hr. The rat's behavior was simultaneously tracked using a video camera mounted on the recording box. EEG, LFP, and EMG signals were continuously recorded and digitalized using a CED Power 1401 converter and Spike2 software (Cambridge Electronic Design, UK). During the recordings, the electrodes were connected through a swiveling commutator to an amplifier (Model 15A54, Grass Technologies, USA). The screw electrode in the occipital skull served as reference for all EEG, LFP, and EMG recordings. Filtering was for the EEG between 0.1 and 300 Hz, for LFP signals a high-pass filter of 0.1 Hz was applied, and for the EMG between 30 and 300 Hz. Signals were sampled at 1 kHz.

Histology

After the last recording session, electrolytic lesions were made at the tip of the electrodes to verify their precise location (dHC and mPFC). Rats were deeply anesthetized with a lethal dose of fentanyl, midazolam, and medetomidin and intracardially perfused with saline (0.9%, wt/vol) followed by a 4 per cent

paraformaldehyde fixative solution. After extraction from the skull, brains were post-fixed in 4 per cent paraformaldehyde fixative solution for 1 day. Brains were then sliced into coronal sections (70 μm) and stained with 0.5 per cent toluidine blue (Figure 1).

Sleep stage characterization

The sleep stages (SWS, IS, and REM sleep) and wakefulness were determined offline for subsequent 10 s epochs through visual inspection. For classification of sleep stages, standard criteria were followed as described by Neckelmann et al. [2] and Bjorvatn et al. [20]. Accordingly, the wake stage was characterized by predominant low-amplitude fast activity associated with increased EMG tonus. SWS was characterized by predominant high-amplitude delta activity (<4.0 Hz) and reduced EMG activity, and REM sleep by predominant theta activity (5.0–10.0 Hz), phasic muscle twitches, and minimum EMG activity. IS was identified by a decrease in delta activity, a progressive increase of theta activity and the presence of sleep spindles (10–16 Hz). Sleep stage classification was independently performed for the frontal and parietal EEG signals and the mPFC and dHC LFP signals. Each single EEG and LFP record was independently classified (together with the associated EMG record) by two experienced experimenters (interscorer agreement > 89.9%). Consensus was achieved afterwards for epochs with discrepant classification. In addition to the classical scoring based on subsequent 10 s intervals, we rescored recordings using 2 s intervals, to examine dissociations of sleep stages at a finer temporal resolution. Analyses based on the scoring of 2 s intervals confirmed essential all results of the classical 10 s scoring and will not be reported here in detail.

Data analyses

Time spent asleep and in the different sleep stages, number of episodes, and average duration of an episode in each sleep stage was calculated for the whole 12 hr recording period. Also, for each sleep stage, the co-occurrence between any two recording sites was calculated by determining the percentage of 10 s epochs with co-occurrence of the specific sleep stage with the number of epochs with occurrence of this sleep stage in at least one of the recordings set to 100 per cent. This report is limited to the congruence between the frontal EEG signal which we used as reference (as it is most commonly used in rodent sleep research) and the three other recording sites.

To examine whether the timing of transitions into or out of specific sleep stages systematically differed between recording sites, we calculated average "delay times" for each recording site. For this purpose, the signal at the four recording sites was scanned, and whenever a transition into the sleep stage of interest occurred at one site, this time point was set to zero. Then, the transition delays for all the remaining channels were calculated based on the difference relative to this reference time point. For each recording site, the delay times to enter a specific sleep stage were averaged across all transitions into this sleep stage.

To characterize sleep stage transitions, power spectra were calculated based on MATLAB (Mathworks, USA) algorithms and the FieldTrip toolbox [21]. To this end, fast Fourier transformation (FFT) was applied to Hanning tapered blocks of 10,000 data points (corresponding to 10 s epochs), to calculate the single-sided amplitude spectrum within 0.1–25 Hz, before and after the onset of a sleep stage of interest. Power values were also used to generate time-frequency plots. Phase coherence between the dHC signal and the signal in each of the three other channels was calculated based on the frequency domain of each signal's Fourier representation computed with FieldTrip (ft_freqanalysis).

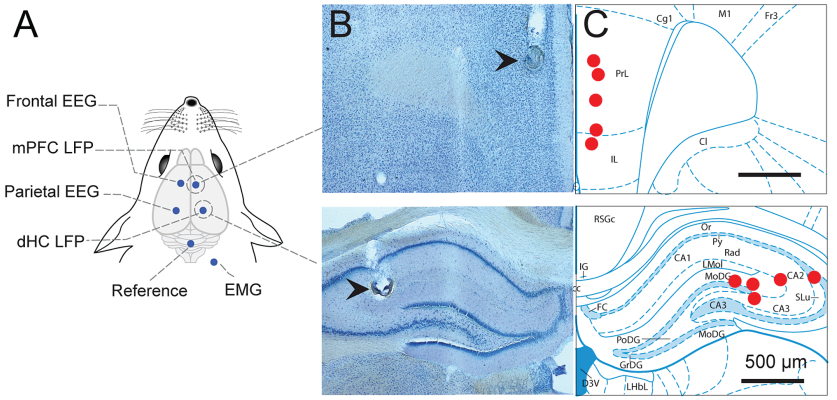

Figure 1. Electrode positions. (A) Schema of recordings. For EEG recordings, two skull electrodes were placed above the frontal and parietal lobe, respectively, of the left hemisphere; for LFP recordings, electrodes were inserted in the mPFC and dHC, respectively, in the right hemisphere. An EMG electrode was implanted into the neck muscle. The reference for EEG, LFP, and EMG recordings was a screw electrode placed above the occipital lobe. (B) Coronal histological sections showing electrode implantation sites (arrows) in the mPFC (left top) and dHC (left bottom). (C) Maps of electrode positions for mPFC (top) and dHC (bottom) LFP recordings (3.0 and −3.22 mm anteroposterior referenced to Bregma, respectively) across five animals. EEG = Electroencephalogram; LFP = Local field potential; EMG = Electromyogram; mPFC = Medial prefrontal cortex; dHC = Dorsal hippocampus.

To calculate EMG amplitude, the signal was root mean squared (rms), then filtered using a third-order low pass Butterworth filter of 0.2 Hz, and down-sampled to a rate of 100 Hz.

Statistical analyses

Kolmogorov–Smirnov test was used to assure normality of the distribution for each parameter. Differences in sleep stage classifications between recording sites were assessed using repeated measures analyses of variance (ANOVA) with a recording Site factor (frontal EEG, mPFC LFP, parietal EEG, dHC LFP) which was followed by post hoc paired sample *t*-tests, to specify significant differences between any two of the recording sites. For comparisons of mean power spectra, mean coherence, and mean EMG rms amplitude measures over time, nonparametric permutation tests were used with 2000 iterations [22]. A *p*-value of <0.05 was considered significant.

Results

Characterization of sleep stages from skull EEG and cortical and hippocampal LFP recordings

Sleep architecture was determined using the frontal EEG and EMG recordings. During the 12 hr recording period, the rats spent (mean ± SEM) 262.4 ± 14.0 min (corresponding to 36.3 ± 5.0% of the recording time) awake and 458.7 ± 13.8 min (63.7 ± 1.9%) asleep, with 364.1 ± 14.0 min (50.5 ± 5.0%) spent in SWS, 15.6 ± 2.1 min (2.2 ± 0.3%) in IS, and 79.0 ± 3.3 min (11.0 ± 0.5%) in REM sleep (Table 1). Figure 2A shows example recordings from the different recording sites for one animal. We took the frontal EEG signal as reference and determined the congruence (i.e. co-occurrence) of sleep stages between the frontal EEG and each of the three other recording sites (i.e. the parietal EEG and the LFP signals from mPFC and dHC). The congruence in wake and sleep stage occurrence during the total 12 hr period was high for time in wake (>92.0%) and SWS (>96.5%), somewhat lower for REM sleep (>87.0%), and distinctly lower for IS (<36.5%) where congruence was lowest for the mPFC LFP recordings (2.5 ± 1.6%, Figure 2B).

The time spent awake and in the different sleep stages for each of the recording sites was then subjected to ANOVA which revealed significant differences among the recording sites for time awake ($F(3, 12) = 4.02$, $p = 0.034$), time in IS ($F(3, 12) = 11.95$, $p = 0.001$), and in REM sleep ($F(3, 12) = 5.66$, $p = 0.012$), whereas time in SWS did not differ among recording sites ($F(3, 12) = 2.26$, $p = 0.134$; Figure 2C). Post hoc analyses of wake time indicated slightly longer wake times in mPFC than dHC LFP recordings ($t(4) = 2.91$, $p = 0.044$). Time in IS was longer in both frontal and parietal EEG signals compared with both mPFC and dHC LFP signals ($t \geq 5.8$, $p \leq 0.05$, for all comparisons, Figure 2C). IS was not detectable in mPFC recordings in three animals, and in dHC recordings in one animal. Time spent in REM sleep was longer in dHC than in mPFC LFP recordings, and also longer than in parietal EEG recordings ($t \geq 2.96$, $p \leq 0.041$, for all comparisons).

Table 1. Sleep architecture during the 12 hr recording period based on the frontal EEG recordings

Sleep architecture during the 12 hr recording				
Stage	Latency (min)	No. of episodes	Time in min	Time in %
Wake	0 ± 0	161.4 ± 15.1	262.4 ± 14.0	36.4 ± 5.0
SWS	34.7 ± 4.8	172.6 ± 15.0	364.1 ± 14.0	50.5 ± 5.0
IS	118.1 ± 18.6	49.8 ± 5.1	15.6 ± 2.1	2.2 ± 0.3
REM sleep	120.0 ± 17.0	47.8 ± 5.0	79.0 ± 3.3	11.0 ± 0.5

Latency is given with reference to start of the recording period. Average time spent in the different sleep stages is given in minutes and per cent of total 12 hr recording time. $n = 5$.
SWS = Slow-wave sleep; IS = Intermediate stage; REM = Rapid eye movement.

There were also distinct differences between the recording sites in the average duration of SWS periods ($F(2.81, 2930.4) = 60.2$, $p < 0.001$) and REM sleep periods ($F(3, 759) = 14.1$, $p < 0.001$, Figure 2D). SWS periods were generally longer in the EEG than LFP signals, and shortest in the mPFC LFP signal ($t \geq 13.3$, $p \leq 0.03$, for respective comparisons). REM sleep duration was also shortest in the mPFC signal ($t \geq 4.9$, $p \leq 0.001$, for all comparisons).

Wake–sleep transitions

Generally, the disparate appearance of sleep stages at the different recording sites concentrated on periods of transition between sleep stages. To examine whether the timing of wake-to-sleep transitions depended on the recording site, we analyzed in which of the four recording sites an ongoing wake epoch ended first (set to t = 0), and determined for each of the remaining recording sites the time interval it took to also finish the wake period and to enter sleep. The main result of this analysis was that the frontal EEG transited from wake into sleep significantly earlier than all other recording sites ($F(2.629, 1614.2) = 27.64$, $p < 0.001$, for ANOVA Site main effect, $t \geq 7.14$, $p \leq 0.001$ for respective pairwise comparisons, Figure 3A). However, although significant, the time differences were overall moderate (on average < 3.5 s) and below the 10 s resolution of visual sleep stage scoring. A corresponding analysis for sleep-to-wake transitions revealed that the frontal EEG was also the first to transit from sleep into wakefulness with this effect reaching significance for the comparisons with the mPFC LFP and parietal EEG signals ($F(3, 2022) = 9.09$, $p < 0.001$, for Site main effect, $t \geq 4.34$, $p \leq 0.001$, for pairwise comparisons).

Appearance of IS and REM sleep

IS episodes were overall rather short (0.31 ± 0.04 min) and most often identified in the frontal EEG recordings (Figure 2C). IS preceded REM sleep epochs in 71.9 ± 5.1% (frontal EEG), 3.45 ± 2.18% (mPFC LFP), 61.7 ± 7.5% (parietal EEG), and 18.6 ± 11.8% (dHC LFP) of all REM sleep epochs. We determined for the periods when the frontal EEG indicated IS, the occurrence of SWS and REM sleep at the other recording sites. During these periods (with the frontal EEG indicating IS), at the other recording sites, overall more SWS than REM sleep occurred, with no significant difference in SWS percentage among the recording sites ($p > 0.75$, for Site main effect, Figure 4B). On the other hand, the percentage of REM sleep during these periods was highest in the dHC recordings, and significantly higher when compared with the mPFC LFP and parietal EEG ($F(2, 8) = 10.20$, $p = 0.006$, for Site main effect, $t \geq 4.35$, $p \leq 0.012$ for respective pairwise comparisons).

To directly examine sleep stage dynamics at the transition into REM sleep, we identified REM sleep onsets in any of the four

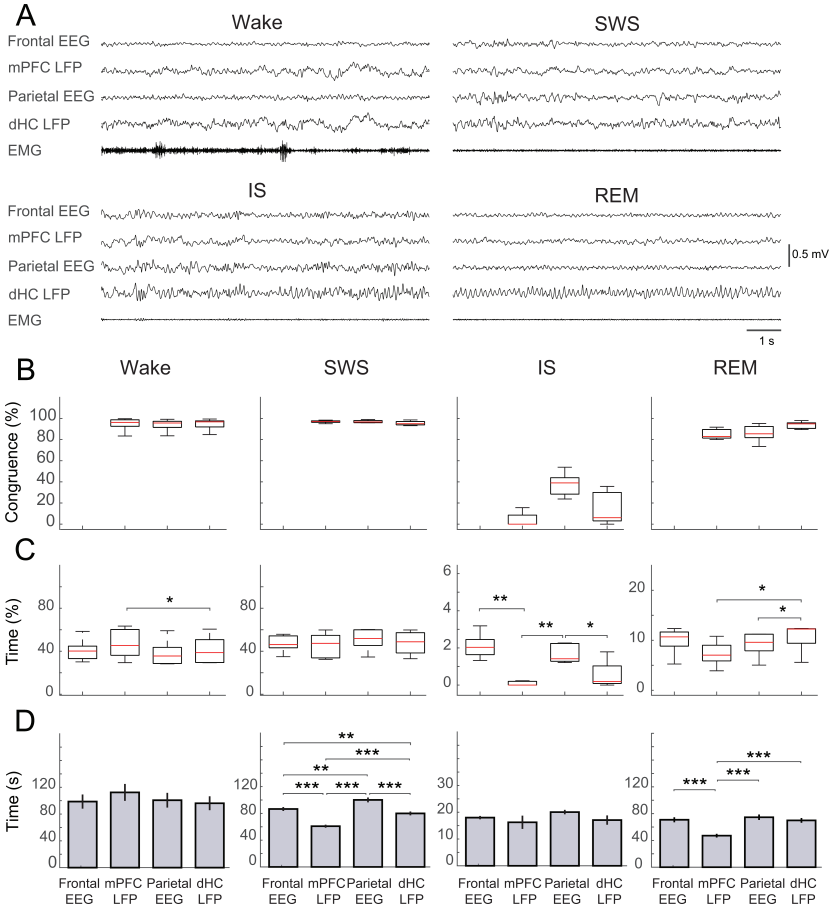

Figure 2. Sleep stage characterization in EEG and LFP recordings. (A) Examples of 10 s epoch recordings of (from top to bottom) frontal EEG, mPFC LFP, parietal EEG, and dHC LFP during wakefulness (top left), SWS (top right), IS (bottom left), and REM sleep (bottom right). (B) Comparison of sleep stage occurrence in the different recordings. Occurrence of sleep stages in mPFC LFP, parietal EEG, and dHC LFP signals is expressed as percentage of congruence with the occurrence of the sleep stages in the frontal EEG signal used as reference. (C) Distribution of time spent awake, in SWS, IS and REM sleep and (D) average duration of episodes awake, in SWS, IS, and REM sleep for the different recording sites. Box-whisker plots indicating median, upper quartile and lower quartile, top and bottom of the box, respectively. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ for pairwise comparison. EEG = Electroencephalogram; LFP = Local field potential; EMG = Electromyogram; mPFC = Medial prefrontal cortex; dHC = Dorsal hippocampus; SWS = Slow-wave sleep; REM = Rapid eye movement; IS = Intermediate stage.

recording sites and assessed how long it took in the respective three other recording sites to enter REM sleep (Figure 3B). This analysis revealed that REM sleep started overall substantially earlier in the dHC recordings compared with all other recording sites, with the greatest difference between REM sleep occurrence in dHC and parietal EEG recordings where REM sleep occurred with an average delay of 17.2 ± 1.1 s (with reference to dHC REM onset; $F(2.53, 387.2) = 38.21$, $p < 0.001$, for ANOVA Site main effect, $t \geq 4.23$, $p \leq 0.001$, for all pairwise comparison with dHC recordings, see Supplementary Figure S2 for results from an analysis

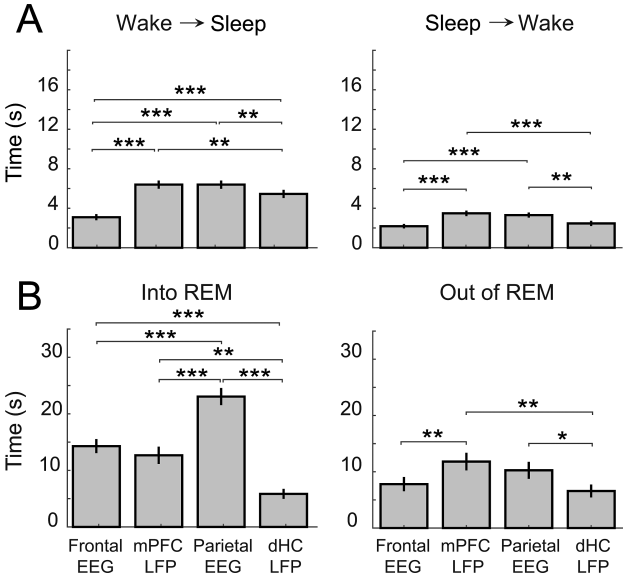

Figure 3. Transitions into and out of sleep and REM sleep. Timing of (A) wake-to-sleep (left) and sleep-to-wake transitions (right) and (B) of transitions into REM sleep (left) and out of REM sleep (right) at the different recording sites (frontal EEG, mPFC LFP, parietal EEG and dHC LFP). In these analyses, the earliest transitions into the specified brain state occurring at a certain recording site were set to 0 s and, then, the delay time was calculated till this transition occurred at each of the other recording sites. The y-axes indicate the mean (±SEM) delay time (across all detected transitions) for each recording site. Note, overall short delay times for wake-to-sleep transitions with the frontal EEG indicating first signs of sleep following a wake period. Note also that in dHC LFP recordings REM sleep is entered substantially earlier than at all other recording sites. $^{**}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ for pairwise comparisons. EEG = Electroencephalogram; LFP = Local field potential; mPFC = Medial prefrontal cortex; dHC = Dorsal hippocampus; REM = Rapid eye movement.

based on the more fine-grained scoring of 2 s epochs). In 36.6 per cent of all REM sleep episodes detected (53 cases of 145), REM sleep onset in dHC recordings preceded that at all other sites (whereas in only eight cases REM sleep started simultaneously at all sites) and in 16.5 per cent of all REM sleep episodes detected (24 cases of 145), REM sleep in the dHC LFP started later than in one of the other sites. In addition, REM sleep occurred significantly earlier (by on average 8.8 ± 1.3 s) in the frontal than in the parietal EEG ($t(153) = 6.81$, $p < 0.001$). A corresponding analysis of transitions out of REM sleep revealed that REM sleep, on average also ended earliest in dHC LFP recordings. Although the respective time difference was rather small (on average < 5 s), the effect reached significance in comparison with the mPFC LFP and parietal EEG signal ($F(2.74, 421.7) = 4.29$, $p = 0.007$, for Site main effect, $t \geq 2.05$, $p \leq 0.042$, for respective pairwise comparisons Figure 3B).

We further analyzed the sleep stages in the other recordings sites when REM sleep had started first in dHC recordings. The frontal EEG indicated IS during almost 80 per cent of this time, whereas the LFP from mPFC indicated SWS most of the time, and in the parietal EEG IS and SWS each covered about half of the time (see Figure 4C also for pairwise statistical comparisons). Finally, we examined the time course of the dissociation between REM sleep onset in cortical EEG and dHC LFP recordings across the 12 hr recording period (summarized in Supplementary Figure S1). These analyses revealed that the number of REM epochs increased across this period. However, the number of REM sleep epochs with an earlier onset in dHC LFP than cortical EEG recordings remained constant and, accordingly, the proportion of such epochs with an earlier onset in hippocampal LFP recordings decreased across this time ($H(2) = 6.36$, $p = 0.042$, for Kruskal–Wallis one-way ANOVA effect of time).

Theta activity and muscle atonia at early hippocampal REM sleep onsets

We further examined those transitions into REM sleep ($n = 53$) which occurred earlier in dHC LFP recordings than at the other recordings sites. For these cases, we calculated average power spectra for EEG and LFP signals, for 10 s intervals before and after REM sleep onset, respectively. Averaging was done either time-locked to REM onset as identified in the dHC LFP recordings or time-locked to REM onset as identified in each of the respective other three recording sites (Figure 5A–C). Comparing these two ways of time-locking revealed distinct differences for the frontal and parietal EEG, i.e. power was higher in a broad

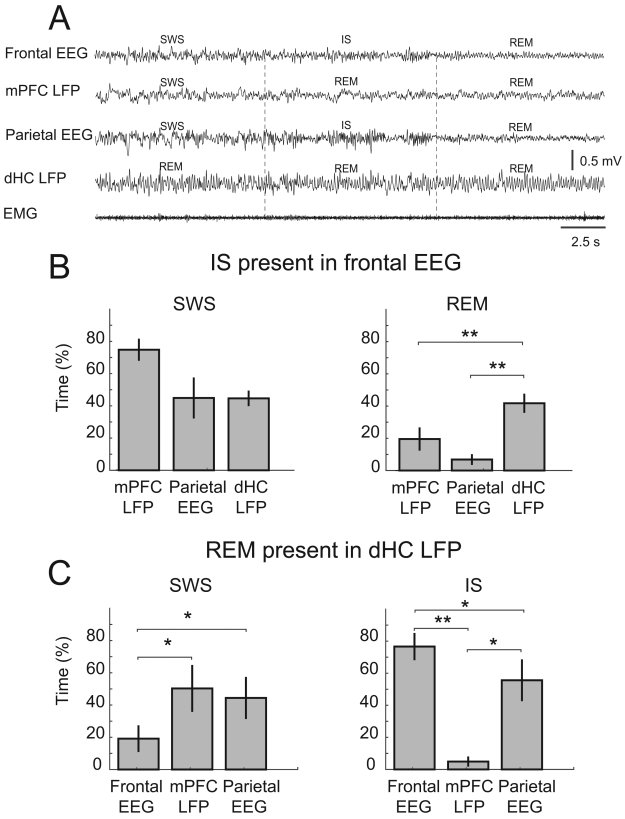

Figure 4. Appearance of REM sleep. (A) Example recordings of frontal EEG, mPFC LFP, parietal EEG, dHC LFP, and EMG signals over three consecutive 10 s intervals. Note, while REM sleep (as identified by high theta activity) is present in dHC LFP recordings already in the first 10 s interval, at the other sites consolidated REM sleep is reached not until the third 10 s interval. EMG activity decreases already during the first 10 s interval. (B) Percentages of SWS (left) and REM sleep (right) at the different recording sites, for intervals of IS in the frontal EEG. (C) Percentages of SWS (left) and IS (right) at the different recording sites, when REM sleep had started already in dHC LFP recordings. Means (±SEM) across all epochs are indicated. *$p < 0.05$; **$p < 0.01$ for pairwise comparison. EEG = Electroencephalogram; LFP = Local field potential; EMG = Electromyogram; mPFC = Medial prefrontal cortex; dHC = Dorsal hippocampus; SWS = Slow-wave sleep; REM = Rapid eye movement; IS = Intermediate stage.

frequency range including delta (1.0–4.0 Hz) and spindle (10–16 Hz) frequencies before and (though less consistently) also after the REM onsets when these REM onsets were determined in the respective EEG recordings in comparison to the spectra aligned to REM onset as defined in dHC LFP recordings. Notably this increase spared the 5.0–10.0 Hz theta band. Moreover, analyzing the coherence between recordings for cases where dHC LFP entered REM sleep first in the same way revealed a significantly reduced coherence in theta activity, particular between the dHC LFP and frontal EEG, when recordings were time-locked to the REM onset in the dHC recording (Figure 5D). Together these findings suggest that during the intervals of early local REM sleep in dHC recordings, there is also high theta activity in the cortical EEG activity that is synchronized to the hippocampal theta. However, the detection of REM sleep in the EEG signal is hampered by strong concurring SWS-related oscillatory activity.

For the cases where REM sleep onset in dHC recordings preceded REM onset in the three other recording sites, we also assessed the time course of muscle atonia as another hallmark of REM sleep. Generally, root mean square (rms) EMG activity, as expected, distinctly decreased from the 10 s interval before REM sleep onset to the 10 s interval after REM sleep onset. In the

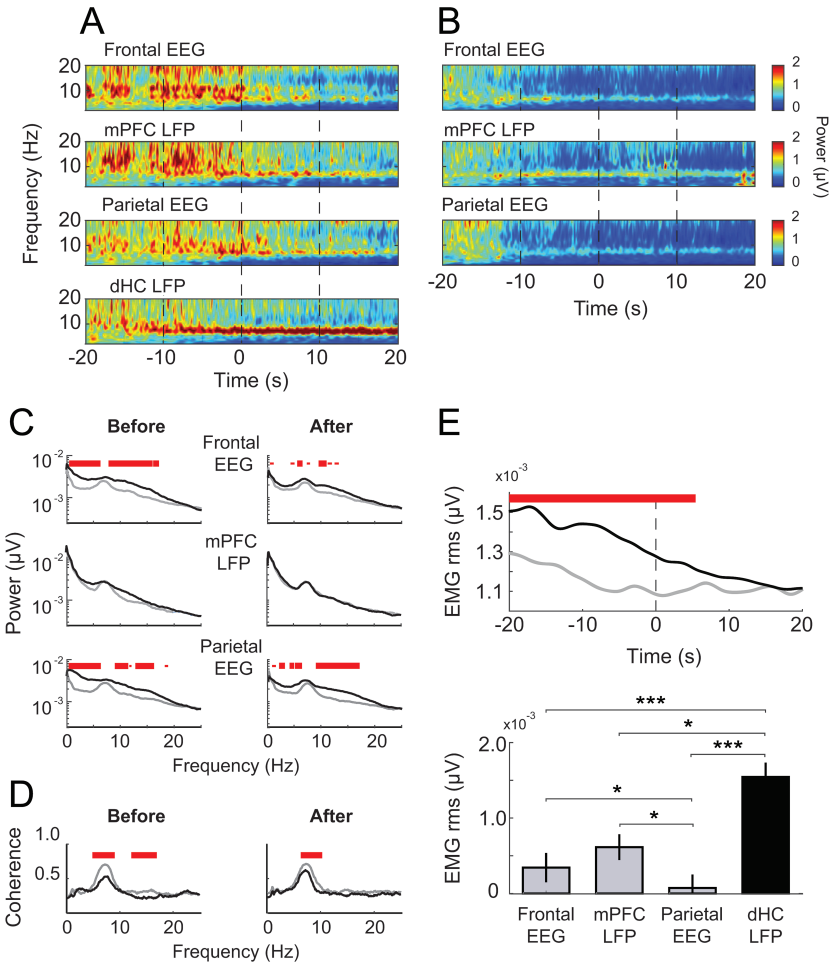

Figure 5. Early transitions into REM sleep at dHC LFP recordings. (A, B) Average time-frequency plots (power—color coded) in the different frequency bands for frontal EEG, mPFC LFP, parietal EEG, and dHC LFP signals, during a ±20 s interval around REM sleep onset ("0 s") for the cases when REM sleep started first in dHC LFP recordings ($n = 53$). Averages in (A) are time-locked to REM onset as identified in dHC LFP recordings, and in (B) to REM onset as identified in each of the other three recordings sites. (C) Power spectra for 10 s epochs before (left) and after (right) REM sleep onset for the cases when REM sleep started first in dHC LFP recordings, time-locked to REM onset as identified in dHC LFP recordings (black lines), and time-locked to REM onset as identified in each of the respective other three sites (frontal EEG, mPFC LFP, and parietal EEG; grey lines). (D) Coherence between the dHC LFP signal and the frontal EEG for 10-s epochs before (left) and after (right) REM sleep onset for the cases when REM sleep started first in dHC LFP recordings, time-locked to REM onset as identified in dHC LFP recordings (black line) and time-locked to REM onset as identified in the frontal EEG (grey). (E) Upper panel: Average EMG root mean square signal during a ±20 s interval around REM sleep onset ("0 s") for the cases when REM sleep started first in dHC LFP recordings ($n = 53$). Averages were either time-locked to the REM sleep onset as determined in dHC LFP recordings (black solid line) or to the REM sleep onset as determined in the parietal EEG (grey line). (C, D, and E) Significant differences in power, coherence, and EMG rms, respectively, are indicated by horizontal thin ($p < 0.05$) and thick ($p < 0.01$) red lines. Bar graph below shows mean differences in EMG rms activity between the 10 s intervals before minus after REM sleep onset for these cases, with REM sleep onset determined either in frontal EEG, mPFC LFP, parietal EEG, or dHC LFP recordings. Note, highest value, i.e. strongest decrease in EMG activity for defining REM sleep onset with reference to dHC LFP signal (black bar). *$p < 0.05$; ***$p < 0.001$ for pairwise comparisons. EEG = Electroencephalogram; LFP = Local field potential; EMG = Electromyogram; mPFC = Medial prefrontal cortex; dHC = Dorsal hippocampus; rms = root mean square.

cases with an earlier REM sleep onset in dHC recordings, this decrease from before to after REM sleep onset was significantly higher when the rms EMG signal was time-locked to the onset as determined in the dHC recordings, compared with time-locking the signal to REM sleep onset as determined in any of the other recording sites ($F(2.2, 84) = 9.74$, $p < 0.001$, for Site main effect, $t \geq 6.6$, $p \leq 0.03$ for pairwise comparisons, Figure 5E). Thus, earlier REM onsets in dHC recordings were also accompanied by earlier muscle atonia. A complementary analysis on the cases where REM sleep occurred in dHC LFP recordings occurred later than at least one of the other three recording sites, did reveal hints that in these cases atonia is specifically coupled to REM occurrence in the hippocampal recording (Supplementary Figure S3).

Discussion

We compared in rats the expression of sleep stages in EEG recordings over frontal and parietal cortex and in LFP recordings from mPFC and dHC, and found distinct differences between cortical and hippocampal signals that mainly pertained to the timing of REM sleep. In dHC LFP recordings, REM sleep epochs in many cases started substantially earlier than at the other recording sites preferentially covering neocortical activity, which confirms recent findings by Emrick et al. [18]. The early start of REM sleep in hippocampal recordings, moreover, was accompanied by a REM sleep-typical decrease in muscle tone. We also found differences in the occurrence of SWS at the different recording sites. However, compared with those found for REM sleep, these were overall marginal. In fact, determination of SWS in neocortical and hippocampal signals was hallmarked by a very high congruence of greater than 95 per cent. Our findings underline that differences in the regional expression of sleep stages need to be considered when it comes to characterizing the function of sleep stages, especially of REM sleep.

The high congruence of SWS at the different recording sites with no differences in the time spent in SWS in neocortical and hippocampal signals suggests that SWS reflects a rather unitary phenomenon that catches widespread areas of the brain. Classification of SWS relies mainly on the occurrence of slow waves including the <1.0 Hz slow oscillation. These oscillations are generated in thalamo-cortical networks [23–26]. Beyond synchronizing activity in these regions, the oscillations are also known to synchronize activity in several other brain regions including the hippocampus, thereby allowing precisely timed interactions between these regions [19, 27–29]. However, despite the high congruence in the occurrence of SWS at the different sites, there were subtle differences. At a first glance, it appears surprising that in the mPFC LFP signal the mean duration of SWS epochs was on average slightly shorter than at the other sites, because the prefrontal cortex is thought to be a major source of slow waves [30]. However, an LFP recording from deep layers of the mPFC is expected to be most sensitive to and to pick up only locally generated slow potential changes, whereas the amplitude of slow wave potentials originating from other sites is comparatively low at this site. By contrast, skull EEG electrodes, although receiving an overall diminished potential, pick up slow-wave signals from rather broad cortical areas. Consistent with this explanation, we found that the prefrontal EEG signal was the first to indicate the occurrence of SWS. Again, it is to emphasize that these differences were marginal and appear to mainly reflect the different sensitivity of LFP and EEG recordings to the slow-wave signal.

Contrasting with the SWS-related findings, the observed differences in REM sleep occurrence appeared to reflect a disparate regulation of this sleep stage in neocortical and hippocampal networks, which were most obvious at the transition into this sleep stage. This was evident already in the analyses of IS which in rats is defined as a transition stage between SWS and REM sleep, mainly characterized by the simultaneous occurrence of spindle-like activity and theta activity. Apart from the fact that co-occurrence of IS at the different sites was quite low (<37%), we found that IS epochs mainly occurred in EEG recordings covering frontal cortical signal, and that while the frontal cortex was in IS, the hippocampal LFP signaled already the presence of REM sleep much more often than the other recording sites (Figure 4B). Conversely, during early REM sleep onsets in hippocampal LFP recordings, the frontal EEG signaled the presence of IS in almost 80 per cent of the cases (Figure 4C), altogether suggesting that the spread of hippocampal theta activity might contribute to classification of IS in the cortical signal. Indeed, due to its amalgamate nature and the resulting difficulties to determine this sleep stage, in many studies IS is not considered as a separate stage from SWS.

The view of a disparate regulation REM sleep in hippocampal and neocortical networks is corroborated by our finding that REM sleep onset in hippocampal LFP recordings on average substantially preceded REM onsets at the other recoding sites. This finding confirms and extends findings from a previous study [18], which overall reported an even stronger asynchrony in the occurrence of REM sleep comparing skull EEG recordings with dorsal hippocampal LFP recordings. Of note, in that study hippocampal LFP recordings were referenced to an electrode in neocortical deep white matter, which contrasts with the present recordings employing an occipital skull electrode. Although widely used in standard LFP recordings, such reference electrode might bias hippocampal LFP recordings due to EEG activity picked up from underlying cerebellum [31]. However, comparing our present dHC LFP recordings with those in other studies using different electrode montages did not reveal obvious alterations, e.g. with regard to the occurrence of spindles and theta activity. Also, theta activity (used as core signal for the determination of REM sleep) showed up in very much the same way when, for exploratory purposes, we re-referenced the dHC LFP signal to the medial prefrontal LFP electrode. Nevertheless, although a substantial bias seems unlikely, the precise contribution of cerebellar EEG activity during sleep to the dHC LFP signal using an occipital skull reference is presently unclear. It is hence the more important that the central findings of our study quite well agree with those of Emrick et al., despite their use of a rather different reference for LFP recordings. Note, our findings exclude an independent regulation of REM sleep in the hippocampus because in the hippocampus REM sleep much more often preceded that followed the occurrence of REM sleep in neocortex. The signal hallmarking REM sleep is 5.0–10.0 Hz theta activity which, however, also occurs during (active) wakefulness [32, 33]. Generation of the theta rhythm involves the medial septum along with the diagonal band of Broca which directly projects to the hippocampus, with the hippocampal network representing the major theta current generator [33, 34]. In this way, the first appearance of REM sleep in hippocampal networks and before the appearance in neocortex might partly be a consequence of

this direct innervation of the hippocampus from theta generating structures. However, the early appearance of REM sleep in hippocampal recordings, in our study, was also coupled to a distinct decrease in muscle tone (Figure 5E), another major feature of REM sleep, with this coupling pointing to the involvement of brainstem mechanisms in the disparate regulation of hippocampal REM sleep. The meso-pontine area of the brainstem, including *REM-off* and *REM-on* networks, has been proposed as a switch between REM sleep and SWS [35]. Different populations of the *REM-on* network project to the basal forebrain (including theta generating structures of the medial septum and diagonal band of Broca) and to medullary nuclei and the spinal cord where they contribute to establishing muscle atonia [35–37]. Thus, projections of these brainstem *REM-on* networks are likely capable of mediating a concurrent increase in hippocampal theta activity and muscle atonia.

Interestingly, in the cases where REM sleep occurred earlier in hippocampal networks, our spectral analyses of the EEG signal during this interval revealed enhanced power in wide frequency ranges including the 0.5–4.0 Hz slow wave activity and the 10–16 Hz spindle activity ranges characteristic for SWS, but sparing the 5.0–10.0 Hz theta range (Figure 5C). Notably, this increase spared the 5.0–10.0 Hz theta range reflecting that the EEG recordings at that time also expressed high theta activity which—as revealed by coherence analyses—appeared to be synchronized in phase with the hippocampal theta rhythm (Figure 5D). Thus, when REM sleep occurs earlier in the hippocampus than neocortex, this appears to be due to SWS-related activity still capturing neocortical networks, in the presence of REM-related theta activity that in the EEG, at that time, probably represents volume-conducted hippocampal activity [38, 39]. This conclusion is further supported by our analysis of the time course in dissociation of cortical and hippocampal REM sleep onset, indicating enhanced proportion of REM sleep epochs with earlier onset in hippocampal networks in the beginning of the recording (light) period when sleep and pressure were high. Thus, the expression of theta activity per se in the cortical EEG during this time of local hippocampal REM sleep appears not to be hindered by the simultaneous appearance of slow wave and spindle frequency activity [40]. The mechanisms that then, with some delay, make neocortical networks to ultimately synchronize to the hippocampal theta rhythm remain to be clarified.

In sum, our data are consistent with the concept that sleep and SWS for the most part present as global phenomena with a common impact on different brain regions. However, the occurrence of REM sleep underlies region-specific regulatory mechanisms, in as much this sleep stage in many cases begins substantially earlier in hippocampal than neocortical networks. Future studies need to characterize the mechanisms mediating this dissociation between hippocampal and neocortical networks, and the question to what extent this dissociation might become stronger with increased propensity of sleep and SWS. Whatever the case, the present findings might be also of relevance for the understanding of the functions (like memory formation) that have been associated with the stage of REM sleep and involve respective structures of interest [41, 42].

Supplementary Material

Supplementary material is available at *SLEEP* online.

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Notes

Conflict of interest statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Rechtschaffen A, Kales A. *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects*. Bethesda, MD: U. S. National Institute of Neurological Diseases and Blindness, Neurological Information Network; 1968.
2. Neckelmann D, *et al*. The reliability and functional validity of visual and semiautomatic sleep/wake scoring in the Møll-Wistar rat. *Sleep*. 1994;**17**(2):120–131.
3. Tobler I. Is sleep fundamentally different between mammalian species? *Behav Brain Res*. 1995;**69**(1-2):35–41.
4. Lesku JA, *et al*. A phylogenetic analysis of sleep architecture in mammals: the integration of anatomy, physiology, and ecology. *Am Nat*. 2006;**168**(4):441–453.
5. Gottesmann C, *et al*. Intermediate stage of sleep in the cat. *J Physiol (Paris)*. 1984;**79**(5):365–372.
6. Glin L, *et al*. The intermediate stage of sleep in mice. *Physiol Behav*. 1991;**50**(5):951–953.
7. Benington JH, *et al*. Scoring transitions to REM sleep in rats based on the EEG phenomena of pre-REM sleep: an improved analysis of sleep structure. *Sleep*. 1994;**17**(1):28–36.
8. Krueger JM, *et al*. Sleep as a fundamental property of neuronal assemblies. *Nat Rev Neurosci*. 2008;**9**(12):910–919.
9. Nobili L, *et al*. Dissociated wake-like and sleep-like electrocortical activity during sleep. *Neuroimage*. 2011;**58**(2):612–619.
10. Vyazovskiy VV, *et al*. Local sleep in awake rats. *Nature*. 2011;**472**(7344):443–447.
11. Vyazovskiy VV, *et al*. The dynamics of cortical neuronal activity in the first minutes after spontaneous awakening in rats and mice. *Sleep*. 2014;**37**(8):1337–1347.
12. Nir Y, *et al*. Regional slow waves and spindles in human sleep. *Neuron*. 2011;**70**(1):153–169.
13. Maquet P. The role of sleep in learning and memory. *Science*. 2001;**294**(5544):1048–1052.
14. Rasch B, *et al*. About sleep's role in memory. *Physiol Rev*. 2013;**93**(2):681–766.
15. Diekelmann S, *et al*. The memory function of sleep. *Nat Rev Neurosci*. 2010;**11**(2):114–126.
16. Watson BO, *et al*. Sleep, memory & brain rhythms. *Daedalus*. 2015;**144**(1):67–82.
17. Sarasso S, *et al*. Hippocampal sleep spindles preceding neocortical sleep onset in humans. *Neuroimage*. 2014;**86**:425–432.

18. Emrick JJ, *et al*. Different simultaneous sleep states in the hippocampus and neocortex. *Sleep*. 2016;**39**(12):2201–2209.
19. Mölle M, *et al*. Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. *J Neurophysiol*. 2006;**96**(1):62–70.
20. Bjorvatn B, *et al*. EEG power densities (0.5-20 Hz) in different sleep-wake stages in rats. *Physiol Behav*. 1998;**63**(3):413–417.
21. Oostenveld R, *et al*. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci*. 2011;**2011**:156869.
22. Maris E, *et al*. Nonparametric statistical testing of EEG- and MEG-data. *J Neurosci Methods*. 2007;**164**(1):177–190.
23. Steriade M, *et al*. Thalamocortical oscillations in the sleeping and aroused brain. *Science*. 1993;**262**(5134):679–685.
24. Hughes SW, *et al*. Cellular mechanisms of the slow (<1 Hz) oscillation in thalamocortical neurons in vitro. *Neuron*. 2002;**33**(6):947–958.
25. Steriade M. Grouping of brain rhythms in corticothalamic systems. *Neuroscience*. 2006;**137**(4):1087–1106.
26. Crunelli V, *et al*. The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat Neurosci*. 2010;**13**(1):9–17.
27. Siapas AG, *et al*. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron*. 1998;**21**(5):1123–1128.
28. Wierzynski CM, *et al*. State-dependent spike-timing relationships between hippocampal and prefrontal circuits during sleep. *Neuron*. 2009;**61**(4):587–596.
29. Maingret N, *et al*. Hippocampo-cortical coupling mediates memory consolidation during sleep. *Nat Neurosci*. 2016;**19**(7):959–964.
30. Riedner BA, *et al*. Temporal dynamics of cortical sources underlying spontaneous and peripherally evoked slow waves. *Prog Brain Res*. 2011;**193**:201–218.
31. Canto CB, *et al*. The sleeping cerebellum. *Trends Neurosci*. 2017;**40**(5):309–323.
32. Bland BH. The physiology and pharmacology of hippocampal formation theta rhythms. *Prog Neurobiol*. 1986;**26**(1):1–54.
33. Buzsáki G. Theta oscillations in the hippocampus. *Neuron*. 2002;**33**(3):325–340.
34. Pignatelli M, *et al*. Neural circuits underlying the generation of theta oscillations. *J Physiol Paris*. 2012;**106**(3-4):81–92.
35. Lu J, *et al*. A putative flip-flop switch for control of REM sleep. *Nature*. 2006;**441**(7093):589–594.
36. Krenzer M, *et al*. Brainstem and spinal cord circuitry regulating REM sleep and muscle atonia. *PLoS One*. 2011;**6**(10):e24998.
37. Fraigne JJ, *et al*. REM sleep at its core – circuits, neurotransmitters, and pathophysiology. *Front Neurol*. 2015; **6**:1–9.
38. Scheffzük C, *et al*. Selective coupling between theta phase and neocortical fast gamma oscillations during REM-sleep in mice. *PLoS One*. 2011;**6**(12):e28489.
39. Scheffzük C, *et al*. Global slowing of network oscillations in mouse neocortex by diazepam. *Neuropharmacology*. 2013;**65**:123–133.
40. Lecci S, *et al*. Coordinated infraslow neural and cardiac oscillations mark fragility and offline periods in mammalian sleep. *Sci Adv*. 2017;**3**(2):e1602026.
41. Ribeiro S. Sleep and plasticity. *Pflugers Arch*. 2012;**463**(1):111–120.
42. Calais JB, *et al*. Experience-dependent upregulation of multiple plasticity factors in the hippocampus during early REM sleep. *Neurobiol Learn Mem*. 2015;**122**:19–27.

Supplemental material

Figure S1: Number of REM sleep episodes determined in the frontal EEG recordings (left panel) and the proportion of these REM sleep episodes starting earlier in dHC LFP recordings (right panel) for succeeding 4-hour bins across the 12-hour recording (light) period. Note, whereas the number of REM sleep episode increases across the 12-hour period ($F(2,18) = 7.55$, $p = 0.004$, for ANOVA Time main effect), the proportion of REM sleep episodes with an earlier onset in dHC LFP recordings decreased across this period ($H(2) = 6.36$, $p = 0.042$, for one-way Kruskal-Wallis ANOVA on non-normally distributed data. The y-axes indicate the mean (± SEM). * $p < 0.05$ for ANOVA main effect of Time. REM: Rapid-eye movement.

Figure S2: Transitions into REM sleep at the different recording sites (frontal EEG, mPFC LFP, parietal EEG and dHC LFP). The left panel show results from an analysis based on scoring of 10-s intervals, the right panel shows the same analysis based on scoring of 2-s interval. In these analyses, the earliest transitions into the specified brain state occurring at a certain recording site was set to 0 s and, then, the delay time was calculated till this transition occurred at each of the other recording sites. The y-axes indicate distribution of delay time for each recording site. The y-axes indicate the mean (± SEM) delay time (across all detected transitions) for each recording site. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ for pairwise comparison. ($F(2.44, 374.3) = 32.70$, $p < 0.001$, for ANOVA Site main effect, $t \geq -5.38$, $p \leq 0.012$, for all pairwise comparison with dHC recordings). Note that in dHC LFP recordings REM sleep is entered substantially earlier than at all other recording sites. REM: Rapid-eye movement; EEG: Electroencephalogram; LFP: Local field potential; EMG: Electromyogram; mPFC: Medial prefrontal cortex; dHC: Dorsal hippocampus

Figure S3: Left panel: Average EMG root mean square (rms) signal during a ± 20-s interval around REM sleep onset ('0 s') for the cases when REM sleep do not started first in dHC LFP recordings (n = 24) time-locked to REM onset as identified in dHC LFP recordings (black lines) and time-locked to REM onset as identified in that channel (of the respective 3 other sites - frontal EEG, mPFC LFP, parietal EEG) where REM sleep was detected first (grey lines). Significant differences in EMG rms are indicated by horizontal thin ($p < 0.05$) red lines. Right panel: Bar graph shows mean differences in EMG rms activity between the 10-s intervals before minus after REM sleep onset for these different types of alignments. Note, with REM onset time-locked to the dHC LFP, the decrease in EMG activity is on average slightly weaker than with time-locking EMG activity to the channel in which REM occurred first, which rules out that the occurrence of atonia is specifically linked to REM sleep occurrence in hippocampal networks also in these cases. EMG: Electromyogram; rms: root mean square; REM: Rapid-eye movement; dHC: Dorsal hippocampus; LFP: Local field potential; n.s.: non-significant.

Figure S1

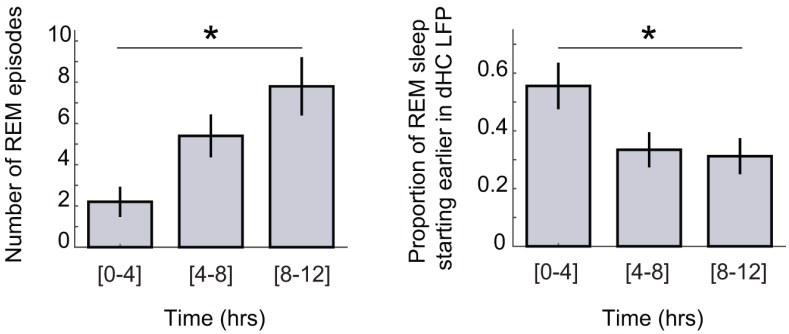


Figure S2

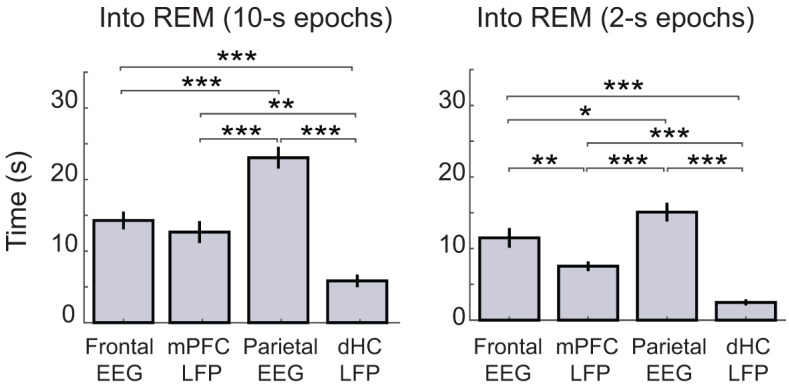
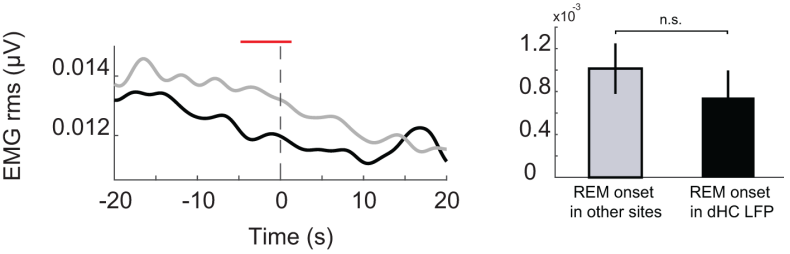


Figure S3


EMG rms (μV)
0.014
0.012
-20
-10
0
10
20
Time (s)
x10^-3
n.s.
1.2
0.8
0.4
0
REM onset in other sites
REM onset in dHC LFP

Study 4 – Temporal association between sleep slow oscillations, spindles and ripples

Temporal associations between sleep slow oscillations, spindles and ripples

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Abstract

The systems consolidation of memory during slow wave-sleep (SWS) is thought to rely on a dialogue between hippocampus and neocortex that is regulated by an interaction between neocortical slow oscillations (SOs), thalamic spindles, and hippocampal ripples. Here, combining frontal and parietal surface EEG with local field potential (LFP) recordings in medial prefrontal cortex (mPFC) and dorsal hippocampus (dHC), we examined the occurrence rates of and the temporal relationships between these oscillatory events in rats, to identify the possible direction of interaction between these events under natural conditions. Consistent with a top-down driving influence, EEG SO upstates are associated with an increase in spindles and hippocampal ripples. These associations were missing for SO upstates identified in mPFC LFP recordings. Ripples in dHC recordings always followed the onset of spindles consistent with spindles timing ripple occurrence. Moreover, comparing ripple activity during co-occurring SO-spindle events with that during isolated SOs or spindles, revealed that ripple dynamics during SO-spindle events are mainly determined by the spindle, with only the SO downstate providing a global inhibitory signal to both thalamus and hippocampus. As to bottom-up influences, we found an increase in hippocampal ripples ~200 ms before the SO downstate, but no similar increase preceding SO downstates for spindles. Overall, the temporal pattern is consistent with a loop-like scenario where, top-down, SOs can trigger thalamic spindles which, in turn, regulate in hippocampal networks the occurrence of ripples. Ripples, bottom-up, and independent from thalamic spindles, can contribute to the emergence of neocortical SOs.

Key words: rat, slow wave sleep, hippocampus, medial prefrontal cortex.

Statement of Significance

The systems consolidation of memory during sleep is thought to be established through the coupling between neocortical slow oscillation, spindles originating from thalamus, and hippocampal ripples. Examining the occurrence rates and temporal relations among these oscillatory events in EEG and local field potential recordings in spontaneous conditions, we provide support for a loop-like neocortical-hippocampal interaction where the neocortical SO downstate, top-down, leads to a global inactivation of the loop. The following SO-upstate phase is characterized by a spindle-regulated increase in ripples in hippocampal circuitry. Bottom-up, hippocampal ripples can trigger neocortical SOs thereby bypassing spindle-generating thalamic networks.

Introduction

Sleep has been identified as a state that supports the systems consolidation of hippocampal memory[1–3]. In particular, during slow-wave sleep (SWS), the hippocampus and neocortex establish a dialog where the depolarizing upstates of the slow oscillations (SOs) coordinate the occurrence of thalamic spindles in synchrony with hippocampal ripples, the latter accompanying the reactivations of hippocampal memory representations during SWS[4–6]. Ripples nesting in spindle oscillations have been proposed as a mechanism promoting the hippocampo-to-neocortical transmission of reactivated memory information and the more gradual redistribution of representations towards neocortical networks[7–12]. The SO (~1 Hz) is a global and synchronized cortical phenomenon that preferentially originates in prefrontal cortex, substantially involving subcortical structures like the thalamus [13–16], and typically travels towards posterior cortex, reaching also the hippocampus[11,17]. The shorter downstate of the SO is associated with generalized hyperpolarization and reduced neuronal firing whereas the longer SO upstate goes along with synchronized membrane depolarization and increased neuronal firing, and also drives the generation of thalamic spindles [16,18–20]. Spindles (10 – 15 Hz), originating from GABAergic networks of the reticular thalamic nucleus, spread via thalamo-cortical fibers to the entire neocortex, but they also reach the hippocampus where they are phase-locking ripples[9–11,13,21]. Ripples are high-frequency bursts (100 – 200 Hz) that occur in the CA1 subregion in conjunction with a CA3-generated sharp wave, and typically accompany the reactivation of neuronal ensembles that are activated and used for encoding the representation during prior wake phases[22–24].

Although there is a continuing controversy about the role SOs, spindles and ripples play in memory consolidation[e.g. 25–27], a large body of evidence supports the view that these oscillatory

events are involved in memory formation (summarized in [6,28,29]). Indeed, several studies consistently revealed an association of memory formation with an increased co-occurrence of these oscillatory events, where ripples tend to nest in the excitable phases of the spindle, and such spindle-ripples events tend to nest into the upstate of the SO[8,11,12,30–33]. In light of the strong implications of these findings for memory processing during SWS, here we aimed at a characterization of the dialogue between neocortex and hippocampus during SWS in terms of SO, spindle, and ripple events, under natural conditions. Of special interest was the temporal relationship between these events - what comes first – which in connection with the spatial distribution of the events across the different brain regions allows to specify the direction of the interaction between these events. For this purpose, we recorded in rats EEG signals via skull electrodes over the frontal and parietal cortex and, local field potentials (LFPs) from medial prefrontal cortex (mPFC) and dorsal hippocampus (dHC). Our results are consistent with the view that top-down, the SO downstate provides a suppressive signal that synchronizes thalamic spindles and hippocampal ripples, whereas the SO upstate drives mainly thalamic spindles which, in turn, regulate hippocampal ripple occurrence. Hippocampal ripples might, bottom-up, contribute to the occurrence of neocortical SO events. Differing from previous findings, we do not find, under natural conditions, hints for a contribution of spindles to the generation of SO events.

Methods

Animals

Five male Long Evans rats (Janvier, Le Genes-Saint-Isle, France, 280 – 340 g, 14 – 18 weeks old) were used. The rats were kept in temperature (22 ± 2 °C) and humidity (45 – 65 %) controlled cages, on a 12-h light/dark cycle with the lights off at 19:00 h. Water and food were available *ad libitum*. All experimental procedures were approved by the University of Tübingen and the local institutions in charge of animal welfare (Regierungspräsidium Tübingen, Germany). The animals had been used in a previous study[34].

Surgeries

Animals were anesthetized with an intraperitoneal injection of fentanyl (0.005 mg/kg of body weight), midazolam (2.0 mg/kg), and medetomidin (0.15 mg/kg). They were placed into a stereotaxic frame and were supplemented with isoflurane (0.5%) if necessary. The scalp was exposed and five holes were drilled into the skull. Three EEG screw electrodes were implanted: one frontal electrode (AP: +2.6 mm, ML: -1.5 mm, relative to Bregma), one parietal electrode (AP: -2.0 mm, ML: -2.5 mm, relative to Bregma), and one occipital reference electrode (AP: -10.0 mm, ML: 0.0 mm, relative to Bregma). Additionally, two platinum electrodes were implanted to record LFP signals (also referenced to the occipital skull electrode): one into the right medial prefrontal cortex (mPFC; AP: +3.0 mm, ML: +0.5 mm, DV: -3.6 mm), and one into the left dorsal hippocampus (dHC; AP: -3.1 mm, ML: +3.0 mm, DV: -3.6 mm). Electrode positions were confirmed by histological analysis (Figure S1). For EMG recordings, in all animals a stainless steel wire was implanted in the neck muscle. Electrodes were connected to a

six-channel electrode pedestal (PlasticsOne, USA) and fixed with cold polymerizing dental resin, and the wound was sutured. Rats had at least 5 days for recovery.

Recordings

Rats were habituated to the recording box (dark grey PVC, 30 x 30 cm, height: 40 cm) for two days, twelve hours per day. On the third day, animals were recorded for twelve hours, during the light phase. The animal's behaviour was continuously tracked using a video camera mounted on the recording box. EEG, LFP and EMG signals were continuously recorded and digitalized using a CED Power 1401 converter and Spike2 software (Cambridge Electronic Design, Cambridge, UK). During the recordings, the electrodes were connected through a swiveling commutator to an amplifier (Model 15A54, Grass Technologies, USA). EEG signals were amplified and filtered between 0.1 – 300 Hz. LFP signals were amplified and filtered between 0.1 – 1000 Hz. EMG signals filtered between 30 – 300 Hz. The signals were sampled at 1 kHz.

Sleep stage determination

Sleep stages and wakefulness were determined off-line based on EEG and EMG recordings, using standard visual scoring procedures for consecutive 10-s epochs as previously described[34,35] (Table 1). Three sleep stages were discriminated: slow wave sleep (SWS), intermediate stage (IS) and REM sleep. Wakefulness was identified by mixed-frequency EEG and sustained EMG activity, SWS by the presence of high amplitude low activity (delta activity: < 4.0 Hz) and reduced EMG tone, REM sleep by low-amplitude EEG activity with predominant theta activity (5.0 – 10.0 Hz), phasic muscle twitches and decrease of EMG tone. IS was identified by a decreased delta activity, progressive increase of theta activity and presence of sleep spindles.

Recordings were scored by two experienced experimenters (interrater agreement >89.9 %). Afterward consensus was achieved for epochs with divergent scoring.

Event detections

To identify SOs, standard procedures were used as described in detail previously[3,30]. In brief: EEG and LFP signals were filtered between 0.3 and 4.5 Hz, and an SO event was selected in the EEG if the following criteria were fulfilled: *(i)* two consecutive negative-to-positive zero crossings of the signal occurred at an interval between 0.4 and 2.0 s, *(ii)* of these events in an individual rat and channel, the 35% with the highest negative peak amplitude between both zero crossings were selected, and *(iii)* of these events the 45% with the highest negative-to-positive peak-to-peak amplitude were selected. Because in the LFP the SO shows up in opposite polarity, LFP signals were inverted (multiplied by -1) before applying the detection algorithm. The criteria resulted in the detection of SOs with downstate peak amplitudes exceeding -80 µV in the EEG and 110 µV in LFP recordings, and peak-to-peak amplitudes exceeding 120 µV in the EEG and 160 µV in LFP recordings.

Spindle detection was also based on procedures described previously e.g.[31]. The EEG signal was filtered between 10.0 and 16.0 Hz. Then, the envelope was extracted via the absolute value, i.e., the instantaneous amplitude, of the Hilbert transform on the filtered signal, followed by an additional smoothing (moving average with 200-ms window size). A spindle was identified when the absolute value of the transformed signal exceeded 1.5 standard deviations (SD) of the mean signal in the respective channel, during the animal's SWS epochs, for at least 0.4 s and not more than 2.0 s. Spindle onset was defined by the time when the signal the first time exceeded the 1.5-SD threshold. The spindle power was calculated as the integral of the

envelope of the Hilbert-transformed signal between spindle onset and end. For calculating Hilbert transformations the MATLAB function *Hilbert* was used. The envelope was extracted using the MATLAB function *abs*, which returns the absolute value (modulus), i.e., the "instantaneous amplitude" of the transformed signal.

Ripples were identified only in dorsal hippocampal (dHC) LFP recordings (as described in[31]). The signal was filtered between 150.0 – 250.0 Hz. As for spindle detection, the Hilbert transform was calculated and the signal was smoothed using a moving average (window size 200 ms). A ripple event was identified when the Hilbert transform value exceeded a threshold of 2.5 SDs from the mean signal during an animal's SWS epochs, for at least 25 ms (including at least 3 cycles) and for not more than 500 ms.

Co-occurrence of events

For analyzing the temporal relationships between SOs, spindles, and ripples we calculated event correlation histograms, with one of the event types used as reference (e.g., SOs) and one of the respective other two event types (spindles or ripples) used as target event. For calculating event correlation histograms only epochs were considered in which a target event occurred within a ±1.5–s window around the reference event. Table 2 summarizes the proportion of reference events co-occurring (in this interval) with one of the respective target events, separately for the three types of events of interest (SOs, spindles, ripples). To analyze the occurrence of spindle and ripple events with reference to the SO, the respective target events were time-locked to the SO downstate peak (t = 0 s) representing the most distinct and optimal time reference for scaling the SO cycle. The SO upstate peak is typically much flatter and more variable and has been proven to provide only a very imprecise reference for averaging and event time-locking[36,37]. For

the analogous analyses with spindles and ripples as reference events, the spindle onset and the maximum trough of a ripple, respectively, were used for time-locking target events. Window sizes (around t = 0 s) was always 3 s (±1.5 s), and bin size was 100 ms. To calculate the event rate for SOs, the downstate peaks of all detected events were taken. For spindle and ripple activity, all detected spindle and ripple peaks and troughs were taken. (Exploratory analyses on spindles revealed basically identical results when counting one event per spindle). The counts in every bin were divided by the number of the reference events (used for time-locking one of the respective other two event types), and then divided by the bin width to give event rate per second (Hz).

Phase-locking analyses

Supplementing event correlation histograms, we calculated the "preferred cycle phase", for the temporal association of spindles and hippocampal ripples, respectively, with the SO, as well as for the temporal association of ripples with the spindle oscillation. For determining the temporal associations with the SO cycle, each detected SO associated with a spindle and ripple, respectively, was filtered between 0.3 and 4.5 Hz and the Hilbert transform was calculated. Then, the instantaneous phase of the SO at spindle onset and ripple maximum, respectively, was extracted. Correspondingly, for determining the temporal associations of ripples with the spindle cycle, each spindle that co-occurred with a ripple was first filtered between 10.0 and 16.0 Hz, then the Hilbert transform was calculated, and the instantaneous phase of the spindle at the time of a ripple was extracted. For calculating the average preferred phase, we used the function *CircHist* of the CircStat toolbox[38,39].

Power spectral analyses

In addition to event-based analyses, we calculated time-frequency plots of LFP power (in dHC recordings) to analyze the co-occurrence of SO-spindle events with ripples. For this purpose time-frequency analysis was performed per SO and spindle event. The function *mtmconvol* of the FieldTrip toolbox[40] was used for frequencies from 150.0 to 250.0 Hz in steps of 1 Hz using a sliding Hanning tapered window with a variable, frequency-dependent length that always comprised ten cycles. Time-locked time-frequency analysis of all events were normalized by dividing the values with the average power during the baseline between -2.0 to -1.0 s before the event (using the FieldTrip function *ft_freqbaseline, baselinetype: 'relative'*), and then averaged across all events (using the FieldTrip function *ft_freqgrandaverage*).

Statistical analyses

Kolmogorov–Smirnov tests were used to assure normality of the distribution for each parameter. Differences in SOs and spindles among the different recording sites were assessed using repeated measures analyses of variance (ANOVA) with "recording site" as factor (frontal EEG, mPFC LFP, parietal EEG, dHC LFP), followed by *post hoc* paired sample *t*-tests, to specify significant differences between any two of the recording sites. For the evaluation of event correlation histograms each bin was compared to a baseline interval which was the 1-s interval form -2.0 s to -1.0 s prior to the reference event at 0 s. For LFP recordings, these analyses were restricted to a ±0.8-s interval around the reference event. Additionally, we tested the significance of the event correlation histograms against a randomized event distribution using procedures as described by[30]. These analyses revealed essentially similar results and, hence, are not reported here. For statistical evaluation of ripple-related power spectra, the normalized power values were averaged

across the 150 – 250 Hz frequency band and for subsequent 100-ms bins of the event-locked time-frequency plot, and compared to baseline values (-2.0 to -1.0 s prior to the reference event) using paired-sample *t-tests*.

Histology

After the last recording session, rats were terminally anesthetized with fentanyl (0.01 mg/kg of body weight), midazolam (4.0 mg/kg), and medetomidin (0.3 mg/kg). The electrodes positions were marked by electrolytic lesion (10 μA, 30 s; Figure S1). Rats were perfused with physiological saline (200 – 300 mL) followed by 4 % paraformaldehyde (PFA, 200 – 300 mL). After decapitation, the brains were removed and post-fixed in 4 % PFA for one day. Coronal sections of 60 μm were cut using a vibratome, stained with 0.5 % toluidine blue and examined under a light microscope.

Results

Event detection during SWS in the EEG, and cortical and hippocampal LFP

We analyzed brain oscillations during all SWS epochs recorded for each rat in a 12-hour session during the light phase. Figure S1 shows examples of parietal EEG and dHC LFP recordings during these SWS epochs for individual rats. The rats spent on average 364.1 min in SWS (Table 1). Table 2 summarizes occurrence (absolute numbers, densities) of SO, spindle, and ripple events and their co-occurrence in the different recording channels. SO density was highest in the parietal EEG and distinctly lower in the frontal EEG and dHC LFP ($F(3, 12) = 3.7$, $p = 0.043$, see Figure 1 for pairwise comparisons). SO duration was shorter in LFP than EEG recordings, and shortest in the mPFC LFP ($F(3, 12) = 26.9$, $p < 0.001$). SO amplitude was higher in the parietal than frontal EEG and higher in the dHC than mPFC LFP ($F(3,12) = 7.0$, $p = 0.006$, Figure 1).

The number of spindles identified ranged between 399.6 ± 109.2 in the frontal EEG and 176.2 ± 43.2 in the mPFC LFP (Table 2). Both spindle density and duration were higher in the frontal EEG than all other sites ($F(3,12) = 13.9$, $p < 0.001$ and $F(3,12) = 26.7$, $p < 0.001$, respectively, Figure 2). Spindle power was lowest in mPFC and highest and most variable in dHC LFP recordings ($F(3,12) = 6.0$, $p < 0.009$). Spindle frequency was generally higher in EEG than LFP recordings ($F(3,12) = 19.6$, $p < 0.001$). In dHC LFP recordings, we detected 1498.6 ± 298.5 ripples with an average density of 10.5 ± 0.9 ripples per minute, duration of 101.4 ± 4.2 ms, and power of 1.1 ± 0.2 mV^2s^{-1}.

Temporal association between SOs and spindles

The percentage (of the total number) of SOs that co-occurred, in an interval ±1.5 s around the SO downstate peak, with a spindle was between 14.8 ± 1.1 % in the frontal EEG and 6.1 ± 0.3 % in mPFC LFP recordings (Table 2). Event correlation histograms of spindle events time-locked to the SO downstate peak confirmed a clear relationship in both frontal and parietal EEG recordings such that spindle occurrence was diminished for a more or less extended interval around the SO downstate peak, and distinctly increased during the subsequent SO upstate, reaching a maximum ~500 ms after the SO downstate peak (see Figure 3, also for statistical comparisons). The SO-upstate related increase in spindle occurrence was likewise demonstrated in phase-locking analyses (Figure 3, right panels). Surprisingly, there was no distinct temporal association between SOs and spindles in the mPFC LFP (Figure 3C), or dHC LFP (Figure 3D). Extended analyses showed that mPFC SOs also did not modulate spindle occurrence in the other recordings, except for a slight increase in spindles in the parietal EEG during the SO upstate (Figure S2).

A complementing pattern with an increase in the occurrence of SO downstates preceding spindle onset in the EEG, was revealed when, conversely, spindles were taken as reference of events correlation histograms for SO events. However, with the alignment to spindle onset the temporal relationships between SOs and spindles generally appeared to be more variable (Figure S3). Additional exploratory analyses revealed overall similar relationships to the SO for slow spindles determined in the 7 – 10 Hz band.

Temporal association between SOs and ripples

The percentage of SOs co-occurring with hippocampal ripples was comparable for all channels: 39.3 ± 2.3 % in frontal EEG, 40.1 ± 2.5 % in parietal EEG, 36.7 ± 2.6 % in mPFC LFP, and 38.3 ± 2.6 % in dHC LFP recordings (Table 2). Event correlation histograms of ripple events, referenced to the SO downstate peak, indicated a suppression of hippocampal ripples around the downstate peak of SOs in the frontal and parietal EEG, followed by an increased ripple occurrence during the SO upstate (Figure 4A). These upstate-related increases in ripple occurrence were also revealed in phase-locking analyses of SO-ripple co-occurrence (Figure 4A, right panels). The parallel downstate-related decrease and upstate-related increase in ripples in mPFC recordings did not reach significance. Instead, there was a slight but significant increase in ripples preceding (by ~400 ms) the SO downstate in mPFC recordings.

SOs identified in dHC recordings displayed a distinct dynamic of accompanying ripple activity (Figure 4A). While showing the typical upstate-related increase in ripple events, hippocampal SOs were accompanied by a second increase in ripples that preceded the SO downstate peak and was even more pronounced than the upstate-related increase. This ripple increase preceding the SO did not reflect an upstate-related ripple increase of a foregoing SO, because a comparison of isolated SOs with SOs occurring in a train of several succeeding SOs revealed the ripple increase preceding the SO downstate to be even more distinct for SOs occurring in isolation (Figure S4). Moreover, the number of SOs with ripples preceding and following the downstate was significantly lower than the number of SOs with either a preceding ripple or a following upstate-related ripple (Figure S4B), indicating that the two types of ripples were independently occurring during the hippocampal SO cycle.

Event correlation histograms of SO events referenced to dHC ripples, confirmed that ripples were preceded by an increase in SO events as defined by the downstate peak, in the frontal and parietal EEG and dHC LFP, and there was also a suppression of such SO events in the EEG and mPFC LFP during an ongoing ripple (Figure 4B). In addition, in these histograms, hippocampal ripples were followed, with a delay of 200 – 500 ms, by an increase in SO events in the frontal and parietal EEG consistent with a bottom-up influence of ripples on SO occurrence.

Temporal dynamic between spindles and ripples

The percentage of spindles co-occurring (±1.5-s around spindle onset) with hippocampal ripples averaged between 45.0 ± 4.0 % (mPFC LFP) and 51.7 ± 5.9 % (dHC LFP, Table 2). Conversely, the percentage of ripples in dHC co-occurring with spindles averaged between 7.2 ± 0.6 % (mPFC LFP) and 17.4 ± 2.6% (frontal EEG). Figutr 5A shows event correlation histograms for ripple events time-locked to (the onset of) spindles identified in the four different recordings. A distinct relationship was observed only for spindles in the parietal EEG such that here spindle onsets were followed, with a delay of ~300 ms, by an increased occurrence of ripples. There was a parallel increase in ripples for spindles in the dHC LFP which approached significance. No consistent patterns occurred in frontal EEG and mPFC LFP recordings. A supplementary phase-coupling analysis confirmed in 4 of the 5 rats significant spindle-ripple nesting such that the occurrence of ripples concentrated on the excitable phase of the spindle oscillation, particularly for spindles identified in dHC recordings[11,12]. Histograms of spindle occurrence time-locked to ripples confirmed that ripples were preceded by an increase in spindle events starting 300-100 ms before, in all the recordings (Figure 5B). The pattern is overall consistent with a driving influence of spindles on ripple occurrence in hippocampal networks.

Triple co-occurrence of slow oscillations, spindles and hippocampal ripples

We finally examined the co-occurrence of SOs with spindles *and* hippocampal ripples which has been proposed as a mechanism regulating information flow during the systems consolidation of memories[11,12]. Spindles, in these analyses, were detected in dHC LFP recordings, because analyses accounting for spindles detected in other channels did not reveal channel specific differences in associated ripple activity, and because evidence from foregoing studies suggested that coupling between spindles and hippocampal ripples is strongest for spindles detected in the hippocampus, in comparison with spindles identified in cortical LFP or EEG recordings e.g.[10,12]. An event-based analysis indicated that the number of SO events co-occurring with spindle and ripple events was overall low, reaching a maximum of 3.6 ± 0.4 % in dHC recordings, and thus did not provide sufficient statistical power for a fine-grained analysis of temporal relationships. Given that the determination of the three kinds of events of interest was based on more or less arbitrary amplitude criteria, we therefore decided, with regard to hippocampal ripples, to shift the focus of analysis to the signal power in the respective 150 – 250 Hz frequency band.

In a first analysis focussing on the role of SOs, we compared average power spectra of the dHC LFP in a ±0.3-s interval around the maximum trough of the spindle (identified in the dHC), between spindles that did and did not co-occur with an SO event. With respect to SOs, analyses were performed collapsed across events identified in all four channels. The spectra indicated an increase in 150 – 250 Hz ripple power oscillating around the maximum trough of the spindle ($p < 0.01$) which did not differ between spindles occurring in isolation and spindles co-occurring with an SO (Figure 6), suggesting that the presence of an SO does not substantially

add to the spindle-related modulation of ripple power. Spindles co-occurring with SOs and isolated spindles did not differ in terms of duration, frequency or power (all $p > 0.1$).

In a second analysis concentrating on the role of spindles, we compared average power spectra of the dHC LFP in a ±0.8 s-interval around the SO downstate peak, between SOs that did and did not co-occur with a spindle (identified in the dHC LFP). Figure 7 summarizes results of this analysis (see Figure S7 for an exploratory analysis where ripple activity was related to spindle activity in the respective channel of SO detection). The spectra indicated a suppression of ripple power around the SO downstate peak that was most distinct for isolated SOs (Figure 7). Importantly, ripple power was distinctly higher during SOs that co-occurred with spindles than during SOs occurring in isolation, with this difference being restricted ($p < 0.05$) to a 100-ms bin around the downstate peak in the analyses across all channels, as well as in a separate analysis of SOs identified in the dHC LFP (Figure 7). SOs co-occurring with spindles and isolated SOs did not consistently differ in terms of amplitude and duration ($p > 0.1$). Together, these findings go beyond our event-based approach (above) in indicating that the spindle oscillation is the primary factor driving hippocampal ripple activity even in the presence of a SO upstate, whereas the direct hippocampal influence of the SO appears to be restricted to its suppression of ripple activity during the hyperpolarizing downstate.

Discussion

We examined the communication between neocortex and hippocampus as established during SWS in rats through the interaction of neocortical SOs, spindles, and hippocampal ripples. Combining concurrent LFP recordings from mPFC and dHC and EEG recordings from frontal and parietal sites we aimed at an integrative assessment of the oscillatory events of interest and

their temporal relationships in natural conditions. As to top-down modulations, we found that SO downstates in the EEG are associated with a parallel decrease in spindles and hippocampal ripple activity whereas the SO upstate was associated with increases in spindle and ripple activity. Notably, this dynamic was not obtained in mPFC LFP recordings. Spindle onsets were followed by an increase in hippocampal ripple activity with, this increase not depending on whether or not the spindle co-occurred with a SO suggesting that, once a spindle is released and reaches the hippocampus, it dominates the regulation of hippocampal ripple activity. As to bottom-up influences, we found an increase in hippocampal ripples preceding (~200 ms) the SO downstate, whereas no similar increase preceding SO downstates was found for spindles, which in combination suggests that ripples directly contribute to the occurrence of neocortical SOs. Overall, in comparison with foregoing studies, our approach revealed a more complete picture of the temporal relationships between the three oscillatory events of interest, i.e., a picture suggesting a loop-like scenario where top-down, the SO-downstate sets the frame for a global time-window for processing memory information (Figure 8). In this window, the transition into the SO upstate drives thalamic spindles which, in turn, time the occurrence of ripples and associated replay of memory information in hippocampal networks. Bottom-up, ripples might contribute to the emergence of a neocortical SO.

By determining the precise temporal relationships we aimed to reveal hints about the direction of information flow between neocortex and hippocampus during memory processing in SWS. Focussing on the oscillatory configuration under natural conditions, we refrained from experimentally manipulating one of the oscillations. This approach comes with the price that our data do not allow for strictly causal inferences between the rhythms, although the identified temporal relationships allow to exclude certain causal interactions. Importantly, we here

deliberately supplemented our LFP recordings by surface EEG recordings, in order to support the translation of our findings to the conditions in healthy humans only allowing for EEG recordings. Indeed, relevant electrophysiological results from rodent sleep studies are often ignored in human research simply because of the lack of precise knowledge about how an intracortical LFP event appears in the surface EEG recording. Generally, the comparison of EEG signals, e.g., over frontal cortex, with LFP signals from mPFC in the present study revealed that SOs and spindles as picked up in the EEG are not necessarily related to corresponding oscillations of the LFP in underlying cortex. Thus, LFP recordings reflect the much more localized generation of these oscillations, particularly of spindles, which agrees with previous work[41–43]. Indeed, also human studies revealed that many sleep spindles have an extremely small spatial extent and are thus picked up only by methods with extremely small receptive fields, like MEG and intracortical LFP recordings[44–49].

In keeping with the majority of studies in the field, we concentrated on an event-based analysis of SOs, spindles and ripples, with the numbers of events detected during SWS closely comparable to those in previous studies[4,7,8,30]. Of note, whereas the proportion of spindles co-occurring with an SO was generally >65 %, conversely, the proportion of SOs co-occurring with a spindle was generally rather low (<15 %; Table 2) which may be taken to question the concept of a strong driving influence of SOs on the thalamic generation of spindles. However, spindle generating mechanisms undergo fast refractoriness which prevents that each SO can trigger a spindle event[50,51]. In addition, methodological factors play a role: The localized nature of spindle events might have prevented detection of events co-occurring with an SO and, also, events might have been missed due to too high detection criteria. In the case of spindles, the commonly used detection procedures have indeed been found to lack convergent validity and to differ in how

they extract the EEG events contributing to spectral peaks[52–54]. Event detection criteria mainly based on amplitude-thresholds, are thus arbitrary to a certain extent and difficult to compare between event types like SOs and spindles. Implicating an all-or-none conceptualization of the event of interest, such event-detection approach may not sufficiently reflect that SO and spindle generation can capture and synchronize more or less extended networks resulting in LFP and EEG oscillations of smaller or greater amplitude. Generally, for these reasons, it seems justified to supplement an event-based analysis by power spectral analyses, which we did here to examine the triple-co-occurrence of SOs, spindles and ripples.

Our EEG recordings confirmed previous findings of a robust increase in spindle activity accompanying the early upstate of SOs[31,42,55] which supports the view that membrane depolarization of cortico-thalamic projections during the SO upstate are driving the generation of spindle activity in thalamic networks[13,18]. A clear coupling of spindles to SO upstates was not observed in hippocampal LFP recordings, which is likewise compatible with the notion that such coupling originates in cortico-thalamic feedback loops. SO and spindles in hippocampal LFP recordings likely represent travelling waves that reach these networks via thalamic and cortical projections[56–58]. The hippocampus itself is not capable of generating SOs[59].

Interestingly, a coupling of spindles to SO upstates was also entirely absent in mPFC recordings. This finding might surprise at a first glance, as the majority of SOs arise from prefrontal cortical networks[17]. However, the observation well agrees with intracranial recordings in humans where such SO-spindle coupling was similarly weakened or even completely absent specifically in recordings from prefrontal regions[41]. It might reflect anatomical conditions with only weak cortico-thalamic projections conveying frontal depolarization to thalamic spindle generators[60]. SOs arising from medial prefrontal cortex may primarily propagate intracortically

towards posterior areas, which is consistent with our observation that SO upstates in mPFC recordings were associated with an increased spindle activity in the parietal EEG.

Not only spindles but also hippocampal ripples nested into the SO upstates, with this upstate-related increase being preceded by a dip in ripple activity during the prior SO downstate. Ripple occurrence distinctly increased also following the onset of spindles in the parietal EEG and hippocampal LFP, and hippocampal ripples were preceded by increased spindle activity in all channels. Moreover, hippocampal ripple power was increased during spindles regardless of whether the spindles co-occurred with an SO or not. On the other side, ripple activity was significantly higher when a spindle identified in hippocampal recordings co-occurred with a SO than during an isolated SO. Altogether these observations suggest that spindles reaching the hippocampus are the primary regulator of ripple activity in these networks, even in the presence of an SO. The influence of the SO, in this constellation, appears to be mainly restricted to a downstate-related suppression of ripples, indicating that the downstates of these global SOs also effectively inactivate hippocampal circuitry[61]. Consistent with a spindle-mediated regulation of hippocampal ripples, mPFC recordings in which an SO upstate-related modulation of spindle activity was missing, did also not reveal any upstate-associated modulation of hippocampal ripple activity. Moreover, in a previous study, optogenetically induced spindles identified in hippocampal LFP recordings synchronized hippocampal ripple activity regardless of whether or not the spindle was induced during an SO upstate[12]. The pathways of hippocampal spindle effects on ripple activity are unclear, but likely involve the nucleus reuniens of the thalamus[58,62,63].

Our data also provide cues about possible bottom-up contributions of hippocampal ripples to neocortical SOs. Hippocampal ripples were consistently followed by an increased

occurrence of SO downstates. This relationship was likewise evidenced when ripples were aligned to SO downstates in dHC recordings, and such increase in ripples also preceded the SO downstates identified in mPFC recordings. These findings concur with previous studies suggesting that hippocampal ripples can directly prime the occurrence of cortical downstates by activating inhibitory cortical networks, especially in prefrontal cortex[32,64,65]. Interestingly, immediately during a hippocampal ripple the occurrence of cortical SO downstates was suppressed, suggesting a rebound mechanism that produces the increase in SOs with a delay of about 200 ms. Such mechanism would also be consistent with the fact that during the SO downstate, cortical inhibitory interneurons themselves are inactive[20].

Surprisingly, we did not find clear hints at increases in spindle events that preceded increases in cortical SO events. In previous studies, the stimulation of thalamic spindle activity consistently induced neocortical SOs[12,66]. In combination, these data suggest that thalamic spindles, in principle, can contribute to SO generation, although this rarely happens in natural "unstimulated" conditions as examined here. This conclusion fits with evidence that spindle-generating networks appear to go into refractoriness distinctly faster than SO-generating networks[51,67]. It highlights the importance to examine the oscillatory interactions of interest in natural conditions. In sum, the temporal relationships revealed here suggest the presence of a loop-like scenario with a top-down global inactivation of the loop during the SO downstate, followed by a spindle regulated increase in ripples (and associated memory processing) in hippocampal circuitry during the SO upstate (Figure 8). Bottom-up, hippocampal ripple can trigger SOs and this influence appears to bypass spindle-generating thalamic networks.

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References

1. Diekelmann S, Born J. The memory function of sleep. *Nat Rev Neurosci*. 2010;11(2):114-126. doi:10.1038/nrn2762

2. Dudai Y, Karni A, Born J. The consolidation and transformation of memory. *Neuron*. 2015;88(1):20-32. doi:10.1016/j.neuron.2015.09.004

3. Sawangjit A, Oyanedel CN, Niethard N, Salazar C, Born J, Inostroza M. The hippocampus is crucial for forming non-hippocampal long-term memory during sleep. *Nature*. 2018;564(7734):109-113. doi:10.1038/s41586-018-0716-8

4. Rasch B, Born J. About sleep's role in memory. *Physiol Rev*. 2013;93(2):681-766. doi:10.1152/physrev.00032.2012

5. Watson BO, Buzsáki G. Sleep, memory & brain rhythms. *Daedalus*. 2015;144(1):67-82. doi:10.1162/DAED_a_00318

6. Skelin I, Kilianski S, McNaughton BL. Hippocampal coupling with cortical and subcortical structures in the context of memory consolidation. *Neurobiol Learn Mem*. 2019;160:21-31. doi:10.1016/j.nlm.2018.04.004

7. Siapas AG, Wilson MA. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron*. 1998;21(5):1123-1128. doi:10.1016/S0896-6273(00)80629-7

8. Sirota A, Csicsvari J, Buhl D, Buzsáki G. Communication between neocortex and hippocampus during sleep in rodents. *Proc Natl Acad Sci*. 2003;100(4):2065-2069. doi:10.1073/pnas.0437938100

9. Clemens Z, Mölle M, Erőss L, Barsi P, Halász P, Born J. Temporal coupling of

parahippocampal ripples , sleep spindles and slow oscillations in humans. *Brain*. 2007;130:2868-2878. doi:10.1093/brain/awm146

10. Clemens Z, Mölle M, Erőss L, et al. Fine-tuned coupling between human parahippocampal ripples and sleep spindles. *Eur J Neurosci*. 2011;33:511-520. doi:10.1111/j.1460-9568.2010.07505.x

11. Staresina BP, Bergmann TO, Bonnefond M, et al. Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nat Neurosci*. 2015;18(11):1679-1686. doi:10.1038/nn.4119

12. Latchoumane C-F V., Ngo H V, Born J, Shin H-S. Thalamic spindles promote memory formation during sleep through triple phase-locking of cortical, thalamic, and hippocampal rhythms. *Neuron*. 2017;95(2):424-435. doi:10.1016/j.neuron.2017.06.025

13. Steriade M, Contreras D, Curro Dossi R, Nuñez A. The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci*. 1993;13(8):3284-3299. doi:10.1523/JNEUROSCI.13-08-03284.1993

14. Hughes SW, Cope DW, Blethyn KL, Crunelli V. Cellular mechanisms of the slow (<1 Hz) oscillation in thalamocortical neurons in vitro. *Neuron*. 2002;33(6):947-958. doi:10.1016/S0896-6273(02)00623-2

15. Crunelli V, Hughes SW. The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat Neurosci*. 2010;13(1):9-17. doi:10.1038/nn.2445

16. Neske GT. The slow oscillation in cortical and thalamic networks: mechanisms and functions. *Front Neural Circuits*. 2016;9:88. doi:10.3389/fncir.2015.00088

17. Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G. The sleep slow oscillation as a traveling wave. *J Neurosci*. 2004;24(31):6862-6870. doi:10.1523/JNEUROSCI.1318-04.2004

18. Steriade M, Mccormick DA, Sejnowski TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science*. 1993;262(5134):679-685. doi:10.1126/science.8235588

19. Destexhe A, Contreras D, Steriade M. Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. *J Neurosci*. 1999;19(11):4595-4608. doi:10.1523/JNEUROSCI.19-11-04595.1999

20. Niethard N, Ngo H-V V., Ehrlich I, Born J. Cortical circuit activity underlying sleep slow oscillations and spindles. *Proc Natl Acad Sci*. 2018;115(39):E9220-E9229. doi:10.1073/pnas.1805517115

21. Kim D, Hwang E, Lee M, Sung H, Choi JH. Characterization of topographically specific sleep spindles in mice. *Sleep*. 2015;38(1):85-96. doi:10.5665/sleep.4330

22. Buzsáki G, Horváth Z, Urioste R, Hetke J, Wise K. High-frequency network oscillation in the hippocampus. *Science*. 1992;256(5059):1025-1027. doi:10.1126/science.1589772

23. Khodagholy D, Gelinas JN, Buzsáki G. Learning-enhanced coupling between ripple oscillations in association cortices and hippocampus. *Science*. 2017;358(6361):369-372. doi:10.1126/science.aan6203

24. Diba K, Buzsáki G. Forward and reverse hippocampal place-cell sequences during ripples. *Nat Neurosci*. 2007;10(10):1241-1242. doi:10.1038/nn1961

25. Ackermann S, Hartmann F, Papassotiropoulos A, de Quervain DJF, Rasch B. No associations between interindividual differences in sleep parameters and episodic memory

consolidation. *Sleep*. 2015;38(6):951-959. doi:10.5665/sleep.4748

26. Kim J, Gulati T, Ganguly K. Competing roles of slow oscillations and delta waves in memory consolidation versus forgetting. *Cell*. 2019;179(2):514-526. doi:10.1016/j.cell.2019.08.040

27. Ngo H-V V., Born J. Sleep and the balance between memory and forgetting. *Cell*. 2019;179(2):289-291. doi:10.1016/j.cell.2019.09.007

28. Inostroza M, Born J. Sleep for preserving and transforming episodic memory. *Annu Rev Neurosci*. 2013;36(1):79-102. doi:10.1146/annurev-neuro-062012-170429

29. Klinzing JG, Niethard N, Born J. Mechanisms of systems memory consolidation during sleep. *Nat Neurosci*. 2019;22(10):1598-1610. doi:10.1038/s41593-019-0467-3

30. Mölle M, Yeshenko O, Marshall L, Sara SJ, Born J. Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. *J Neurophysiol*. 2006;96(1):62-70. doi:10.1152/jn.00014.2006

31. Mölle M, Eschenko O, Gais S, Sara SJ, Born J. The influence of learning on sleep slow oscillations and associated spindles and ripples in humans and rats. *Eur J Neurosci*. 2009;29(5):1071-1081. doi:10.1111/j.1460-9568.2009.06654.x

32. Maingret N, Girardeau G, Todorova R, Goutierre M, Zugaro M. Hippocampo-cortical coupling mediates memory consolidation during sleep. *Nat Neurosci*. 2016;19(7):959-964. doi:10.1038/nn.4304

33. Helfrich RF, Mander BA, Jagust WJ, Knight RT, Walker MP. Old brains come uncoupled in sleep: slow wave-spindle synchrony, brain atrophy, and forgetting. *Neuron*. 2018;97(1):221-230. doi:10.1016/j.neuron.2017.11.020

34. Durán E, Oyanedel CN, Niethard N, Inostroza M, Born J. Sleep stage dynamics in neocortex and hippocampus. *Sleep*. 2018;41(6). doi:10.1093/sleep/zsy060

35. Neckelmann D, Olsen OE, Fagerland S, Ursin R. The reliability and functional validity of visual and semiautomatic sleep/wake scoring in the Moll-Wistar rat. *Sleep*. 1994;17(2):120-131. doi:10.1093/sleep/17.2.120

36. Mölle M, Marshall L, Gais S, Born J. Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. *J Neurosci*. 2002;22(24):10941-10947.

37. Buzsáki G. *Rhythms of the Brain*. New York: Oxford University Press; 2006.

38. Zittrell F. CircHist: Circular histogram in MATLAB. *GitHub*. 2019. doi:10.5281/zenodo.3445084

39. Berens P. CircStat : A MATLAB Toolbox for Circular Statistics . *J Stat Softw*. 2009;31(10). doi:10.18637/jss.v031.i10

40. Oostenveld R, Fries P, Maris E, Schoffelen J-M. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci*. 2011;2011:156869. doi:10.1155/2011/156869

41. Andrillon T, Nir Y, Staba RJ, et al. Sleep spindles in humans: Insights from intracranial EEG and unit recordings. *J Neurosci*. 2011;31(49):17821-17834. doi:10.1523/JNEUROSCI.2604-11.2011

42. Nir Y, Staba RJ, Andrillon T, et al. Regional slow waves and spindles in human sleep. *Neuron*. 2011;70(1):153-169. doi:10.1016/j.neuron.2011.02.043

43. Ayoub A, Mölle M, Preissl H, Born J. Grouping of MEG gamma oscillations by EEG sleep spindles. *Neuroimage*. 2012;59(2):1491-1500.

doi:10.1016/j.neuroimage.2011.08.023

44. Dehghani N, Cash SS, Chen CC, et al. Divergent cortical generators of MEG and EEG during human sleep spindles suggested by distributed source modeling. Valdes-Sosa PA, ed. *PLoS One*. 2010;5(7):e11454. doi:10.1371/journal.pone.0011454

45. Dehghani N, Cash SS, Rossetti AO, Chen CC, Halgren E. Magnetoencephalography demonstrates multiple asynchronous generators during human sleep spindles. *J Neurophysiol*. 2010;104(1):179-188. doi:10.1152/jn.00198.2010

46. Dehghani N, Cash SS, Halgren E. Emergence of synchronous EEG spindles from asynchronous MEG spindles. *Hum Brain Mapp*. 2011;32(12):2217-2227. doi:10.1002/hbm.21183

47. Dehghani N, Cash SS, Halgren E. Topographical frequency dynamics within EEG and MEG sleep spindles. *Clin Neurophysiol*. 2011;122(2):229-235. doi:10.1016/j.clinph.2010.06.018

48. Hagler DJ, Ulbert I, Wittner L, et al. Heterogeneous origins of human sleep spindles in different cortical layers. *J Neurosci*. 2018;38(12):3013-3025. doi:10.1523/JNEUROSCI.2241-17.2018

49. Ujma PP, Hajnal B, Bódizs R, Gombos F, Erőss L. The laminar profile of sleep spindles in humans. *bioRxiv*. 2019:563221. doi:https://doi.org/10.1101/563221

50. Destexhe A, Contreras D, Steriade M. Mechanisms underlying the synchronizing action of corticothalamic feedback through inhibition of thalamic relay cells. *J Neurophysiol*. 1998;79(2):999-1016. doi:10.1152/jn.1998.79.2.999

51. Ngo H-V V., Miedema A, Faude I, Martinetz T, Mölle M, Born J. Driving sleep slow

oscillations by auditory closed-loop stimulation-A self-limiting process. *J Neurosci*. 2015;35(17):6630-6638. doi:10.1523/JNEUROSCI.3133-14.2015

52. Ujma PP, Gombos F, Genzel L, et al. A comparison of two sleep spindle detection methods based on all night averages: individually adjusted vs. fixed frequencies. *Front Hum Neurosci*. 2015;9:52. doi:10.3389/fnhum.2015.00052

53. Cox R, Schapiro AC, Manoach DS, Stickgold R. Individual differences in frequency and topography of slow and fast sleep spindles. *Front Hum Neurosci*. 2017;11:433. doi:10.3389/fnhum.2017.00433

54. Bódizs R, Körmendi J, Rigó P, Lázár AS. The individual adjustment method of sleep spindle analysis: Methodological improvements and roots in the fingerprint paradigm. *J Neurosci Methods*. 2009;178(1):205-213. doi:10.1016/j.jneumeth.2008.11.006

55. Mölle M, Bergmann TO, Marshall L, Born J. Fast and slow spindles during the sleep slow oscillation: disparate coalescence and engagement in memory processing. *Sleep*. 2011;34(10):1411-1421. doi:10.5665/SLEEP.1290

56. Vertes RP, Hoover WB, Szigeti-Buck K, Leranth C. Nucleus reuniens of the midline thalamus: link between the medial prefrontal cortex and the hippocampus. *Brain Res Bull*. 2007;71(6):601-609. doi:10.1016/j.brainresbull.2006.12.002

57. Wolansky T, Clement EA, Peters SR, Palczak MA, Dickson CT. Hippocampal slow oscillation: a novel EEG state and its coordination with ongoing neocortical activity. *J Neurosci*. 2006;26(23):6213-6229. doi:10.1523/JNEUROSCI.5594-05.2006

58. Varela C, Kumar S, Yang JY, Wilson MA. Anatomical substrates for direct interactions between hippocampus, medial prefrontal cortex, and the thalamic nucleus reuniens. *Brain*

Struct Funct. 2014;219(3):911-929. doi:10.1007/s00429-013-0543-5

59. Isomura Y, Sirota A, Özen S, et al. Integration and segregation of activity in entorhinal-hippocampal subregions by neocortical slow oscillations. *Neuron*. 2006;52(5):871-882. doi:10.1016/j.neuron.2006.10.023

60. Carman JB, Cowan WM, Powell TPS. Cortical connexions of the thalamic reticular nucleus. *J Anat*. 1964;98:587-598.

61. Behrens CJ, Van Den Boom LP, De Hoz L, Friedman A, Heinemann U. Induction of sharp wave-ripple complexes in vitro and reorganization of hippocampal networks. *Nat Neurosci*. 2005;8(11):1560-1567. doi:10.1038/nn1571

62. Vertes RP, Hoover WB, Do Valle AC, Sherman A, Rodriguez JJ. Efferent projections of reuniens and rhomboid nuclei of the thalamus in the rat. *J Comp Neurol*. 2006;499(5):768-796. doi:10.1002/cne.21135

63. Cassel JC, Pereira de Vasconcelos A, Loureiro M, Cholvin T, Dalrymple-Alford JC, Vertes RP. The reuniens and rhomboid nuclei: neuroanatomy, electrophysiological characteristics and behavioral implications. *Prog Neurobiol*. 2013;111:34-52. doi:10.1016/j.pneurobio.2013.08.006

64. Logothetis NK, Eschenko O, Murayama Y, et al. Hippocampal–cortical interaction during periods of subcortical silence. *Nature*. 2012;491(7425):547-553. doi:10.1038/nature11618

65. Xia F, Richards BA, Tran MM, Josselyn SA, Takehara-Nishiuchi K, Frankland PW. Parvalbumin-positive interneurons mediate neocortical-hippocampal interactions that are necessary for memory consolidation. *Elife*. 2017;6:e27868. doi:10.7554/eLife.27868

66. Lewis LD, Voigts J, Flores FJ, et al. Thalamic reticular nucleus induces fast and local

modulation of arousal state. *Elife*. 2015;4:e08760. doi:10.7554/eLife.08760

67. Antony JW, Piloto L, Wang M, Pacheco P, Norman KA, Paller KA. Sleep spindle refractoriness segregates periods of memory reactivation. *Curr Biol*. 2018;28(11):1736-1743. doi:10.1016/j.cub.2018.04.020

Figure Captions

Figure 1. **Characterization of slow oscillations (SOs)**. (A) Grand mean (±SEM) SO in the unfiltered signal from all recording sites time-locked to SO downstate peak (for n see Table 2). (B) *Top left*, SO density (events/min) calculated as the number of SO detected in each recording site divided by the time in SWS. *Top right,* SO duration (in s) measured as the time between two succeeding negative-to-positive zero crossings of the SO cycle. *Bottom left,* SO amplitude (in mV) measured as the downstate-to-upstate peak-to-peak amplitude. Box-whisker plots indicate median, upper (top) and lower (bottom) quartiles. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ for pairwise comparison, $n = 5$.

Figure 2. **Characterization of spindles.** (A) Grand mean (± SEM) spindles in the unfiltered signal from all recording sites time-locked to the maximum trough of a spindle (for n see Table 2). (B) *Top* left, spindle density (events/min), i.e., the number of spindles in each channel divided by the time in SWS. *Top right,* spindle duration (in s), i.e., time between onset and end of a spindle. *Bottom left*, spindle power (in mV^2s^{-1}), i.e., the integral of the Hilbert-transformed signal between spindle onset and end. *Bottom right,* spindle frequency (in Hz). Box-whisker plots indicate median, upper (top) and lower (bottom) quartiles. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ for pairwise comparison, $n = 5$.

Figure 3. Temporal association between SOs and spindles. Left panels: Event correlation histogram of spindle events time-locked to the SO downstate peak (0 s, vertical dashed lines) in (A) frontal EEG, (B) parietal EEG, (C) mPFC LFP, and (D) dHC LFP signals. Event rate (in Hz) refers to spindle events quantified by all peaks and troughs of an identified spindle. Mean

(±SEM) rates across all SO epochs with co-occurring spindles from 5 rats are shown. Bin size: 100 ms. Graphs above the histograms show means (±SEM) for the respective reference SOs, time-locked to the SO downstate peak. Significant increases (red) or decreases (blue) in event rates are indicated (thin lines: $p < 0.05$; and thick lines: $p < 0.001$, for pairwise comparison with a 1-s baseline interval (-2.0 to -1.0 s)). Right panels: Results from complementary phase-locking analyses. Circular histogram of preferred phase for spindle onsets during SO cycle (12 bins, 30° each, SO downstate peak is at 180^0 in EEG and at 0^0 in LFP recordings). Red dashed line and red range of the circle represent average phase and 95% confidence interval. ** $p < 0.01$; *** $p < 0.001$ for Rayleigh test which solely tests for deviance from an overall uniform phase distribution of spindle onsets. Note, event correlation histograms and phase-locking analyses indicate clear modulation of spindle occurrence during SO downstate and upstate in frontal and parietal EEG signals. This modulation is absent (and not significant) in the event-correlation histograms of the LFP signal, especially from mPFC.

Figure 4. Temporal association between slow oscillations (SOs) and hippocampal ripples. (A) Left panels: Event correlation histograms of ripple events time-locked to the SO downstate peak (0 s, vertical dashed lines) in (top, left) frontal EEG, (top right) parietal EEG, (bottom left) mPFC LFP, and (bottom right) dHC LFP signals. Event rate (in Hz) refers to ripple events quantified by all ripple troughs and peaks. Mean (±SEM) rates across all SO epochs with co-occurring ripples from 5 rats are shown. Graphs above the histograms show means (±SEM) for the respective reference SOs, time-locked to the SO downstate peak. Right panels: Results from complementary phase-locking analyses. Circular histogram of preferred phase for ripple occurrence during SO cycle (12 bins, 30° each, SO downstate peak is at 180^0 in EEG and at 0^0 in

LFP recordings). Red dashed line and red range of the circle represent average phase and 95% confidence interval. ** $p < 0.01$; *** $p < 0.001$ for Rayleigh test of deviance from an overall uniform phase distribution of ripples. Note, event correlation histograms and phase-locking analyses indicate a decrease in ripple occurrence around the SO downstate peak followed by an increase in ripple activity, for SOs in both EEG channels. Also, note increase in ripple activity before the SO downstate peak in both LFP channels. (B) Event correlation histograms of SO events time-locked to (the maximum trough) of hippocampal ripples (0 s, vertical dashed lines). SO events were identified in (top, left) frontal EEG, (top right) parietal EEG, (bottom, left) mPFC LFP, and (bottom right) dHC LFP signals. Event rate (in Hz) refers to SO events quantified by their downstate peak. Mean (±SEM) rates across all ripple epochs with co-occurring SOs from 5 rats are shown. Graphs above the histograms show mean (±SEM) for the respective reference ripples, time-locked to the maximum ripple troughs. Bin size for event correlation histograms: 100 ms. Significant increases (red) and decreases (blue) in event rates are indicated (thin lines: $p < 0.05$; and thick lines: $p < 0.01$, for pairwise comparison with a 1-s baseline interval (-2.0 to -1.0 s)).

Figure 5. Temporal association between spindles and hippocampal ripples. (A) Event correlation histograms of ripple events time-locked to the onset of spindles (0 s, vertical dashed lines) identified in (top, left) frontal EEG, (top right) parietal EEG, (bottom, left) mPFC LFP, and (bottom right) dHC LFP signals. Event rate (in Hz) refers to ripple events quantified by all ripple troughs and peaks. Mean (±SEM) rates across all spindle epochs with co-occurring ripples from 5 rats are shown. Graphs above the histograms show mean (±SEM) root mean square amplitude of the respective reference spindles, time-locked to the spindle onset. (B) Event

correlation histograms of spindle events time-locked to the maximum trough of ripples identified in dHC recordings (0 s, vertical dashed lines). Spindle events were identified in (top, left) frontal EEG, (top right) parietal EEG, (bottom, left) mPFC LFP, and (bottom right) dHC LFP signals. Event rate (in Hz) refers to spindle events quantified by all spindle troughs and peaks. Mean (±SEM) event rates across all ripple epochs with co-occurring spindle events from 5 rats are shown. Graphs above the histograms show dHC LFP grand averages (±SEM) time-locked to the maximum ripple troughs. Bin size for histograms is 100 ms. Significant increases (red) and decreases (blue) in event rates are indicated (t: $p < 0.1$; thin lines: $p < 0.05$; and thick lines: $p < 0.001$, for pairwise comparison with a 1-s baseline interval (-2.0 to -1.0 s)).

Figure 6. Hippocampal ripple power during spindles. Top panels: Grand average (±SEM) spindle from unfiltered dHC LFP signal during a ±0.3-s interval around the maximum trough of the spindle (0 s) for spindles co-occurring with an SO (left, $n = 658$) and isolated spindles occurring in the absence of an SO event (right, $n = 1770$). Spindles were detected in dHC LFP recordings. SOs were detected in all four channels (i.e., frontal and parietal EEG, mPFC and dHC LFP recordings). Please, refer to Figure S5 for a separate analysis on SOs only identified in dHC LFP recordings). Co-occurrence of an SO was indicated when an SO downstate occurred within the ±1.5-s interval around the spindle maximum trough. Bottom panels: Time-frequency plots of power in the 150 – 250 Hz frequency band of the dHC LFP signal time-locked to the maximum trough of reference spindle (0 s). Power is color-coded and given as normalized value, i.e., divided by the average power during a baseline interval (-2.0 to -1.0 s). Significant differences (increases) from baseline values are indicated underneath (*p*-values for paired-sample

t-test, uncorrected). There were no differences in power between spindles co-occurring with SOs and spindle occurring alone.

Figure 7: Hippocampal ripple power during slow oscillations (SOs). Top panels: Grand average (±SEM) SOs from unfiltered signals during a ±0.8 s-interval around the downstate peak of the SO (0 s) for SOs co-occurring with a spindle (left) and SOs occurring in the absence of a spindle event (right). SOs were detected in all four channels (frontal EEG: dark green, parietal EEG: light green, mPFC LFP: dark purple and dHC LFP: light purple. Please, refer to Figure S6 for a separate analysis on SOs only identified in dHC LFP recordings). Spindles were always detected in dHC LFP recordings. The co-occurrence of a spindle was indicated when a spindle onset occurred within the ±1.8 s-interval around the SO downstate peak. Middle panels: root mean square amplitude for the dHC LFP signal filtered in the spindle frequency band (10.0 – 16.0 Hz). Bottom panels: Time-frequency plot of power in the 150.0 – 250.0 Hz frequency band of the dHC LFP signal time-locked to the downstate peak of reference SO (0 s). Power is color-coded and given as normalized value, i.e., divided by the average power during a baseline interval (-2.0 to -1.0 s). Significant differences (increases: red, decreases; blue) from baseline values are indicated underneath (*p*-values for paired-sample *t*-test, uncorrected). Additional comparison (not shown) between SOs co-occurring with spindles and SOs occurring alone indicated significantly increased ripple power ($p < 0.05$) in a 100-ms bin around the downstate peak. Note, ripple power was persistently increased during SOs co-occurring with spindles, but shows only a brief transient increase during upstates of SOs occurring alone.

Figure 8. Loop-like interaction of oscillatory events regulating the information flow between hippocampus and neocortex during slow wave sleep. Top-down, the neocortical slow oscillation (SO, blue) by its hyperpolarizing downstate provides a global signal inactivating the whole loop thereby setting the temporal frame for memory processing that starts with the transition into the subsequent SO upstate. The SO upstate primarily acts on thalamic networks (blue arrow) to drive spindle activity in thalamo-cortical networks (with a peak ~500 ms following the downstate peak). Thalamic spindles that reach the hippocampus in turn act on these networks (green arrow) to increase ripples and associated memory replay, with this effect starting ~100 ms after spindle onset. Bottom-up, hippocampal ripples increase the occurrence of SO, especially in prefrontal cortex, as indicated by increased occurrence of SO downstates ~200 ms following a ripple. Note, the present data about temporal relationships between SOs, spindles and ripples, can only be used to exclude directions of causality, but not to infer causality in the interaction between these oscillatory events. Against this backdrop, the model represents an attempt to integrate the present findings about temporal relationships with findings in the literature about underlying causal mechanisms.

Table 1

Stage	Latency (min)	Number of episodes	Time (min)	Time (%)
Wake	-	161.4 ± 15.1	262.4 ± 14.0	36.4 ± 5.0
SWS	34.7 ± 4.8	172.6 ± 15.0	364.1 ± 14.0	50.5 ± 5.0
IS sleep	118.1 ± 18.6	49.8 ± 5.1	15.6 ± 2.1	2.2 ± 0.3
REM sleep	120.0 ± 17.0	47.8 ± 5.0	79.0 ± 3.3	11.0 ± 0.5

Table 1. Sleep architecture during the 12-h recording period in the light phase. Latency is given with reference to start of the recording period; time spent in the different sleep stages in minutes and percent of the total 12-h period. *n* = 5. SWS = Slow wave sleep; IS = Intermediate stage; REM = Rapid eye movement.

Table 2

	Frontal EEG		Parietal EEG		mPFC LFP		dHC LFP	
	Absolute number of slow oscillations (SOs)							
	3175.4 ± 810.6		3507.6 ± 920.8		3252.2 ± 850.6		3128.6 ± 809.8	
	SO density (#/min)							
	20.9 ± 0.5		22.9 ± 0.5		21.0 ± 0.7		20.6 ± 0,5	
	% SOs co-occuring with spindles							
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Frontal EEG	14.80	1.06	14.76	1.07	11.44	0.68	12.29	0.47
Parietal EEG	11.46	0.80	11.19	0.91	8.53	0.78	10.27	0.51
mPFC LFP	7.04	0.46	6.95	0.52	6.14	0.34	5.69	0.46
dHC LFP	8.40	1.06	8.75	1.06	7.20	0.95	7.68	1.45
	% SOs co-occuring with ripples							
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
dHC LFP	39.27	2.28	40.09	2.52	36.71	2.63	38.32	2.61

	Frontal EEG		Parietal EEG		mPFC LFP		dHC LFP	
	Absolute number of spindles							
	399.6 ± 109.2		317.4 ± 85.0		176.2 ± 43.2		226.4 ± 70.3	
	Spindle density (#/min)							
	2.7 ± 0.1		2.0 ± 0.1		1.2 ± 0.1		1.5 ± 0.2	
	% Spindles co-occuring with SOs							
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Frontal EEG	81.20	2.09	80.05	1.94	80.05	1.17	78.05	2.14
Parietal EEG	81.35	2.24	80.80	2.42	79.58	1.82	81.11	1.30
mPFC LFP	69.55	0.92	66.70	3.37	74.76	4.59	72.38	1.75
dHC LFP	75.01	1.55	77.02	2.43	68.12	4.13	71.81	2.16
	% Spindles co-occuring with ripples							
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
dHC LFP	47.7	4.4	46.3	4.9	45.0	4.0	51.7	5.9

	dHC LFP			
	Absolute number of ripples		**Ripple density (#/min)**	
	1498.6 ± 298.5		10.5 ± 0.9	
	% Ripples co-occuring with spindles		**% Ripples co-occuring with SOs**	
	Mean	*SEM*	*Mean*	*SEM*
Frontal EEG	17.4	2.6	70.1	2.4
Parietal EEG	13.4	2.6	71.4	2.9
mPFC LFP	7.2	0.6	70.7	1.9
dHC LFP	10.4	1.0	74.1	2.4

Table 2. Absolute numbers and co-occurrence of oscillatory events – slow oscillations, spindles, ripples during slow wave sleep.

Figure 1

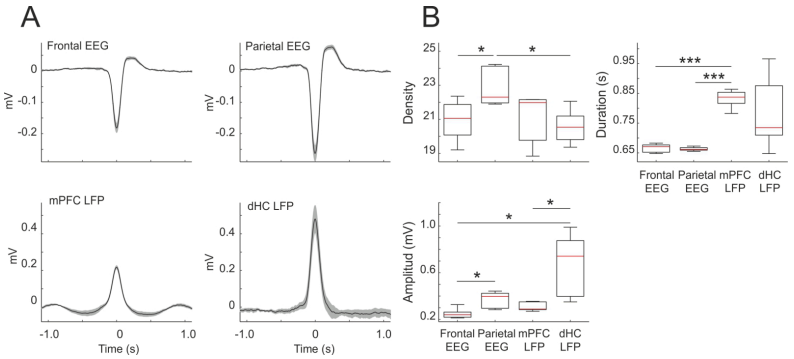

A
Frontal EEG
Parietal EEG
mPFC LFP
dHC LFP
mV
Time (s)
B
Density
Duration (s)
Amplitud (mV)
Frontal EEG
Parietal EEG
mPFC LFP
dHC LFP

Figure 2

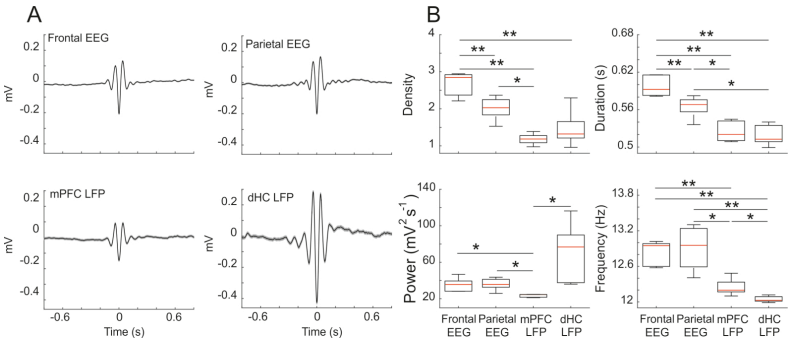

A
Frontal EEG
Parietal EEG
mPFC LFP
dHC LFP
mV
Time (s)
B
Density
Duration (s)
Power (mV^2 s^{-1})
Frequency (Hz)
Frontal EEG
Parietal EEG
mPFC LFP
dHC LFP

Figure 3

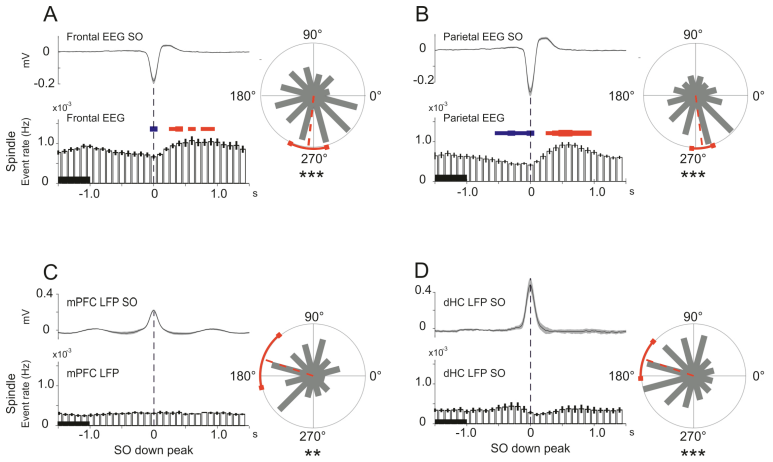

A
Frontal EEG SO
mV
0
-0.2
x10^3
Frontal EEG
Spindle
Event rate (Hz)
1.0
0
-1.0
0
1.0
s
90°
180°
0°
270°
***
B
Parietal EEG SO
Parietal EEG
C
mPFC LFP SO
mPFC LFP
SO down peak
**
D
dHC LFP SO
dHC LFP SO
SO down peak

Figure 4

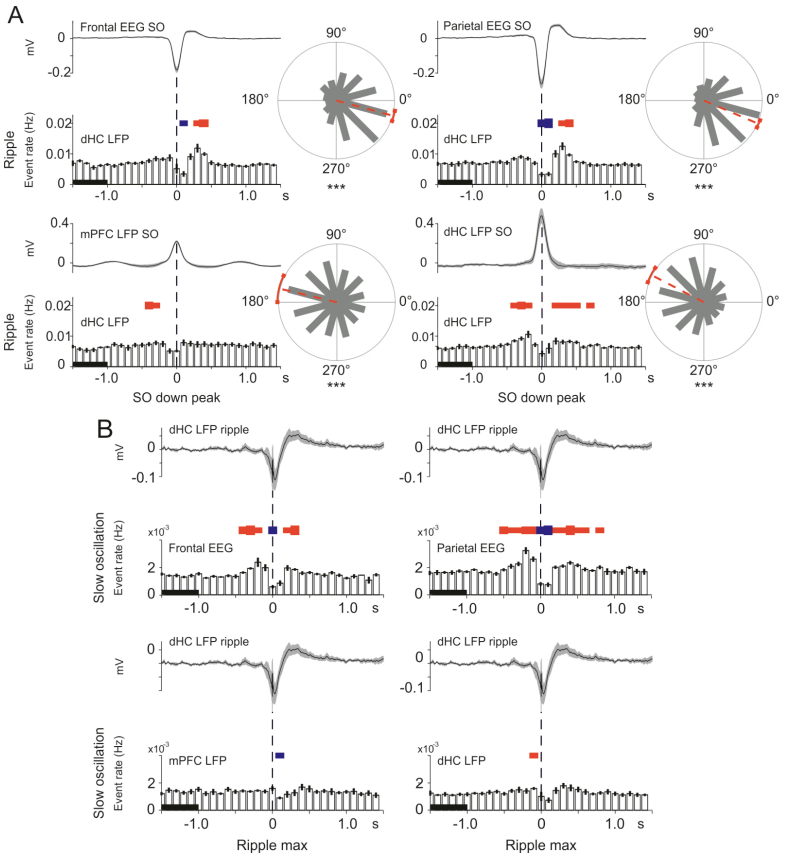

A
Frontal EEG SO
Parietal EEG SO
mPFC LFP SO
dHC LFP SO
dHC LFP
Ripple
Event rate (Hz)
mV
SO down peak
B
dHC LFP ripple
Frontal EEG
Parietal EEG
mPFC LFP
Slow oscillation
Ripple max
90°
180°
0°
270°
***
s

Figure 5

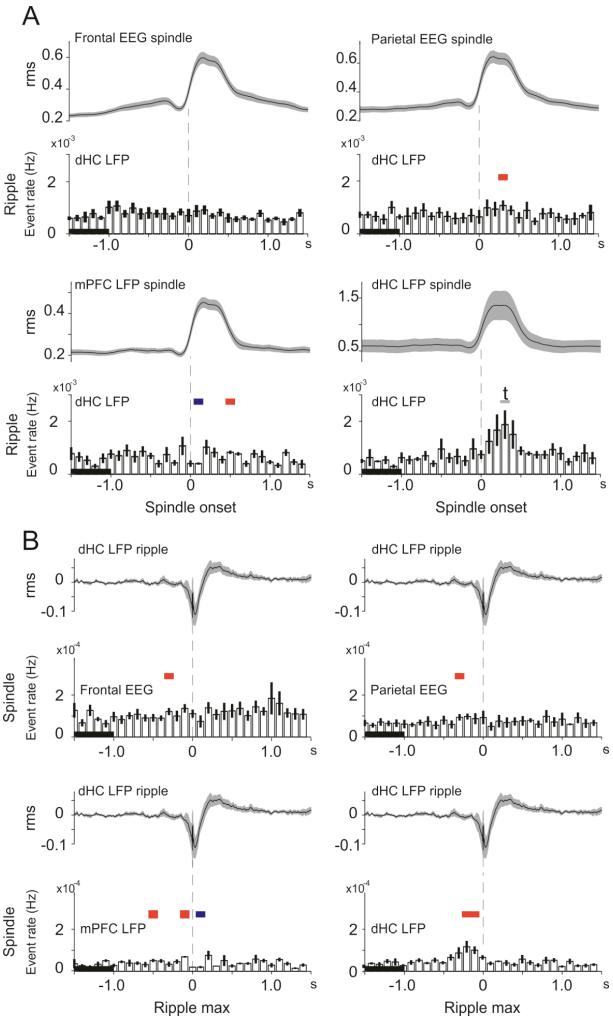

A
Frontal EEG spindle
Parietal EEG spindle
rms
0.6
0.4
0.2
x10-3
dHC LFP
Ripple
Event rate (Hz)
2
0
-1.0
0
1.0
s
mPFC LFP spindle
dHC LFP spindle
1.5
0.5
Spindle onset
B
dHC LFP ripple
0
-0.1
x10-4
Spindle
Frontal EEG
Parietal EEG
mPFC LFP
Ripple max

Figure 6

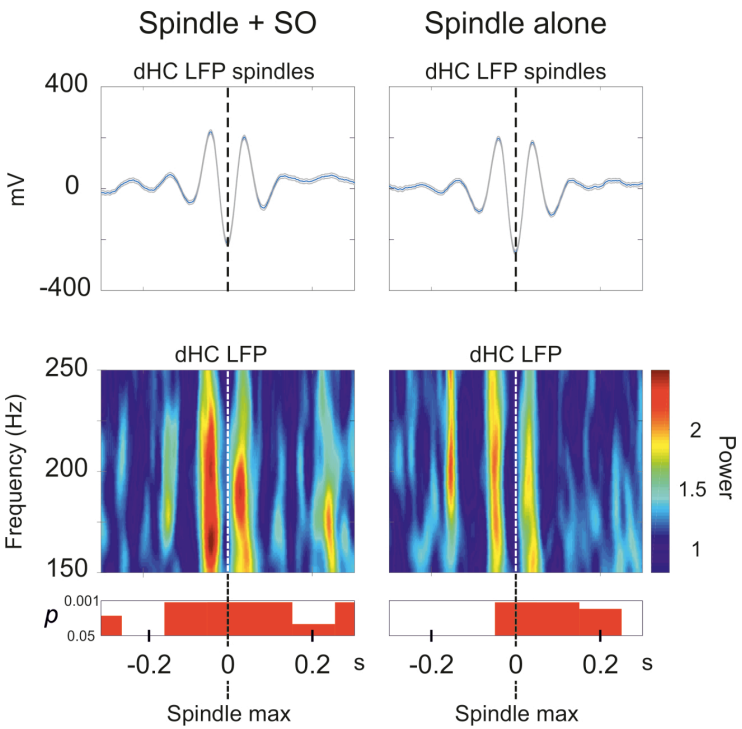

Spindle + SO
Spindle alone
dHC LFP spindles
dHC LFP spindles
400
0
-400
mV
dHC LFP
dHC LFP
250
200
150
Frequency (Hz)
2
1.5
1
Power
p
0.001
0.05
-0.2
0
0.2
s
-0.2
0
0.2
s
Spindle max
Spindle max

Figure 7

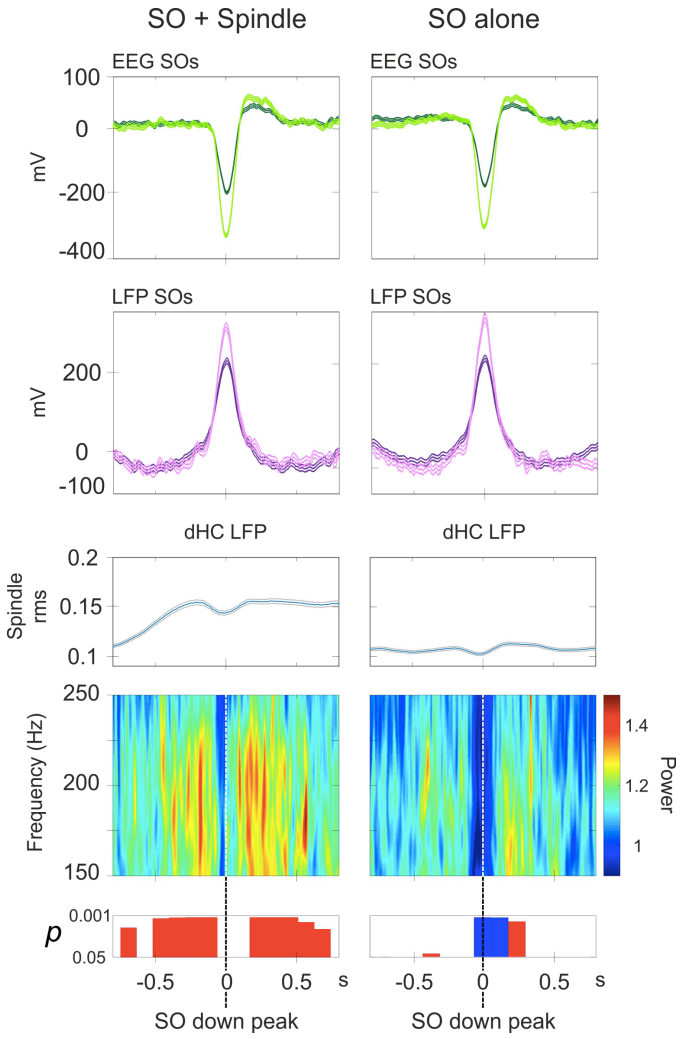
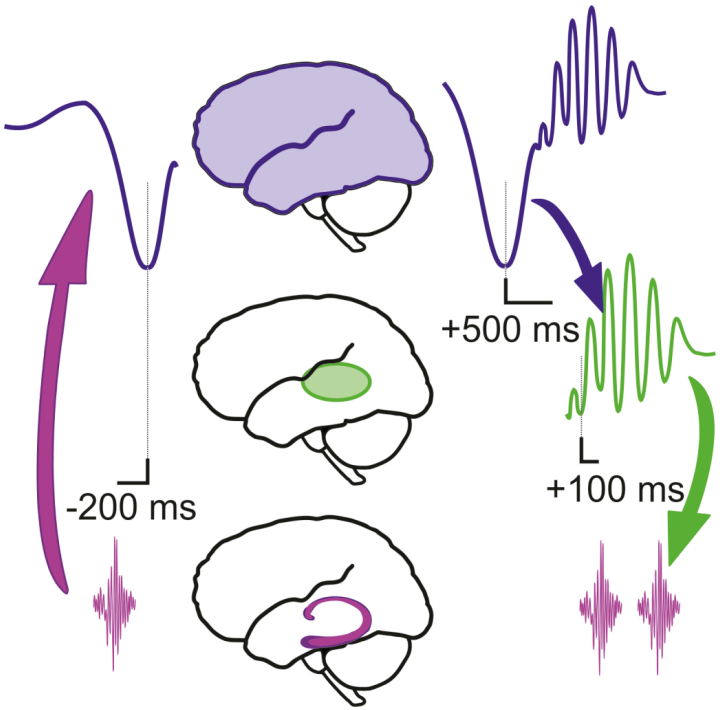

SO + Spindle
SO alone
EEG SOs
EEG SOs
100
0
-200
-400
mV
LFP SOs
LFP SOs
200
0
-100
mV
dHC LFP
dHC LFP
Spindle rms
0.2
0.15
0.1
250
200
150
Frequency (Hz)
1.4
1.2
1
Power
p
0.001
0.05
-0.5
0
0.5
s
SO down peak
-0.5
0
0.5
s
SO down peak

Figure 8



Supplemental Material

Temporal associations between sleep slow oscillations, spindles and ripples

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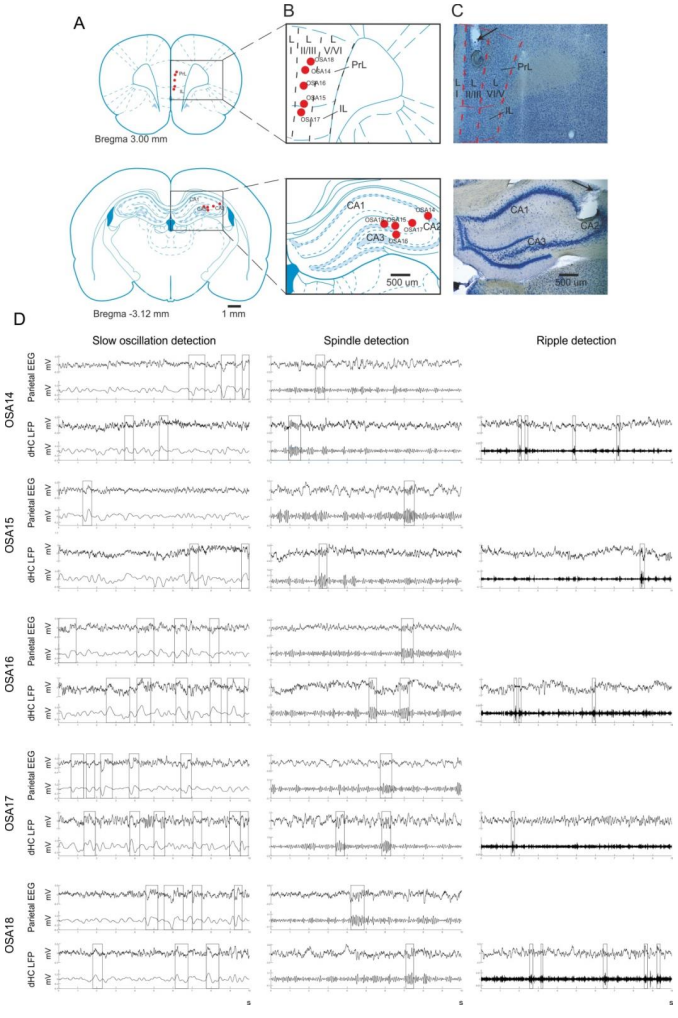
A
B
C
Bregma 3.00 mm
Bregma -3.12 mm
1 mm
PrL
IL
CA1
CA2
CA3
500 um
D
Slow oscillation detection
Spindle detection
Ripple detection
OSA14
OSA15
OSA16
OSA17
OSA18
Parietal EEG
dHC LFP
mV
s

Figure S1. Histological verification and reconstruction of the electrode placement in mPFC and dHC. (A) Coronal rat brain atlas diagrams adapted from Paxinos and Watson (1998) with permission from Elsevier, indicating the electrode position for mPFC (top) and dHC (bottom) LFP recordings. (B) Maps of electrode positions for mPFC (top) and dHC (bottom) LFP recordings (red dots). (C) Coronal view of an example methylene blue-stained section of the mPFC (top), with parcellation of layers I, II/III, and V/VI and electrode position indicated (arrow). Bottom, example section from same animal of dHC. (D) Example traces of LFP recordings from dHC and simultaneous parietal EEG recordings from individual animals (OSA14-OSA18). Unfiltered signals (upper trace) and signals filtered in the frequency band for identifying SOs, spindles, and ripples (lower trace) are shown (identified oscillatory events framed). Note, for example SOs (left panels) can occur in parietal recordings in the absence of any similar SO in dHC recordings excluding strong volume conductance of signal between channels. Recording polarity was determined based on wave shape characteristics of SOs. LFP = Local field potential; mPFC = Medial prefrontal cortex; dHC = Dorsal hippocampus.

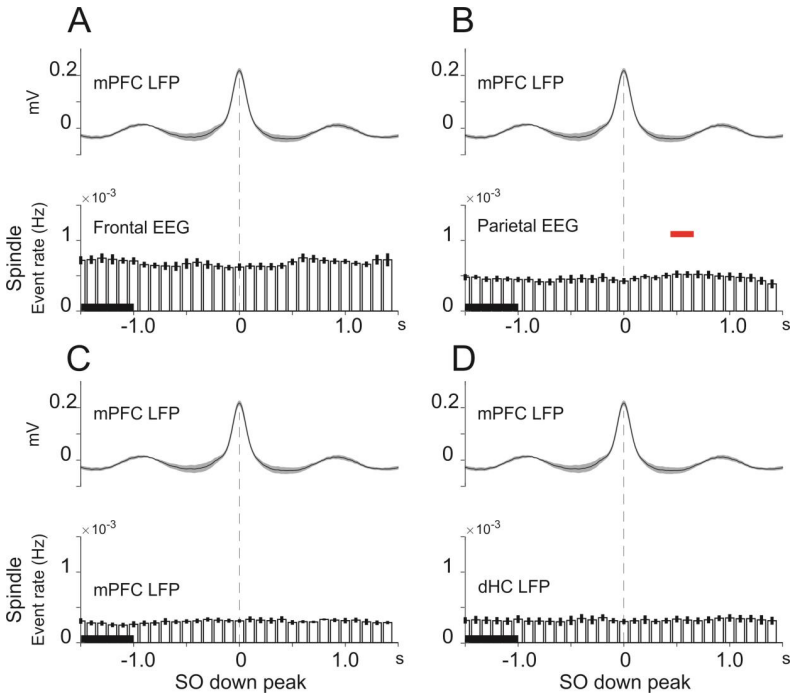

Figure S2. Temporal association between SO events in mPFC and spindles in EEG and LFP recordings. Event correlation histogram of spindle events time-locked to the downstate peak (0 s, vertical dashed lines) of SOs identified in mPFC LFP recordings. Spindle events were identified in (A) frontal EEG, (B) parietal EEG, (C) mPFC LFP, and (D) dHC LFP signals. Event rate (in Hz) refers to spindle events quantified by all peaks and troughs of an identified spindle. Means (±SEM) rates across all SO epochs with co-occurring spindles (in one of the four channels) from 5 rats are shown. Bin size: 100 ms. Graphs above the histograms show the mean

(±SEM) reference SO in mPFC recordings, time-locked to the SO downstate peak. Significant increases in event rates are indicated (red lines: $p < 0.05$, for pairwise comparison with a 1-s baseline interval (-2.0 to -1.0 s)).

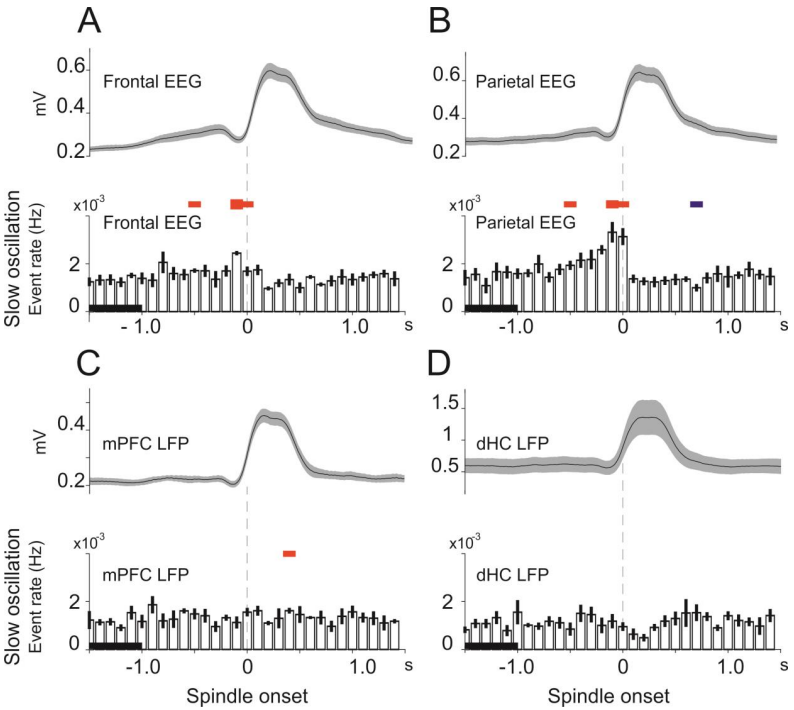

Figure S3. Temporal association between spindles and SOs. Event correlation histogram of SO events time-locked to the onset of spindles (0 s, vertical dashed lines) in (A) frontal EEG, (B) parietal EEG, (C) mPFC LFP, and (D) dHC LFP signals. Event rate (in Hz) refers to SO events identified by their downstate peak. Mean (±SEM) rates across all spindle epochs with co-occurring SOs from 5 rats are shown. Bin size: 100 ms. Graphs above the histograms show mean (±SEM) root mean square amplitude of the respective reference spindles, time-locked to the

spindle onset. Significant increases (red) or decreases (blue) in spindle occurrence are indicated (thin lines: $p < 0.05$; and thick lines: $p < 0.001$, for pairwise comparison with a 1-s baseline interval (-2.0 to -1.0 s)).

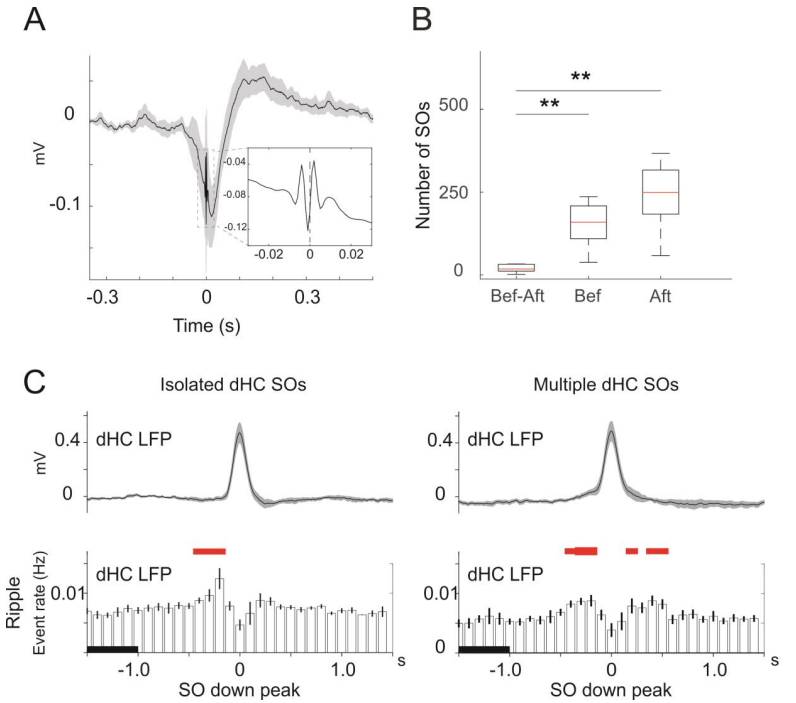

Figure S4. Hippocampal ripples before and after SO downstate peaks. (A) Grand average (±SEM) of ripple from the unfiltered dHC LFP signal time-locked to the maximum ripple trough. Insert illustrates a single ripple. (B) Number of SO events with ripple events before and after the downstate peak (Bef—After), and with ripple event either before (Bef) or after (Aft) the downstate peak. Box-whisker plots indicate median, upper (top) and lower (bottom) quartiles. * $p < 0.05$; ** $p < 0.01$ for pairwise comparison. (C) Event correlation histograms of ripple events time-locked to the SO downstate peak (0 s, vertical dashed lines) for SO events in dHC LFP

recordings that (left) either occurred in isolation (n = 1753.6 ± 495.7), or (right) were preceded or followed (within ± 1.5 ms) by another SO event, i.e., downstate peak (n = 1375.0 ± 343.0). Event rate (in Hz) refers to events quantified by all troughs and peaks of an identified ripple. Mean (±SEM) rates across all respective SO epochs with co-occurring ripples from 5 rats are shown. Graphs above the histograms show means (±SEM) for the respective reference SOs, time-locked to the SO downstate peak. Bin size 100 ms. Significant increases (red) or decreases (blue) in ripple occurrence are indicated (thin lines: $p < 0.05$; and thick lines: $p < 0.001$, for pairwise comparison with a 1-s baseline interval (-2.0 to -1.0 s)). Note, on average stronger increase in ripple activity before the downstate peak of SOs occurring in isolation than before SOs followed by another SO.

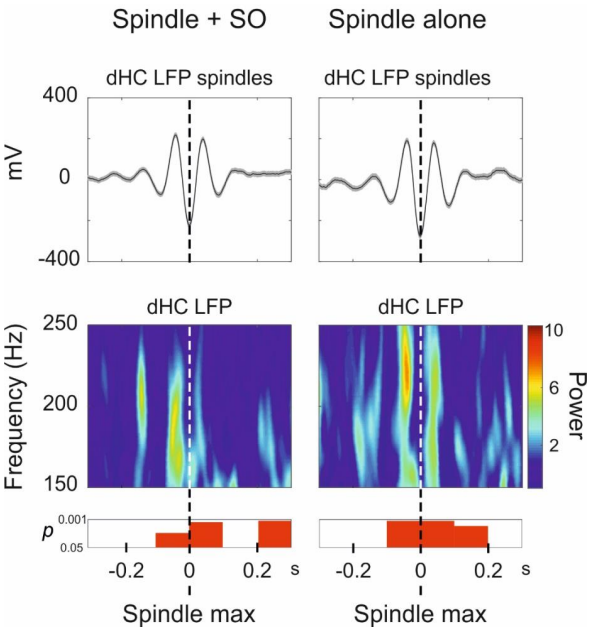

Figure S5. Hippocampal ripple power during spindles. Top panels: Grand average (±SEM) spindle from unfiltered dHC LFP signal during a ±0.3-s interval around the maximum trough of the spindle (0 s) for spindles co-occurring with an SO (left, n = 138) and isolated spindles occurring in the absence of an SO event (right, n = 469). Unlike in Fig. 6 of the main text, here both spindles as well as SOs were detected in dHC LFP recordings. Co-occurrence of an SO was indicated when an SO downstate occurred within the ±1.5-s interval around the spindle maximum trough. Bottom panels: Time-frequency plots of power in the 150–250 Hz frequency band of the dHC LFP signal time-locked to the maximum trough of reference spindle (0 s). Power is color-coded and given as normalized value, i.e., divided by the average power during a

baseline interval (-2.0 to -1.0 s). Significant differences (increases) from baseline values are indicated underneath (*p*-values for paired-sample *t*-test, uncorrected).

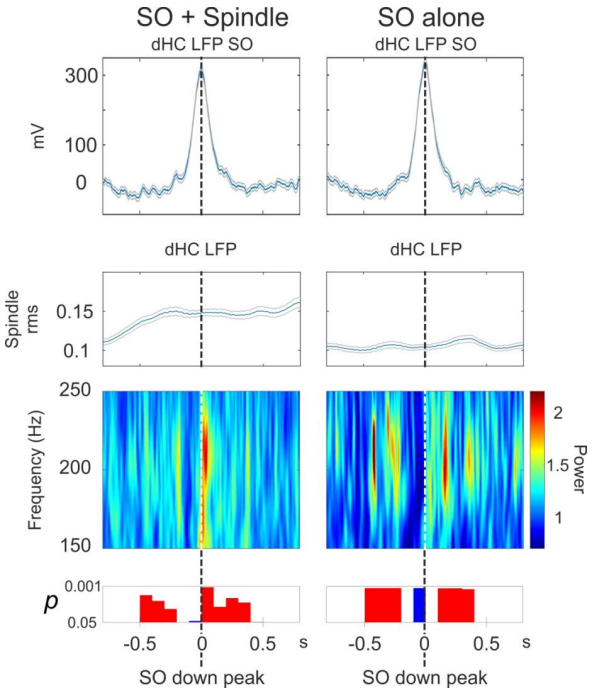

Figure S6. Hippocampal ripple power during slow oscillations (SOs). Top panels: Grand average (±SEM) SOs from unfiltered signals during a ±0.8 s-interval around the downstate peak of the SO (0 s) for SOs co-occurring with a spindle (left) and SOs occurring in the absence of a spindle event (right). Unlike in Fig. 7 of the main text, here, both SOs and spindles were only detected in dHC LFP recordings. The co-occurrence of a spindle was indicated when a spindle onset occurred within the ±1.8 s-interval around the SO downstate peak. Middle panels: root mean square amplitude for the dHC LFP signal filtered in the spindle frequency band (10.0 – 16.0 Hz). Bottom panels: Time-frequency plot of power in the 150.0-250.0 Hz frequency band of

the dHC LFP signal time-locked to the downstate peak of reference SO (0 s). Power is color-coded and given as normalized value, i.e., divided by the average power during a baseline interval (-2.0 to -1.0 s). Significant differences (increases: red, decreases; blue) from baseline values are indicated underneath (*p*-values for paired-sample *t*-test, uncorrected). Note, consistent with the analyses in Fig. 7 of the main text (collapsing SOs across all recording sites), upstates of SOs selectively identified in dHC recordings are associated with increased ripple power, whereas ripple power is decreased around the downstate of these SOs, particularly when they occur in the absence of a spindle. As a result, ripple power ~100 ms around the SO downstate peak is significantly lower ($p < 0.05$) for isolated SOs than SOs co-occurring with a spindle (not shown). Different from the analysis in Fig. 7, the upstate-related increase in ripple power during SOs occurring alone in the dHC LFP signal appears to be more persistent, likely reflecting that once a SO has reached the hippocampus, the upstate per se can effectively contribute to increasing ripple activity.

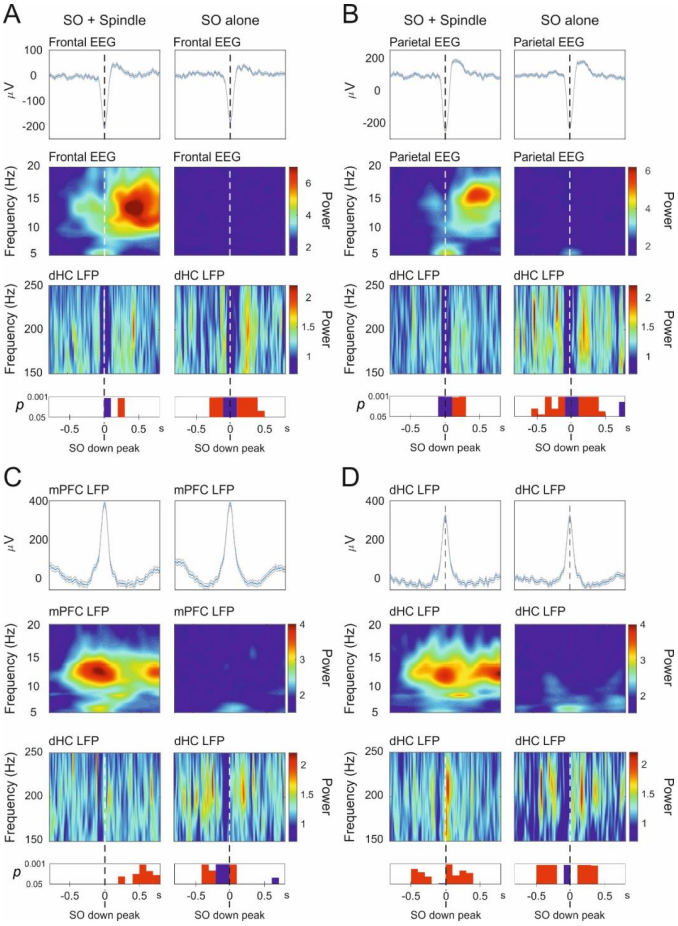

Figure S7. Hippocampal ripple power during slow oscillations (SOs). Top panels: Grand average (±SEM) SOs from unfiltered signals from (A) frontal EEG, (B) parietal EEG, (C) mPFC LFP, and (D) dHC LFP, during a ±0.8 s-interval around the downstate peak of the SO (0 s) for

SOs co-occurring with a spindle (left) and SOs occurring in the absence of a spindle event (right) with the spindle identified in the same channel as the SO. The co-occurrence of a spindle was indicated when a spindle onset occurred within the ±1.8 s-interval around the SO downstate peak. Middle panels: Time-frequency plot of power in the 5.0-20.0 Hz band (covering the spindle band) time-locked to the downstate peak of the reference SO (0 s). Bottom panels: Time-frequency plot of power in the 150.0-250.0 Hz frequency band of the dHC LFP signal time-locked to the downstate peak of reference SO (0 s). Power is color-coded and given as normalized value, i.e., divided by the average power during a baseline interval (-2.0 to -1.0 s). Significant differences in ripple band power (increases: red, decreases: blue) from baseline values are indicated underneath (*p*-values for paired-sample t-test, uncorrected). Except that ripple power around the downstate peak (0 s) of SOs co-occurring with spindles was higher with event detection in the dHC LFP than in EEG recordings ($p < 0.05$), there were no significant differences in ripple power depending on the site of SO/spindle detection.

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I am also grateful to my good old friends in Chile – *por el cariño y apoyo a pesar de la distancia* – and also, to all my new-old friends in Tübingen & Surroundings – from all over the world – for all those shared lovely moments, for making Tübingen a new *Heimat*, for becoming my new family. Specially I would like to thank my dear friend Niels for his support and friendship, for those many years, many beers and many kilometers shared and for the ones to come. *Vielen Dank Chancho*!

For their unconditional love and support, I would like to thank my family – Ana, Carlos, Daniel and my niece Dominique Millaray. *Gracias familia querida por su amor y apoyo*.

Finally, for your love, for being here, for standing by my side, for the loveliest moments we had and the ones that are coming, thank you Oliwia. *Dziękuję bardzo, kochanie, za piękną łódź, którą razem zbudowaliśmy.*