Aus dem

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Sleep's roles in long-term memory consolidation: evidence for a common hippocampal mechanism for forming long-term memory across different memory systems

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Dedicated to my beloved family and my inspiring teachers…….

.....แด่ครอบครัวที่รัก และเหล่าคณาจารย์ผู้เป็นแรงบันดาลใจ

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List of Abbreviations

ANOVA	analysis of variance
BOLD	blood-oxygen-level-dependent imaging
DMS	delayed matching-to-sample task
DNMS	delayed nonmatching-to-sample task
EEG	electroencephalography
e.g.	for example
et al.	and others
fMRI	functional magnetic resonance imaging
GABA	γ-aminobutyric acid
i.e.	in other words
LFP	local field potential
LTP	long-term potentiation
MTL	medial temporal lobes
NMDA	N-methyl D-aspartate
NOR	novel-object recognition
OPR	object-place recognition
REM	rapid eye movement
SD	standard deviation
SEM	standard error of the mean
SOs	slow oscillations
STP	short-term potentiation
SWS	slow-wave sleep

1. Introduction

1.1 Memory processes

Memory is an essential part our lives. New information is acquired and retained over time in the form of memory. An ability to remember past events guides behavioral responses to encounter a new situation. Capability of learning and memorizing is preserved across species even for invertebrates such as nematodes *Caenorhabditis elegans* (Rankin et al.1990, Ardiel and Rankin 2010) and sea slugs *Aplysia* (Kandel 2001). Memory processes compose of three major steps: encoding, consolidation, and retrieval. The brain encodes new information via multimodal sensory inputs and subsequently creates memory representations of the integrated information inputs. Consolidation is a process of transforming newly encoded information into more stable form of memory. During retrieval, memory representations are activated, thereby allowing an access to retrieve information. Memory differs in its capacity and duration for holding newly encoded information. We cannot remember everything we have experienced. Some events, or a part of them, can be retained over the years, yet others has diminished. According to this view, memory is divided into short-term and long-term memory based on stability of stored information.

1.2 Memory systems

Memory is not a simple unitary system. Memory is composed of multiple distinct systems supported by various brain regions. One of the first evidence suggesting the concept of multiple memory systems arose from the study of an amnestic patient H.M. (Scoville and Milner 1957). In attempt to treat his recurrent epileptic seizures, H.M. underwent an experimental brain operation to surgically resect large portions of his bilateral hippocampi. Although his epilepsy was successfully controlled after the surgery, H.M. had lost the ability to form new memory of events (i.e. anterograde amnesia). However, he could remember the events acquired in the period of time before the surgery (i.e. temporally graded retrograde amnesia). His short-term memory as well as intellectual function remained intact. Also, other cognitive functions, such as

motor skill learning, were preserved. The core deficit of his symptom was an inability to transfer information from short-term memory into long-term memory. The description of the patient H.M. has illuminated the science of memory. Based on extensive works on H.M. and several other amnestic patients, the distinction between hippocampus-dependent and non-hippocampus-dependent forms of human memory has been widely accepted. Long-term memory can be divided into two major systems: declarative memory and non-declarative or implicit memory. Declarative memory refers to ability for conscious recollection about events and general knowledge. Processing of this type of memory requires hippocampus and its surrounding medial temporal lobe structures. Non-declarative memory, on the other hand, refers to unconscious memory and memory of skills which is supported by the brain regions outside the hippocampus, for example, striatum and neocortex. These two major types of memory comprise different subtypes of memories (Squire 2004). Declarative memory system can be further divided into episodic memory (i.e. memory for an event in the spatial-temporal context in which it was acquired) and semantic memory (i.e. memory for facts and knowledge about the world independent of personal experience and of the spatial-temporal context) (Tulving 1983). Non-declarative memory such as procedural memory is acquired through a repetition of events and trainings which heavily relies on automatic sensorimotor experience.

1.3 Recognition memory

Recognition memory is a subtype of declarative memory systems which refers to an ability to distinguish a previously encountered stimulus from one that was not presented before. Considerable evidence suggests that recognition memory is dissociated into the two measures of recognition judgments: recollection of details of the events, or recognition of stimulus familiarity (Eichenbaum et al. 2007, Yonelinas et al. 2010). Behavioral tasks assessing recognition memory have become increasingly useful tools for basic and preclinical research investigating the neural mechanisms of memory formation. The most commonly used task to assess recognition memory across species is a novel-object

recognition (NOR) task (also known as a visual paired-comparison task in studies with humans and non-human primates) (Sivakumaran et al. 2018).

1.3.1 Object recognition task in rodents

The novel-object recognition (NOR) task was first developed by Ennaceur and Delacour 1988. The NOR task relies on an animal's spontaneous behavior towards exploring novelty. At the encoding phase of the NOR task, the animal is exposed to two identical objects in an open field. After a retention delay, the animal is re-exposed to the same testing arena containing an object identical to the encoding phase (familiar object) and a novel object. An innate tendency to explore novelty drives the animal's behavior to preferentially explore the novel object over the familiar one. The NOR task has been used in a wide range of research and is currently a benchmark task for assessing recognition memory. The task has been further developed to assess different components of recognition memory, such as spatial memory for the object (i.e. object-place recognition (OPR) task) (Ennaceur et al. 1997), and episodic-like memory (Eacott and Norman 2004, Dere et al. 2005, Kart-Teke et al. 2006). Object recognition tasks can also be used in developmental studies in rodents. However, an expression of recognition memory in developing rats can be shifted from familiarity-based exploration preference at an early development stage to novelty-driven exploration during adolescence (Contreras et al. 2019).

The NOR task and its variations (e.g. OPR task) do not require training or learning of a rule and therefore can be used as a one-trial test to assess recognition memory of a unique event. Also, these tasks do not involve a reinforced learning or punishment, which might confound the interpretation of findings. Since humans and non-human animals do not need a reinforcer for making recognition judgement, these advantages put the object recognition task as an excellent candidate to investigate a "pure" neural basis of recognition memory and a promising approach to the comparative study of memory processes across species.

1.3.2 Social recognition task in rodents

The ability of an animal to distinguish another's distinctive features is fundamental in social species. Recognition of conspecifics (i.e. social recognition memory) is one type of recognition memory which guides an appropriate behavior response during future interactions with conspecifics, for instance, social bonding, mating, defensive behaviors, and social hierarchy. Similar to object recognition memory, rodents exhibit an innate exploration preference to novel conspecifics. The relatively longer exploration towards a novel- compared to a familiar conspecific indicates memory for the previously encountered animal, as referred to as social recognition memory (Thor and Holloway 1982). While social recognition memory in humans and non-human primates primarily relies on visual and auditory cues, chemosensory stimuli such as volatile odorants and pheromones are essential for encoding social information in rodents (Popik et al. 1991, Noack et al. 2010). A social discrimination task was developed to test social recognition memory in rodents (Engelmann et al. 1995, Engelmann et al. 2011). In this task, a juvenile conspecific (i.e. social stimuli) is introduced into the adult home cage. After the encoding phase, the juvenile is kept individually in a new cage to avoid any confounding olfactory stimuli. At the test phase, the same juvenile and a novel juvenile are exposed to the adult conspecific in its home cage. The time investigating the juvenile conspecifics is then calculated as an index of recognition memory performance. A direct interaction between conspecifics in the home cage allows the rodent to have direct access to both volatile and non-volatile components of an individual's olfactory signature which are essential for encoding social information (Noack et al. 2010). In this dissertation work, the social recognition task was conducted in a modified radial arm maze. The maze was modified to investigate the spatial component of social recognition memory, along with providing the rats with direct access to their conspecifics during social exploration. Accordingly, associations between non-spatial social information and spatial memory can be unveiled.

1.3.3 Brain structures involve in recognition memory

Medial temporal lobes (MTL) are crucial for recognition memory. Anatomical organization of the MTL includes the hippocampus, entorhinal cortex, parahippocampal cortex, perirhinal cortex, amygdala, and diencephalon (Squire 2004). There are two major viewpoints regarding the roles of the MTL in recognition memory. One view suggests that recognition memory relies on the integrity of entire medial temporal lobe structures (Squire et al. 2007), while another suggests the distinctive roles of different MTL subregions in processing recognition memory (Eichenbaum et al. 2007). Squire et al. (2007) proposed that the MTL memory system works together and indistinctly contributes to recognition memory. The different roles of MTL structures in memory processes depend on the strength of recognition memory. They suggested that the hippocampus and perirhinal cortex are associated with both components of recognition memory (i.e. recollection and familiarity) and the distinctive responses to stimuli from the hippocampus and perirhinal cortex rather reflect strong and weak memories, respectively. On the other hand, Eichenbaum et al. (2007) suggested that each subregion of the MTL differentially contributes to distinct components of recognition memory. The hippocampus supports recollection of the stimuli together with the context in which they occurred, while the perirhinal cortex signals a sense of familiarity with stimulus features.

Previous studies investigating the involvement of the hippocampus in object recognition memory in rodents revealed conflicting results. Some studies reported that the hippocampus plays an important role in a novel-object recognition (NOR) memory. Rats with bilateral excitotoxic lesions or thermocoagulation lesions of the hippocampus showed impairment of the NOR memory after the long delays but not after the shorter delays (10s and 1 min) (Clark et al. 2000) and the NOR memory deficits were observed only when the extent of damage to the bilateral dorsal hippocampus covered 75-100% of total hippocampal volume, but not 50-75% of total hippocampal volume (Broadbent et al. 2004). Additionally, after repeated exposures to two identical objects (a total of 12 encoding sessions), rats with post-encoding bilateral excitotoxic lesions of hippocampus (more than 90% of total hippocampal volume) were

impaired on the remote NOR memory test (Broadbent et al. 2010). However, emerging evidence suggests a functional double dissociation between the perirhinal cortex and hippocampus in object recognition memory. Winters et al. (2004) showed that the hippocampus is important for spatial but not object recognition memory, and the perirhinal cortex is important for object recognition but not spatial memory. In their study, the NOR task was conducted in a Y-shaped maze designing to minimize the influence of spatial and contextual factors, while a radial arm maze was used to assess spatial memory. Rats with perirhinal lesions could not discriminate the novel object from the familiar object, but they showed intact spatial memory. Conversely, object recognition memory was spared in rats with hippocampal lesions, but their performance on the spatial memory task was impaired. Barker and Warburton (2011) further confirmed the distinct roles between these two brain regions in object recognition memory. They used a standard open field arena as an apparatus to directly compare memory performance of the hippocampal lesioned rats on a series of object recognition tasks. They demonstrated that the perirhinal cortex is essential for the NOR task, while the hippocampus is necessary for object recognition memory only when the task requires an association between an object and its learning contexts. These two studies and others (e.g. Brown and Aggleton 2001, Mumby et al. 2002, Langston and Wood 2010) indicate that the hippocampus and perirhinal cortex are clearly functionally distinct.

It should be taken into account, however, that majority of these studies utilized the permanent lesion approach to investigate the role of hippocampus in the NOR task. Most studies conducted pre-training hippocampal lesions, which accordingly inhibit the hippocampal function also during the pre-encoding habituation and cannot distinguish the contribution of the hippocampus to different processes of object recognition memory (e.g. encoding, consolidation, and retrieval). Also, the size of permanent lesion in the hippocampus was shown to influence the extent of the NOR memory deficits (Broadbent et al. 2004, Ainge et al. 2006). Moreover, it has been suggested that the irreversible damage in specific brain structure could drive compensatory mechanisms of another anatomically connected brain region to compromise its loss of

functional integrity (Goshen et al. 2011). The findings from permanent lesion studies might rather explain how other interconnected brain regions work without the hippocampus. These potentially confounding factors could be one of the explanations of the discrepancies from previous findings. Thus, the contribution of hippocampus in recognition memory remains elusive.

1.4 Pharmacological inactivation of hippocampal function

The permanent lesion techniques have long been used to develop animal models of human amnesia. Although this approach can provide an appropriate estimation of the extent of damaged brain area, it cannot distinguish the contribution of the region of interest to the stage-dependent memory processes. Temporary pharmacological inactivation techniques offer an alternative solution for studying the effects of inactivation in a specific brain region on distinct memory stages. Numbers of studies using temporary inactivation approach have greatly increased over the last decades (Cohen and Stackman 2015) and muscimol is the most commonly used drugs to temporarily inhibit hippocampal functions. A $GABA_A$ receptor agonist drug, muscimol, has a short-term effect into the brain, immediately inhibiting neuronal activity and lasting around 2 hours for a 1µg/µl dosage (Martin 1991, Edeline et al. 2002). After infusion muscimol rapidly spreads to its maximal extent and the distance from the center of infusion site in the hippocampus was 1.80 mm (Corcoran et al. 2005). Muscimol does not affect motor function, which is essential for the behavioral task relying heavily on locomotor activity (e.g. object recognition tasks), and the infusion site can be fully recovered without developing permanent damages (Martin 1991, Martin and Ghez 1999). Unlike the sodium-channel blockers (e.g. tetrodotoxin and lidocaine), muscimol selectively acts on cell bodies within the target region and does not inhibit fibers of passage, thereby producing more local effects on the infusion side (Lomber 1999). Its advantage in reversibly inactivating the local neurons from the region of interest without disrupting axonal transmission from other interconnected brain regions suits with the aim of the dissertation work to investigate functional contribution of the

hippocampus to distinct processes of recognition memory including during sleep consolidation.

1.5 Sleep role's in memory consolidation

Sleep is an adaptive state. It is not mere period of inactivity posing potential threats from the predators but rather has evolutionarily conserved functions. Sleep is necessary for physiological (e.g. body temperature regulation) and cognitive functions (e.g. learning and memory) as well as immune system. The dissertation work focuses on the memory functions of sleep, in particularly the role of sleep in memory consolidation.

1.5.1 Theoretical background

Based on findings in patients with selective hippocampal damages, the systems consolidation theory was first proposed to explain how the brain can form new associative memories without overwriting old memories in the hippocampus-dependent declarative memory system (e.g. Marr 1971, McClelland et al. 1995). This theory assumes that newly encoded information is initially stored in the hippocampus and then gradually transferred to a long-term storage in the neocortex. The hippocampus is thought to be essential for rapid encoding and associations among various components of memory. Its capability of fast and efficient encoding ensures that information is effectively encoded even in one-trial learning. However, the encoded information in the hippocampal networks is sensitive to interference from newly encoded information. In contrast, the neocortex, which acts as a slow learner, relies on gradual changes in synaptic connectivity within a specialized memory system to form long-term memory. Memory consolidation is a process of repeated hippocampal activation which in turn co-activate associative neocortical networks and thereby promotes a gradual redistribution of the memory representations from the hippocampus (short-term store) to the neocortex (long-term store).

Active systems consolidation has extended the standard consolidation theory with a particular emphasize on the role of sleep in memory consolidation. The key concept is that newly encoded memory representations are reactivated during subsequent sleep, thereby facilitating the formation of more stable

memory representations in neocortical networks. Reactivation of learning-associated neuronal ensembles during sleep has been consistently observed both in animals and humans (e.g. Wilson and McNaughton 1994, Peigneux et al. 2004, Ji and Wilson 2007, Bergmann et al. 2012). The memory reactivation mostly occurs during slow-wave sleep (SWS). Three oscillatory hallmarks of SWS, i.e., neocortical slow oscillations, thalamic spindles and hippocampal sharp waves-ripples, have been proposed to orchestrate memory consolidation during sleep. First, the <1 Hz slow oscillations (SOs) represent a synchronized activity of large neuronal populations. SOs consist of alternations between states of membrane depolarization ("up-states") and states of membrane hyperpolarization ("down-states"). During the "up-states" of SOs, increased neuronal firing patterns and network excitability are observed across cortical and thalamo-cortical networks (Steriade 2006, Timofeev and Chauvette 2011). Secondly, sleep spindles are generated in thalamus and are characterized by 10-15 Hz EEG oscillatory activity. The up-states of SOs modulate occurrence of the spindles (Staresina et al. 2015). The coupling between spindles and SO up-states is thought to promote synaptic plasticity in cortical networks (Seibt et al. 2017, Niethard et al. 2018). Hippocampal ripples are the third oscillatory hallmarks of SWS. Ripple oscillations in the hippocampus accompany reactivation of neuronal ensembles. Selective suppression of hippocampal ripples after learning impairs memory consolidation (Girardeau et al. 2009). The hippocampal ripples are nested into the troughs of spindles (Staresina et al. 2015), suggesting a candidate mechanism for spreading the reactivated memory information from the hippocampus to extra-hippocampal networks.

1.5.2 Effects of sleep on recognition memory

Sleep after learning robustly enhances hippocampus-dependent memory (Spencer et al. 2006, Rasch and Born 2013). Although there is also evidence that sleep enhances non-hippocampus dependent memory such as procedural motor skill learning (Robertson et al. 2004, King et al. 2017), performance improvement in some tasks under certain conditions can also be observed when learning is followed by periods of wakefulness (Cai and Rickard 2009,

Rieth et al. 2010). With regard to recognition memory, a previous study in humans indicated that sleep preferentially benefits hippocampus-dependent aspects of recognition memory, as compared with item recognition memory (van der Helm et al. 2011). Consistent with this evidence, studies in rodents revealed that sleep is required for object recognition memory only when the task performance critically relies on hippocampal functions. Previous studies demonstrated a beneficial effect of sleep for object-place recognition (OPR) memory tested up to 24 hours after learning (Ozawa et al. 2011, Binder et al. 2012, Bett et al. 2013, Inostroza et al. 2013, Ishikawa et al. 2014, Oyanedel et al. 2014, Howard and Hunter 2019) but not for novel-object recognition (NOR) memory (Graves et al. 2003, Cai et al. 2009, Inostroza et al. 2013, Ishikawa et al. 2014, Oyanedel et al. 2014). Lesion studies in rodents indicate that performance on the OPR task relies on the hippocampus (Langston and Wood 2010, Barker and Warburton 2011), while normal hippocampal function is not critical for the NOR task (Winters et al. 2004, Barker and Warburton 2011). Hippocampus-dependent OPR task performance is associated with post-encoding slow-wave sleep (SWS) and spindle activity (Binder et al. 2012, Oyanedel et al. 2014). Likewise, episodic-like memory in rats is enhanced after sleep and memory improvement is associated with increased SWS activity (Inostroza et al. 2013, Oyanedel et al. 2014). Moreover, oscillation coupling during SWS appears to be a candidate mechanism for the consolidation of OPR memory. Optogenetic induction of thalamic spindles phase-locked to the SO up-states during post-encoding sleep enhances OPR memory (Latchoumane et al. 2017). Also, reinforcing the coordination of spindle-ripple-SO events by ripple-triggered cortical stimulation applied during post-encoding SWS, distinctly improved OPR memory performance tested on the next day (Maingret et al., 2016). Taken together, previous studies suggest that sleep selectively supports the consolidation of hippocampus-dependent aspects of recognition memory.

1.6 Research questions and objectives

Ample evidence has indicated that sleep preferentially supports consolidation of hippocampus-dependent memory, whereas performance on non-hippocampus-dependent memory tasks does not critically relies on sleep. However, this evidence is based mainly on studies investigating the effect on post-learning sleep on memory performance tested on the next day. It remains unclear whether the enhancing effect of sleep can be observed after a long delay. Therefore, the first study aimed at investigating temporal development of sleep's effects on memory consolidation in both memory domains using two versions of object recognition tasks in rats as a model of human recognition memory. Object-place recognition (OPR) and novel-object recognition (NOR) tasks were used to assess hippocampus-dependent and non-hippocampus-dependent forms of memory, respectively, and to examine whether sleep benefits formation of long-term memory in both memory domains.

Although sleep also benefits non-hippocampus dependent forms of memory (King et al. 2017), the mechanism underlying consolidation of such memories during sleep is poorly understood. The "active system consolidation" has been primarily conceptualized regarding to the hippocampus-dependent memory system (McClelland et al. 1995, Diekelmann and Born 2010). During slow-wave sleep (SWS) repeated reactivation of hippocampal neuronal ensembles that were active during encoding promotes gradual transformation of hippocampal memory representations towards extra-hippocampal long-term storage sites. On the contrary, despite a little evidence, reactivation of task-related neuronal ensembles during sleep have also been found in non-hippocampus-dependent memory (e.g. Pennartz et al. 2004, Lansink et al. 2009, Ramanathan et al. 2015) and might support memory consolidation without relying heavily on hippocampal functions during sleep. Thus, unveiling the role of hippocampus during sleep would provide insight into a mechanism underlying sleep-dependent memory consolidation. Accordingly, the second aim of the first study was to investigate whether the hippocampus also critically contribute to long-term consolidation of non-hippocampus-dependent NOR memory. To determine the role of the hippocampus during sleep, the $GABA_A$

receptor agonist muscimol was used to temporary inactivate hippocampal function during post-encoding sleep, while leaving hippocampal function undisturbed during the encoding and retrieval of NOR memory.

As previously mentioned, sleep is known to support memory consolidation. However, it remains elusive which basic sleep feature (sleep duration or sleep depth) is the most important factor for long-term memory formation. Does memory formation benefit more from a longer sleep duration? or is the depth of sleep more essential for long-term memory consolidation? The aim of the second study was to dissociate effects of duration and depth of sleep on the formation of recent and remote hippocampus-dependent OPR memory. In this study, the depth of sleep was manipulated by extending the time rats spent in sleeping environment. The electroencephalography (EEG) and local field potentials (LFPs) in the hippocampus were recorded to probe the efficacy of the sleep manipulation.

The last study investigated the effect of sleep on memory for the rat's conspecific (i.e. social recognition memory). Similar to object recognition memory, rodents exhibit an innate exploration preference to novel conspecifics. While humans rely heavily on visual and auditory cues for social recognition, chemosensory stimuli are essential for encoding social information in rodents. This study aimed at dissociating interactions between the consolidation of "non-spatial" social information and of spatial memory during sleep. Accordingly, social recognition memory was tested in a modified radial arm maze to unveil the associations between social recognition memory and spatial memory and to examine effects of sleep on consolidation of these two components of episodic memory.

2. Results

2.1) Study 1: The hippocampus is crucial for forming non-hippocampal long-term memory during sleep

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LETTER

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The hippocampus is crucial for forming non-hippocampal long-term memory during sleep

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There is a long-standing division in memory research between hippocampus-dependent memory and non-hippocampus-dependent memory, as only the latter can be acquired and retrieved in the absence of normal hippocampal function[1,2]. Consolidation of hippocampus-dependent memory, in particular, is strongly supported by sleep[3–5]. Here we show that the formation of long-term representations in a rat model of non-hippocampus-dependent memory depends not only on sleep but also on activation of a hippocampus-dependent mechanism during sleep. Rats encoded non-hippocampus-dependent (novel-object recognition[6–8]) and hippocampus-dependent (object–place recognition) memories before a two-hour period of sleep or wakefulness. Memory was tested either immediately thereafter or remotely (after one or three weeks). Whereas object–place recognition memory was stronger for rats that had slept after encoding (rather than being awake) at both immediate and remote testing, novel-object recognition memory profited from sleep only three weeks after encoding, at which point it was preserved in rats that had slept after encoding but not in those that had been awake. Notably, inactivation of the hippocampus during post-encoding sleep by intrahippocampal injection of muscimol abolished the sleep-induced enhancement of remote novel-object recognition memory. By contrast, muscimol injection before remote retrieval or memory encoding had no effect on test performance, confirming that the encoding and retrieval of novel-object recognition memory are hippocampus-independent. Remote novel-object recognition memory was associated with spindle activity during post-encoding slow-wave sleep, consistent with the view that neuronal memory replay during slow-wave sleep contributes to long-term memory formation. Our results indicate that the hippocampus has an important role in long-term consolidation during sleep even for memories that have previously been considered hippocampus-independent.

Since the description of the patient H.M., who underwent bilateral removal of large portions of the hippocampus and suffered from severe anterograde amnesia, the distinction between hippocampus-dependent and non-hippocampus-dependent forms of memory has been widely accepted[1,2,9]. Encoding and retrieval of hippocampus-dependent memories require the hippocampus, whereas this is not the case for non-hippocampus-dependent memory, which is otherwise comprised of rather heterogeneous kinds of memory (motor skills, cue fear conditioning and so on). The 'standard consolidation theory' and recent advances[2,9,10] assume that memory of episodes, and in particular the relations among their elements, are initially encoded into hippocampal networks, but that during consolidation the representations are redistributed over days, weeks, and months to neocortical networks that serve as long-term stores. In this way these memories may become independent of the hippocampus[9,11].

Sleep is known to support memory consolidation[3–5]. Sleep after memory encoding robustly enhances hippocampus-dependent memory, although there is also evidence that sleep enhances non-hippocampus-dependent forms of memory[12]. With regard to hippocampus-dependent memory, an active systems consolidation process has been proposed[10,13,14] on the basis of findings that neural representations of freshly encoded memories are replayed during subsequent slow-wave sleep (SWS)[15,16]. The neural replay originating from hippocampal networks, together with sharp-wave ripples and thalamic spindles, is likely to promote the transmission of memory information and, with repetitive occurrence, the gradual redistribution of the representation towards extrahippocampal networks[17,18].

The ability of sleep to consolidate non-hippocampus-dependent memory is less well understood[4,19,20]. Here, we compare the effects of post-encoding sleep with those of post-encoding wakefulness on consolidation of non-hippocampus-dependent and hippocampus-dependent forms of memory in rats, and examine the temporal evolution of consolidation effects. We used the novel-object recognition (NOR) task and an object–place recognition (OPR) task as tests of non-hippocampus-dependent and hippocampus-dependent memory, respectively (Fig. 1a). Performance on the NOR task relies on the perirhinal cortex, but normal hippocampal function is not necessary for encoding and retrieving NOR memory in rats[6–8,21].

After task encoding, rats either slept or remained awake during a 2-h interval. Retrieval was tested either immediately after the 2-h interval (recent test) or, in order to test long-term memory, 1 week or (for NOR only) 3 weeks later (remote tests; Fig. 1a). At the recent memory test, NOR memory did not differ between the sleep and wake conditions ($P = 0.43$), and exploration discrimination ratios indicated that there was significant NOR memory in both conditions ($P < 0.045$, Fig. 1b). By contrast, OPR memory at the recent test was enhanced in the sleep compared to the wake condition ($P = 0.034$) and was itself significant only after sleep ($P = 0.044$) and not in the wake condition ($P = 0.49$, $F_{1,20} = 4.70$, $P = 0.043$ for NOR/OPR × sleep/wake analysis of variance (ANOVA) interaction). That sleep benefits recent OPR but not NOR memory confirms previous findings in rats[22,23], and has been taken as evidence that sleep preferentially strengthens hippocampus-dependent memory. Total object exploration, total distance travelled and mean speed at retrieval were comparable between sleep and wake conditions (all $P > 0.194$, Extended Data Fig. 1a), excluding confounds by nonspecific changes, for example, in locomotion or motivation.

At the remote test performed after 1 week, NOR memory still did not differ between the sleep and wake conditions ($P = 0.45$), and in both conditions rats showed significant NOR memory ($P < 0.045$). Also, as at the recent test, at the 1-week test rats showed better OPR memory in the sleep than the wake condition ($P = 0.001$), and OPR memory was not significant in the wake condition ($P > 0.308$, $F_{1,15} = 17.26$, $P = 0.001$ for NOR/OPR × sleep/wake interaction; Fig. 1b).

NOR memory faded only when the retrieval delay was extended to 3 weeks. After 3 weeks, rats in the sleep condition but not in the wake condition showed significant NOR memory, and performance was significantly better for rats that had slept after encoding than for rats that had not ($P = 0.031$, $F_{1,17} = 4.696$, $P = 0.045$ for 1/3 weeks × sleep/wake interaction in analysis of NOR data). A supplementary experiment indicated that the decrease in NOR memory after post-encoding

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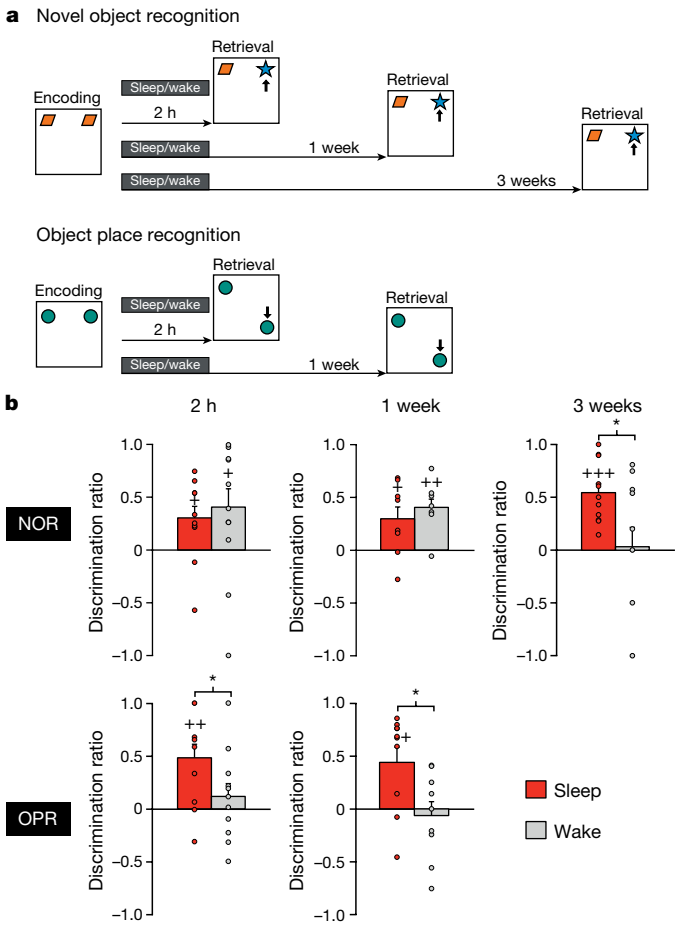

Fig. 1 | Effects of post-encoding sleep versus wakefulness on memory in the NOR and OPR tasks. a, During the encoding phase of both tasks, the rats explored (for 10 min) two identical objects in an arena. Encoding was followed by a 2-h interval in which the rat either slept or remained awake. Retrieval was tested immediately after the 2-h post-encoding interval (recent memory) and 1 week and (for NOR only) 3 weeks later (remote memory). At the retrieval test, the rat explored the arena for 5 min. To test NOR retrieval, one of the two objects (from the encoding phase) was replaced by a novel object (arrow); recognition memory was indicated when the rat spent more time exploring the novel object than the familiar object (discrimination ratio), with exploration during the first minute being most sensitive to exploration of novelty[6]. To test OPR retrieval, one of the objects was displaced (relative to its location at encoding, arrow) and memory for the place was indicated when the rat spent more time exploring the displaced object than the stationary object (which had not moved). **b**, Mean + s.e.m. discrimination ratios during the first minute of exploration for NOR and OPR at the recent (2 h) and remote (1 or 3 weeks) retrieval tests (dot plots overlaid). NOR memory benefited from post-encoding sleep (red bars; compared with wake, grey) only at the 3-week retrieval test, when NOR memory had decayed in the wake condition. By contrast, OPR memory benefited from sleep at both recent and remote testing. $n = 12$, 8 and 11 rats for NOR at 2 h, 1 week and 3 weeks; $n = 11$ and 9 rats for OPR at 2 h and 1 week, respectively. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample t-tests against chance level; $^{*}P < 0.05$ for pairwise t-tests (two-sided) between sleep and wake (see Extended Data Fig. 2 for discrimination ratios during the entire retrieval phase).

wakefulness had already occurred 1 week earlier, at a 2-week retrieval test (Extended Data Fig. 2c). Overall, remote testing confirmed that NOR memory was maintained over time periods of up to one week, even if encoding is followed by a wake period[24]. However, the formation of more persistent long-term NOR memory requires sleep after encoding, with the sleep effect emerging only after 2–3 weeks, which corresponds to the time required for NOR memory in the wake condition to fade.

The consolidating effect of sleep on hippocampus-dependent spatial memory is mediated by repeated reactivations of the newly encoded hippocampal representations during subsequent SWS[15,16,25]. Moreover, hippocampus-dependent and non-hippocampus-dependent memory systems have been found to interact during consolidation[12,26]. Thus, we investigated whether hippocampal activity also critically contributes to the consolidation of non-hippocampus-dependent memory by reversibly inactivating hippocampal function by infusing muscimol into the dorsal hippocampus during sleep after encoding the NOR task.

At remote retrieval testing 3 weeks later, rats who had received muscimol injection into the hippocampus during sleep after learning did not show significant NOR memory ($P = 0.38$), whereas remote NOR memory was preserved in those injected with vehicle at the same time point ($P = 0.001$; $F_{1,14} = 8.99$, $P = 0.01$, for muscimol/vehicle main effect, Fig. 2a). Control parameters such as total object exploration did not differ between conditions, excluding nonspecific changes in motivation or vigilance (Extended Data Fig. 1). This result demonstrates that the hippocampus is crucial for the formation of persistent NOR memory during sleep. Previous studies that suppressed hippocampal activity after encoding in the NOR task had conflicting results[8,27–29], which fuelled a long-standing debate about the possible hippocampal dependency of NOR memory[21,30,31]. These discrepancies can be resolved by our results, which show that formation of persistent long-term NOR memory relies on a hippocampal mechanism that is specifically active during sleep.

To determine whether the hippocampus is specifically involved in sleep consolidation, in a control experiment hippocampi were inactivated during a 2-h post-encoding wake period and retrieval was tested 1 week later. In these rats, NOR memory tended to be enhanced when compared to control animals whose hippocampal function was intact during the wake period after encoding ($F_{1,13} = 4.492$, $P = 0.054$ for muscimol/control main effect; Fig. 2b and Extended Data Fig. 3b), suggesting that, during wakefulness, hippocampal activity normally interferes with NOR memory consolidation[8]. Overall, these results corroborate the notion that persistent long-term NOR memory formation relies on a hippocampal mechanism that is specifically active during sleep, whereas non-hippocampal mechanisms during post-encoding wakefulness enable NOR memory over a period of 1 week.

We investigated whether the hippocampus would also be required for retrieval of long-term NOR memory at 3 weeks. Hippocampal infusion of muscimol before the 3-week retrieval test (in rats that had slept for 2 h after encoding) did not abolish NOR memory, with the rats' performance being closely comparable to that of a vehicle-infused group ($P > 0.70$ for all comparisons, Fig. 2a). This result indicates that whereas the formation of long-term NOR memory during sleep requires the hippocampus, its retrieval is not dependent on hippocampal function. In two further control experiments, muscimol was infused either shortly before a retrieval test that took place 30 min after encoding, or shortly before the encoding phase, with retrieval tested 30 min later (Fig. 2c). The experiments confirmed that short-term retrieval of NOR memory and encoding per se likewise do not depend on hippocampal function ($P = 0.46$ and $P = 0.79$, respectively, for differences between vehicle and muscimol)[6,7]. Together, these results indicate that whereas the sleep-dependent formation of persistent long-term NOR memory requires the hippocampus, the retrieval of these memories is non-hippocampus-dependent at any time after encoding.

The architecture of post-encoding sleep was comparable to that reported in previous studies[23] (Extended Data Table 1). Correlation analyses revealed that remote NOR memory retrieval was strongly associated with measures of spindle activity during SWS, but not with rapid eye movement (REM)-sleep-related measures (Extended Data Table 2). Thus, NOR discrimination ratios at the 3-week retrieval test correlated with the number ($r = 0.719$, $P = 0.029$) and duration of spindles ($r = 0.705$, $P = 0.034$, Fig. 3a), with the latter correlation being most robust in an exploratory analysis focusing on the first 30 min of post-encoding sleep ($r = 0.888$, $P = 0.001$) in which neuronal replay in hippocampal networks, as a possible consolidation mechanism, is typically strongest[32] (see Extended Data Fig. 4 for related OPR data).

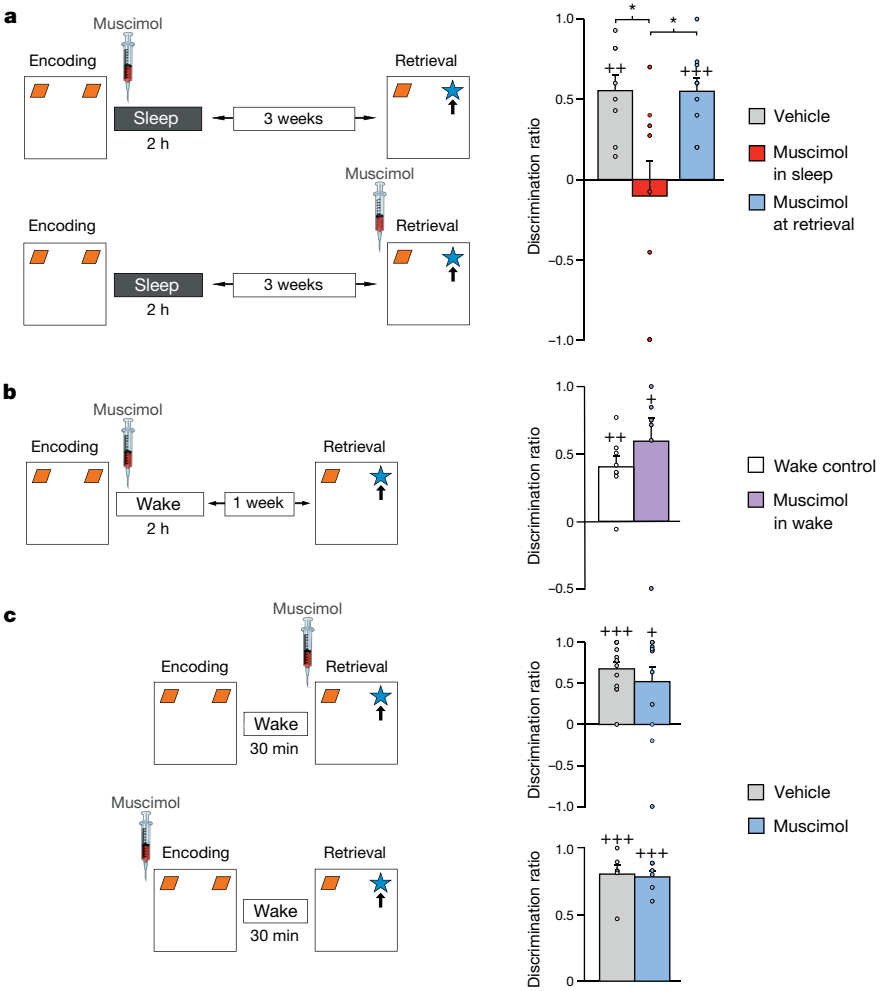

Fig. 2 | Effects of reversibly inactivating the hippocampus on NOR memory. Left, procedures; right, mean + s.e.m. discrimination ratios, with overlaid dot plots. **a**, To suppress hippocampal activity, muscimol was bilaterally infused (over 2 min) into the dorsal hippocampus, either during the post-encoding interval upon the first occurrence of continuous SWS (top; $n = 8$ rats each for muscimol and vehicle), or 15 min before remote retrieval testing 3 weeks after encoding (bottom; $n = 9$ rats). Hippocampal inactivation during post-encoding sleep (red bar) abolished remote NOR memory whereas inactivation before retrieval testing (blue bar) was ineffective. Grey bar, vehicle injection. **b**, Muscimol (purple bar) was infused shortly after encoding while the rats remained awake during the 2-h post-encoding interval ($n = 7$ rats). Retrieval was tested 1 week later. Compared with untreated wake control rats ($n = 8$ rats, empty bar), which had intact hippocampal function and stayed awake during the post-encoding interval, hippocampal inactivation did not disturb but rather tended to enhance NOR performance. Timing (with reference to encoding), dosage and procedures of muscimol infusion were the same as in **a**. **c**, Muscimol (or vehicle) was infused 15 min before retrieval testing of recent NOR memory (top, $n = 12$ rats each) or 15 min before the encoding phase (bottom, $n = 6$ rats each). Retrieval was tested 30 min after encoding (rats stayed awake during this interval). Hippocampal inactivation does not affect retrieval of recent NOR memory either during retrieval or during encoding. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample *t*-test against chance level; $^{*}P < 0.05$ for pairwise tests (two-sided) between conditions.

Intrahippocampal injection of muscimol during post-encoding sleep reduced electroencephalogram (EEG) theta activity ($P = 0.014$), which is thought to be generated in septal–hippocampal circuitry, and accordingly reduced time spent in both REM (0.83 ± 0.83 versus 6.04 ± 1.07 min after vehicle) and preREM sleep (1.97 ± 0.51 versus 5.80 ± 0.70 min after vehicle, both $P < 0.003$). Muscimol did not influence surface EEG activity during SWS (all $P > 0.410$, Extended Data Table 1). However, intrahippocampal local field potential (LFP) recordings from additional rats showed a distinct reduction in the number and density of hippocampal ripples, hippocampal spindle power and slow oscillation amplitude following muscimol infusion during post-encoding sleep ($P = 0.005$, 0.025, 0.013, and 0.007, respectively; Fig. 3b). These changes are consistent with the view that muscimol prevents formation of long-term NOR memory by suppressing hippocampal ripples and associated reactivation of representations during SWS[33], although our findings do not rule out contributions of REM-sleep-related mechanisms[34].

There is ample evidence that the hippocampus is involved in the consolidation of memory classified as hippocampus-dependent, as it can be acquired and retrieved only with normal hippocampal function[9]. We have now shown that normal hippocampal function is also required for the formation of persistent long-term representations on a task that, based on the same criterion, is classified as non-hippocampus-dependent[6,7]. How does the hippocampus contribute to the formation of long-term NOR memory? In the NOR task, representation of the object resides mainly in the perirhinal cortex, whereas the hippocampus encodes spatial context features[7,35]. Accordingly, retrieval in the NOR task also involves hippocampal function—making the task seemingly hippocampus-dependent—when it is performed in a context that is novel to the rat[8,36]. Along this line, we propose that, during sleep, the hippocampus is likely to boost object representation through activation of context-related representations, rather than directly enhancing perirhinal object memory. The observed correlation of long-term NOR performance with post-encoding sleep spindle activity corroborates this view: neuronal reactivations of spatial context representations during sleep occur in the hippocampus, in conjunction with ripples and thalamic spindles[25,32]. Spindles, moreover,

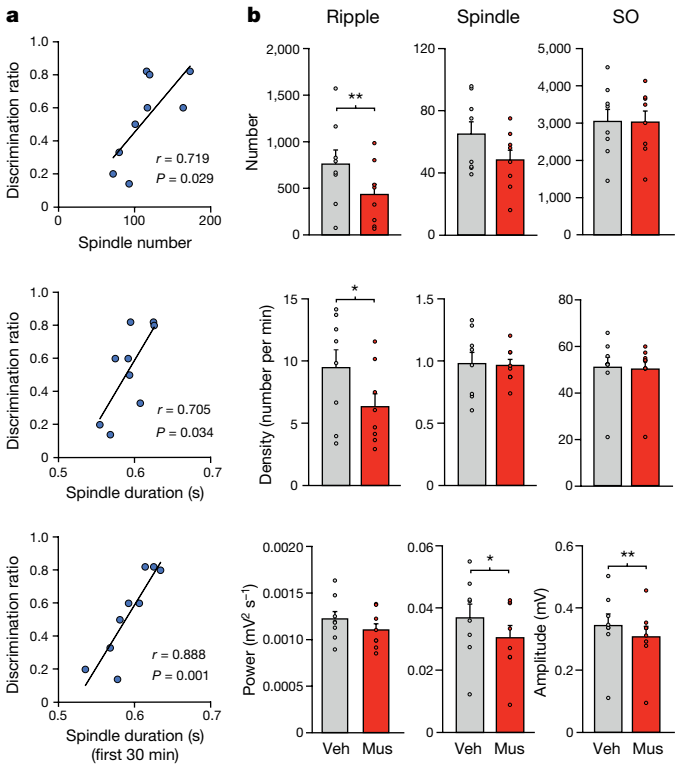

Fig. 3 | Contribution of post-encoding slow wave sleep to remote NOR memory. a, NOR performance (discrimination ratio) at the 3-week retrieval test was correlated with the number of sleep spindles during SWS (top) and spindle mean duration during the 2-h post-encoding interval (middle), as well as with spindle mean duration during the first 30 min of post-encoding sleep (bottom; Pearson's product–moment correlations, $n = 9$ rats). **b**, Intrahippocampal LFPs were recorded in additional rats to examine the effects of bilateral intrahippocampal infusion of muscimol (Mus) (versus vehicle, Veh) on (from left to right) ripples, spindles, and slow oscillations (SO) in hippocampal networks during SWS ($n = 8$ tests per condition). Muscimol decreased the total number and density of ripples, as well as spindle power and slow oscillation amplitude. Data shown as mean + s.e.m. with overlaid dot plots. $^{**}P < 0.01$, $^{*}P < 0.05$ for pairwise two-sided t-tests.

have been identified as a mechanism that favours the spreading of reactivations to extrahippocampal networks[17,37], thereby promoting plastic synaptic changes that can ultimately strengthen these extrahippocampal representations[18,38].

In conclusion, our findings suggest that a common hippocampal mechanism boosts consolidation in both hippocampus-dependent and non-hippocampus-dependent memory systems through the reactivation of contextual features. Indeed, in humans, hippocampal activity during training predicts sleep-dependent consolidation of a motor skill that is considered to be non-hippocampus-dependent[12,26]. From this perspective, the formation of long-term representations during sleep, whether hippocampus-dependent or not, critically depends on their being encoded within a spatiotemporal context—that is, as episodic memories. Because such a mechanism puts the hippocampus-dependent episodic memory system into a supra-ordinate position to organize long-term memory, it has strong implications for current theorizing about interacting 'parallel memory systems'[39]. However, non-hippocampus-dependent memory is heterogeneous, and other memories of this kind need to be studied to scrutinize the proposed general hippocampal mechanism of long-term memory formation.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0716-8.

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METHODS

Animals. Ninety-one adult male Long Evans rats (Janvier, 260–310 g, 10–12 weeks) were used for the experiments. Rats were housed in groups of 2–4 rats per cage, except during the post-surgery recovery period, when they were kept individually on a 12-h light/12-h dark cycle (lights on at 06:00), and had unrestricted access to water and food throughout the experiments. 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 Baden-Württemberg state authority.

No statistical methods were used to predetermine sample size. In all experiments, rats were randomly assigned to experimental groups and conditions before the experiment. The experimenters were not blinded to the experimental group or condition during data collection. However, all behavioural and electrophysiological recordings were analysed offline, with the experimenters blinded to the experimental groups and conditions.

Design and general procedures. Different groups of rats were tested on either the NOR task or the OPR task, using post-encoding retention intervals of 2 h, 1 week and, only for the NOR task, of 3 weeks. Each group of animals was tested on a sleep condition (allowed to sleep during the 2-h post-encoding interval) and a wake condition (stayed awake during this interval). The order of sleep and wake conditions was counterbalanced across animals of a group. For an individual rat, the conditions were separated by an interval that was at least 2 weeks and twice as long as the tested retention interval. Encoding and the subsequent 2-h post-encoding interval took place in both the sleep and wake conditions during the animal's rest phase (between 08:00 and 13:00). In the sleep condition, during the 2-h post-encoding interval, the animals were left undisturbed in a 'post-encoding' box (35 × 35 cm, height: 45 cm) that was made of plastic and contained some bedding materials. Sleep was assessed using video recorded behaviour using standard procedures (see below). In the wake condition, wakefulness was enforced using gentle handling[22,23]. This procedure minimizes stress and confounding influences of locomotion. It involved tapping on the retention box and, if necessary, gently shaking the box. No intense stimulation was used, and video records ensured that signs of startle or freezing behaviour did not occur. In the remote groups tested after 1 and 3 weeks, animals were brought to their home cages after the 2-h post-encoding interval and kept under routine conditions until testing.

Habituation and memory tasks. After handling daily for five consecutive days for 5–10 min, the rats were brought into the test room once every day on three consecutive days for a habituation session. For object familiarization, the rat was placed into an empty cage with an object (not used for the experiments) positioned in the centre of the cage. The rat was allowed to freely explore the object for 10 min. For arena familiarization, the rat was then placed into an empty open field, facing a different wall of the open field at each session to facilitate allocentric navigation, and allowed to explore for 10 min. Immediately afterwards, the rat was left undisturbed in the post-encoding box for 2 h.

On the day after the habituation phase, the experiment started with the encoding phase of the memory task. The encoding phase was identical for the NOR and OPR task, and comprised a 10-min interval during which the rats were allowed to explore two identical objects in the open field. For testing retrieval on the NOR task, one of the two objects of the encoding phase was replaced by a novel object. For testing retrieval on the OPR task, one of the two objects of the encoding phase was moved to a different location. At each test, the rat had 5 min to explore the arena.

The tasks were performed in a room with a noise-generator providing masking noise. The open field (80 cm × 80 cm, height of walls: 40 cm) was made of grey PVC. Through the open upper side of the arena the rat could perceive distal cues (two rectangles at the north wall, two other rectangles at the east wall, and a square at the west wall). Objects for exploration were made of glass, with different colours and shapes, and heavy enough not to be moved by the rat (height: 15–30 cm; base diameter: 7–12 cm). They were positioned at least 10 cm equidistant from the walls to ensure that the animal's preference to stay in corners did not bias exploration times. Pilot studies ensured that the rats could discriminate among the different objects and did not show any preference for one of the objects. The locations of objects during the encoding and retrieval phases were randomized across rats. Each rat's exploration behaviour was monitored by a video camera and analysed offline by an experienced researcher using ANY-maze software (Stoelting Europe). After each phase, the apparatus and objects were cleaned with water containing 70% ethanol.

Inactivating the hippocampus during sleep. To reversibly inactivate the dorsal hippocampus during sleep, we infused the GABA-A receptor agonist muscimol, according to standardized procedures[8,40]. After 5 days of handling, guide cannulae were surgically implanted bilaterally into the dorsal hippocampi, and at least 8 days were allowed for recovery. Muscimol (Sigma, 0.5 μg dissolved in 0.5 μl saline solution, per hemisphere) or an equivalent volume of vehicle (saline solution) was infused bilaterally over 2 min by an automated syringe pump. (In pilot studies with this dosing, no spread of the substance to extrahippocampal regions occurred; Extended Data Fig. 5b.) For substance administration, two 30-gauge injection cannulae were connected to two 10-μl Hamilton microsyringes (Hamilton), with 1-m polyethylene cannula tubing. The injection cannulae protruded 1 mm beyond the tip of the guide cannulae. The injection cannulae were kept in the bilateral guide cannulae for a further 2 min to prevent backflow. The procedure enabled substance administration into freely moving rats without disturbing ongoing sleep. Rats were killed at the end of the experiments for histological confirmation of the infusion sites (Extended Data Fig. 5).

The effects of muscimol and vehicle were compared in a between-subjects comparison in 16 rats (8 per group). To test the effects of hippocampal inactivation during sleep in the 2-h post-encoding interval, substance administration started immediately upon (visual) online detection of continuous SWS for at least 10 s. On average, substance administration took place after 38.30 ± 2.16 min of the post-encoding interval in the muscimol condition and after 40.35 ± 1.33 min in the vehicle condition ($P = 0.42$).

Surgery in experiments with reversible inactivation of the hippocampus. Guide cannulae were implanted under general isoflurane anaesthesia (induction: 1–2%, maintenance: 0.8–1.2% in 0.35 l/min O_2). Preoperatively, fentanyl (0.005 mg/kg), midazolam (2 mg/kg) and medetomidine (0.15 mg/kg) were administered intraperitoneally. Rats were placed in the stereotaxic frame and the skull was exposed. Two stainless steel guide cannulae (7 mm long, 23 gauge, Plastics One) were bilaterally implanted into the dorsal hippocampi (anterior–posterior (AP): −4.3 mm, mediolateral (ML): ±2.8 mm, dorsoventral (DV): −1.3 mm under skull surface, relative to bregma). The cannulae were introduced to this position laterally tilted by 9° with respect to the vertical axis and were affixed to the skull with four bone screws and cold polymerizing dental resin. Dummy cannulae (7 mm long, Plastics One) were inserted into the guide cannulae and removed only for infusions.

For simultaneous EEG recordings in the animals, four screw electrodes were implanted: two frontal electrodes (AP: +2.6 mm, ML: ±1.8 mm, relative to bregma) and two occipital electrodes (AP: −10.0 mm, ML: ±1.8 mm), with the latter serving (for all recordings) as reference and ground, respectively. Additionally, in a subgroup of animals, two platinum electrodes were attached to the guide cannulae to record hippocampal LFP signals (AP: −4.3 mm, ML: ±2.8 mm, DV: −2.3 mm, relative to bregma). Two stainless steel wire electrodes were implanted bilaterally in the neck muscles for electromyography (EMG) recordings. Electrodes were connected to a Mill-Max pedestal and fixed to the skull with cold polymerizing dental resin and the wound was sutured. After the surgery, the rats received a subcutaneous 1-ml injection of saline solution to prevent dehydration, and carprofen (5 mg/kg). Rats were allowed to recover for at least 8 days.

Correct placement of the cannulae and of electrodes for LFP recordings was confirmed by histology after completion of the experiments. For this, the rats were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde (PFA). After decapitation, the brains were removed and immersed in the 4% PFA for at least two days. Coronal sections of 50–70 μm were cut on a vibratome, stained with toluidine blue and examined under a light microscope (Extended Data Fig. 5).

Analysis of memory performance. Exploration was defined by the rat being within 2 cm of an object, directing its nose towards the object and engaging in active exploration behaviours such as sniffing. For each task, the time a rat spent exploring each object during the retrieval test was converted into a discrimination ratio according to the general formula: (time spent at novel − time spent at familiar)/(time spent at novel + time spent at familiar), where 'novel' on the NOR task refers to the novel object and on the OPR task refers to the displaced object. A value of zero indicates no exploration preference, whereas a positive value indicates preferential exploration of the novel configuration, thus indicating memory of the familiar configuration. Additionally, the total time of object exploration (across both objects), distance travelled and mean speed on each task were determined. Statistical comparisons concentrated on cumulative discrimination ratios for the first 1 min and 3 min of the retrieval phase.

Analysis of sleep, EEG, and hippocampal LFP recordings. Sleep during the retention interval was assessed using video recordings and tracking software (ANY-Maze, Stoelting Europe) using standard visual procedures[41]. In brief, 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. The agreement of the procedure with EEG-based scoring of sleep in the present (see below) and previous studies was >92%[22,41]. Scores indicated an average of 46.97 ± 2.86 min spent asleep during the 2-h post-encoding retention interval, with the first bout of sleep occurring 41.24 ± 2.99 min after the encoding phase. There were no significant differences in sleep parameters between NOR and OPR task conditions or retention intervals tested (Extended Data Table 1).

In the experiments testing the effects of reversible inactivation of the hippocampus, sleep was additionally analysed using EEG and EMG recordings. For the recordings, electrodes were connected through a preamplifier headstage (Model HS-18MM, Neuralynx) to a Digital Lynx SX acquisition system (Neuralynx),

amplified, filtered (EEG: 0.01–300.0 Hz; EMG: 30.0–300.0 Hz), and sampled at a rate of 1,000 Hz. Sleep stages (SWS, preREM and REM sleep) and wakefulness were scored offline by visual inspection using 10-s epochs according to standard criteria[42]. In brief, 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 (4.0–8.0 Hz), phasic muscle twitches and minimal EMG activity. PreREM sleep was identified by a decrease in delta activity, a progressive increase in theta activity and the presence of sleep spindles (10.0–16.0 Hz). Sleep stage classification was performed by an experienced experimenter.

EEG signals in these experiments were also used to identify slow oscillations and spindles during SWS. Identification of slow oscillations followed procedures as described[17]. In brief, the EEG signal during all SWS epochs for an animal was filtered between 0.3 and 4.5 Hz. A slow oscillation event was then identified 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, 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. These criteria resulted in the detection of slow oscillations with negative peak amplitudes exceeding −80 μV and peak-to-peak amplitudes exceeding 120 μV. For spindle detection, the EEG signal was filtered between 10.0 and 16.0 Hz. The Hilbert transform was calculated for the filtered signal and smoothed with a moving average (window size 200 ms). A spindle was identified when the absolute value of the transformed signal exceeded 1.5 s.d. of the mean signal during the animal's SWS epochs, for at least 0.4 s and not more than 2.0 s.

The same procedures were applied to identify slow oscillations and spindles in the hippocampal LFP recordings. To identify ripples in these LFP recordings, the signal was filtered between 150.0 and 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 s.d. 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.

Statistical analyses. Statistical analyses were performed using SPSS 21.0 for Windows. To evaluate the discrimination ratios determined for each task, we used ANOVAs that included group factors for the task (NOR/OPR) or the retention interval (1/3 weeks), and repeated-measures factors representing the sleep/wake conditions and discrimination ratios after 1 and 3 min of the retrieval phase. (ANOVAs separately run on 1-min and 3-min values yielded almost identical results and are not reported here.) Muscimol/vehicle comparisons were introduced as group or repeated-measures factors, depending on the experiment. ANOVAs indicating significance for main or interaction effects of interest were followed by post hoc *t*-tests (two-sided). Discrimination ratios were also compared with chance level performance (zero) using one-sample *t*-tests. To analyse the relationship between post-encoding retention sleep and memory performance, Pearson product–moment correlation coefficients were calculated. $P < 0.05$ was considered significant.

Code availability. The codes used in this study are available from the corresponding authors on reasonable request. MATLAB scripts used for analyses of EEG and LFP signals are available at https://github.com/MedPsych/LongTermMemory_Sleep.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The data that support the findings of this study are available from the corresponding authors on reasonable request. Source Data for graphs shown in Figs. 1–3 and Extended Data Figs. 1–4 are available in the online version of the paper.

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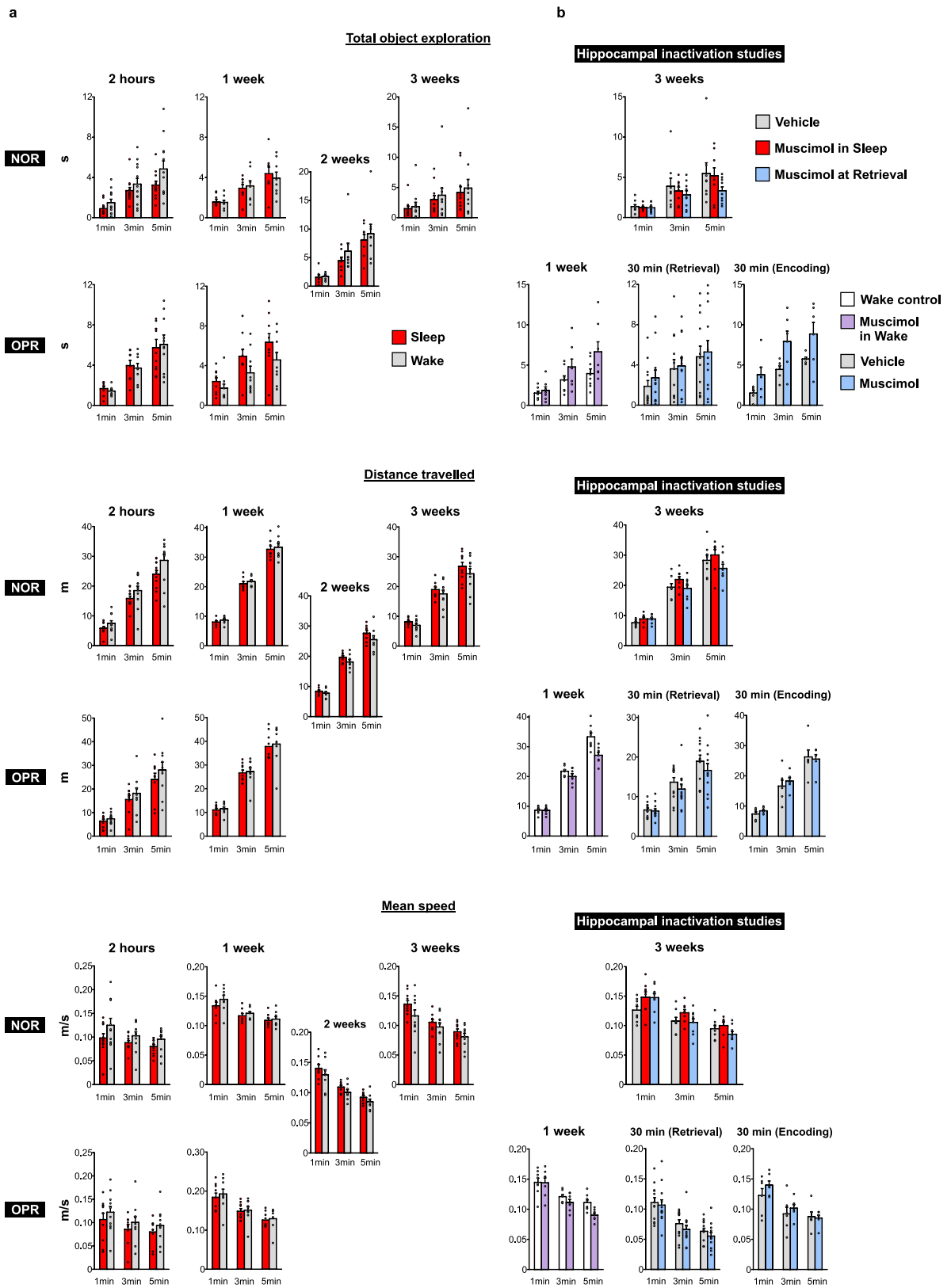
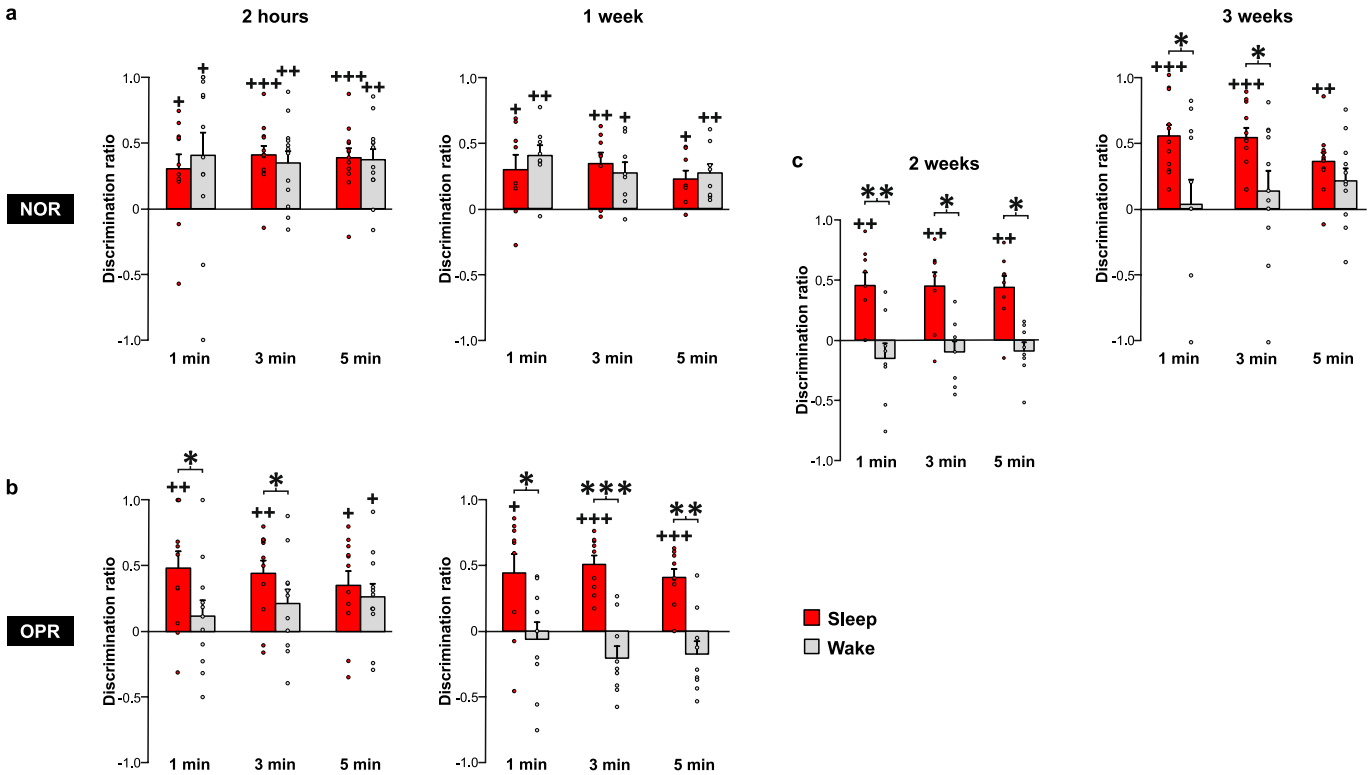
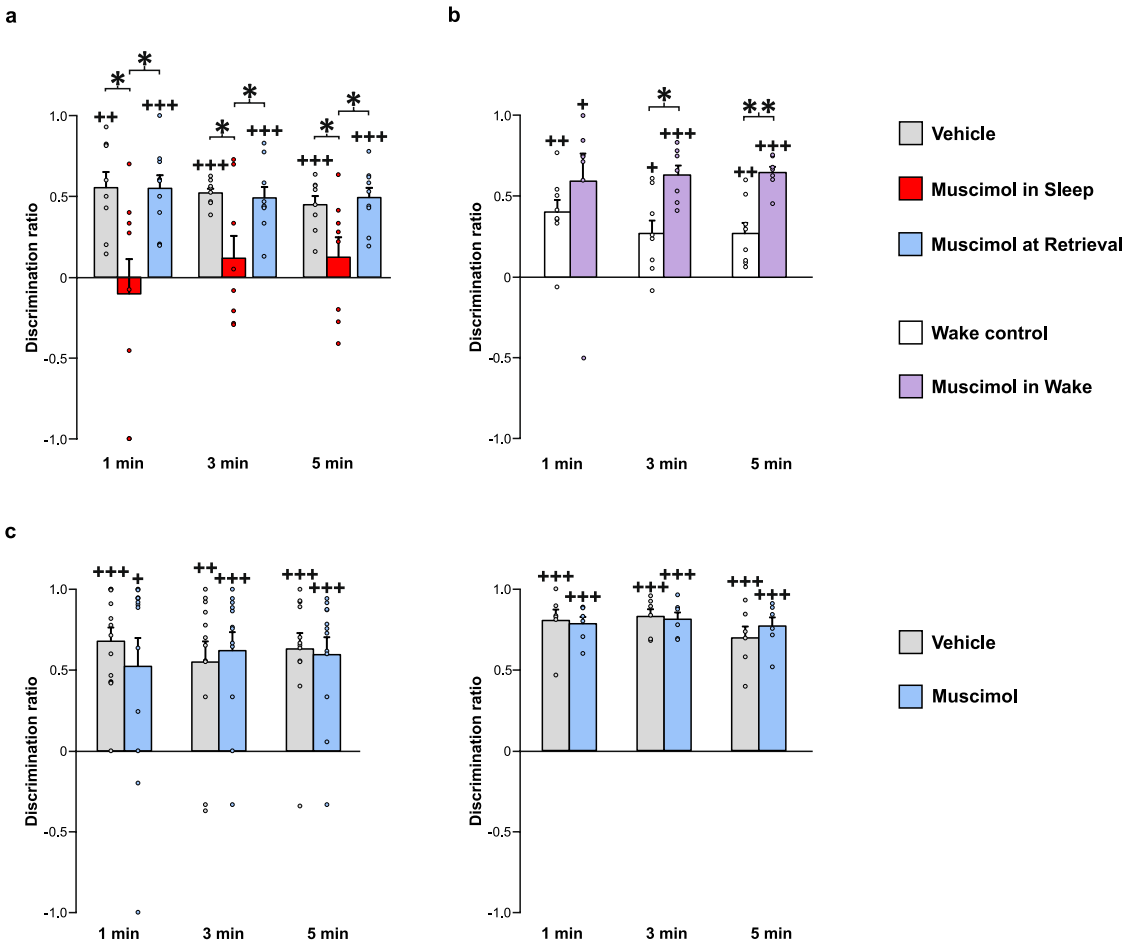


Extended Data Fig. 1 | See next page for caption.

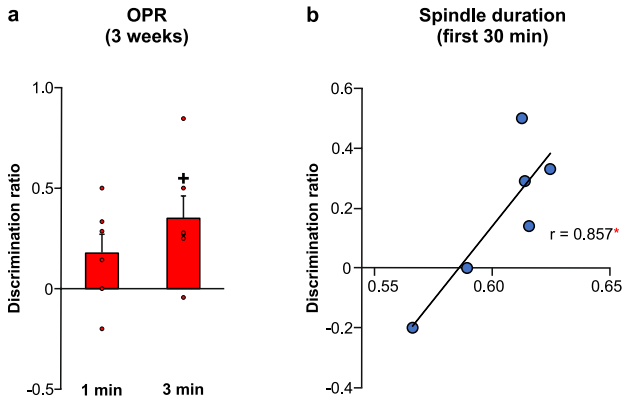
Extended Data Fig. 1 | Control measures for NOR and OPR task performance. Total object exploration (s), total distance travelled (m) and average speed (m s^{-1}) at retrieval testing. Mean values (± s.e.m., dot plots overlaid) for the first 1 and 3 min and for the entire 5 min of the retrieval phase are shown. **a**, Results from main experiments of NOR and OPR memory as illustrated in Fig. 1. Retrieval was tested either immediately after the 2-h retention interval (recent) or 1 week or (for the NOR task only) 3 weeks later (remote). In a supplementary experiment, NOR was tested 2 weeks after encoding (offset downwards). Red, sleep; grey, wake; $n = 12$, 8, 8 and 11 rats for NOR testing after 2 h and 1, 2 and 3 weeks, and $n = 11$ and 9 rats for OPR testing after 2 h and 1 week, respectively. **b**, Results from experiments after bilateral intrahippocampal infusion of muscimol as in Fig. 2. Top, muscimol (versus vehicle, grey bars, $n = 8$ rats) was infused either during the 2-h post-encoding interval (upon first occurrence of SWS, red bars, $n = 8$ rats) or 15 min before retrieval (blue bars, $n = 9$ rats) with the retrieval phase taking place 3 weeks after encoding. Bottom, control studies. Left, muscimol (purple, $n = 7$ rats) was infused shortly after encoding while the rats remained awake during the 2-h post-encoding interval, compared with untreated wake control rats ($n = 8$ rats, empty bars). Retrieval was tested 1 week after encoding (corresponding to Fig. 2b). Right, muscimol (blue bars, versus vehicle, grey bars) was infused either 15 min before retrieval testing ($n = 12$ rats) or 15 min before encoding ($n = 6$ rats) with the retrieval phase taking place 30 min after encoding (corresponding to Fig. 2c). There were no significant differences between sleep and wake or between muscimol and vehicle conditions ($P > 0.194$, for all comparisons based on ANOVA and two-sided post hoc t-tests, see Methods and Figs. 1, 2 for further details).



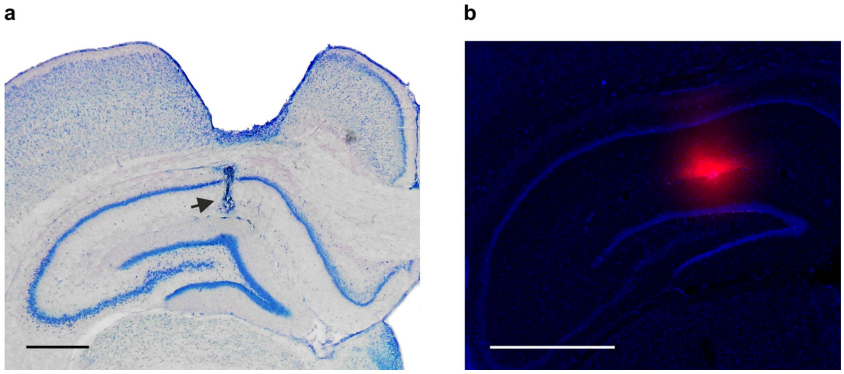
Extended Data Fig. 2 | Performance in recent and remote tests for NOR and OPR tasks. Memory is indicated by mean ± s.e.m. discrimination ratios during the first 1 min, first 3 min, and entire 5 min of the retrieval phase on the NOR and OPR tasks (dot plots overlaid). **a**, NOR was tested with 2-h (recent) and with 1-week and 3-week (remote) retrieval tests. **b**, OPR was tested with 2-h (recent) and 1-week retrieval tests. Whereas OPR memory benefited from sleep (red bars; compared to wake, grey) at both recent and remote (1 week) retrieval tests, NOR benefited from sleep only at the 3-week retrieval test, when NOR memory had decayed in the wake condition. **c**, A supplementary experiment with NOR retrieval tested 2 weeks after post-encoding sleep and wake intervals showed that NOR memory in the wake condition had already faded at this 2-week point, whereas it was preserved in the sleep condition ($F_{1,7} = 14.997$, $P = 0.006$, for sleep/wake main effect; $F_{1,14} = 18.151$, $P = 0.01$ and $F_{1,14} = 0.82$, $P = 0.382$, for 1 versus 2-week comparisons in the wake and sleep conditions, respectively, $F_{1,14} = 12.073$, $P = 0.005$, for 1/2 weeks × sleep/wake interaction; $P > 0.222$ for all comparisons between 2- and 3-week retrieval). In all experiments, recognition memory was assessed by the discrimination ratios during the first 1 and first 3 min of the retrieval period, which typically cover exploration of novelty most sensitively on both the NOR and OPR tasks[6,43–45]. With extended exploration periods, the novelty response often decreases and is thought to become more noisy. Hence, here, the 5-min values were not used for the assessment of recognition memory. $n = 12$, 8, 11 and 8 rats for NOR testing at 2 h, 1 week, 3 weeks and 2 weeks; $n = 11$ and 9 rats for OPR testing at 2 h and 1 week, respectively. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample t-test against chance level; $^{***}P < 0.001$, $^{*}P < 0.05$ for pairwise t-tests (two-sided) between sleep and wake conditions.



Extended Data Fig. 3 | Performance on NOR task for hippocampal inactivation studies. Memory is indicated by mean ± s.e.m. discrimination ratios during the first 1 min, first 3 min, and entire 5 min of the retrieval phase on the NOR task in experiments involving reversible inactivation of the hippocampus (dot plots overlaid). **a**, Muscimol (red bars, $n = 8$ rats, versus vehicle, grey bars, $n = 8$ rats) was infused into the hippocampus in the post-encoding interval upon the first occurrence of continuous SWS, or 15 min before retrieval testing (blue bars, $n = 9$ rats). Retrieval was tested 3 weeks after encoding. **b**, Control study in which muscimol (purple bars, $n = 7$ rats) was infused shortly after encoding while the rats remained awake during the 2-h post-encoding interval, compared with untreated wake control rats ($n = 8$ rats, empty bars). Retrieval was tested 1 week after encoding. Infusion of muscimol during post-encoding wakefulness tended to enhance NOR performance, which suggests that during wakefulness hippocampal activity normally interferes with NOR memory consolidation[8]. It might also reflect compensatory plasticity occurring in extrahippocampal regions upon hippocampal suppression[46]. **c**, Control studies in which muscimol (blue bars, versus vehicle, grey bars) was infused 15 min before retrieval testing of recent NOR memory (left, $n = 12$ rats for each substance condition) or 15 min before the encoding phase (right, $n = 6$ rats for each substance condition). Retrieval was tested 30 min after encoding, with the rats staying awake during this interval. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample t-test against chance level; $^{**}P < 0.01$, $^{*}P < 0.05$ for pairwise t-tests (two-sided) between conditions. See Fig. 2 for further details.



Extended Data Fig. 4 | Remote 3-week OPR testing. OPR memory was tested in $n = 6$ rats, 3 weeks after a 2-h post-encoding sleep interval. These supplementary experiments followed the same procedures as described for the 1-week sleep condition on the OPR task, but included sleep EEG recordings. **a**, OPR memory is indicated by the mean ± s.e.m. discrimination ratio during the first 1 min and 3 min of exploration. $^{+}P = 0.034$, for one-sample *t*-test against chance level. Rats displayed significant OPR memory after 3 min (as well as for the whole 5-min exploration period). **b**, OPR performance (discrimination ratio at 1 min) at the 3-week retrieval test was correlated with sleep spindle duration during the first 30 min of post-encoding sleep ($^{*}P = 0.029$, Pearson's product–moment correlation). A similar correlation with NOR performance at the 3-week retrieval (Fig. 3a) points towards a similar mechanism underlying the formation of long-term NOR and OPR memory during sleep.



Extended Data Fig. 5 | Verification of cannula location and muscimol spreading. a, Coronal brain section showing location of cannula in the dorsal hippocampus (black arrow) together with position of guide cannula in overlying cortex. **b**, Coronal brain section showing spread of muscimol (red) after infusion into the hippocampus. Experiments were repeated in $n = 3$ rats with similar results. The infusion protocol was the same as in the behavioural experiments. In brief, after implantation of the guide cannula in the dorsal hippocampus, animals were infused using the injection cannulae with 0.5 μl fluorophore-conjugated muscimol[47,48]. After infusion, animals were intracardially perfused and brains were post-fixed with PFA 4% for 24 h. Brains were cut on a vibratome to obtain 70-μm-thick sections and stained with DAPI (1:5,000 μl in PBS) for 15 min. Fluorescent images were acquired by epifluorescence microscopy (Axio imager Zeiss, Germany). Scale bars, 1 mm.

Extended Data Table 1 | Sleep parameters

a

Sleep parameter	NOR			OPR	
	2 hours	1 week	3 weeks	2 hours	1 week
Duration (min)	56.87 ± 6.36	53.12 ± 8.92	41.85 ± 7.10	46.94 ± 5.63	40.05 ± 5.01
Latency (min)	31.46 ± 5.77	29.78 ± 8.06	43.24 ± 6.35	44.57 ± 6.73	43.24 ± 4.06

b

Sleep parameter	Latency (min)	Duration (min)		
	SWS	SWS	PreREM	REM
Vehicle	20.80 ± 5.71	47.13 ± 5.44	5.80 ± 0.71	6.04 ± 1.07
Muscimol	17.57 ± 6.16	53.63 ± 11.15	1.97 ± 0.51**	0.83 ± 0.83**

c

SWS parameter	SO density (number/min)	SO amplitude (mV)	Spindle density (number/min)	Spindle power (mV^2/s)	Spindle mean duration (s)
Vehicle	32.61 ± 4.06	0.189 ± 0.021	2.81 ± 0.15	0.026 ± 0.002	0.591 ± 0.011
Muscimol	31.62 ± 3.47	0.187 ± 0.015	2.86 ± 0.10	0.025 ± 0.002	0.598 ± 0.022

a, Sleep duration and latency during the 2-h post-encoding interval for the sleep groups of the main experiments (Fig. 1). In these experiments retrieval was tested either immediately after the 2-h retention interval (test of recent memory) or 1 week or (for the NOR task only) 3 weeks later (tests of remote memory). There were no significant differences between NOR and OPR task conditions or retention intervals. $n = 12, 8$, and 11 rats for NOR testing after 2 h, 1 week and 3 weeks, and $n = 11$ and 9 rats for OPR testing after 2 h and 1 week, respectively. **b**, Post-encoding sleep in the experiments after bilateral intrahippocampal infusion of muscimol (Fig. 2a). Sleep latency, time in SWS, preREM sleep, and REM sleep are indicated ($n = 8$ rats for each condition). **c**, For the same experiments, density and amplitude of slow oscillations (SO) and density, power, and mean duration of spindles identified during SWS are indicated for the vehicle and muscimol conditions. Substances were infused during the 2-h post-encoding interval (upon the first occurrence of SWS). **PreREM $P = 0.002$, REM $P = 0.003$, for pairwise t-tests (two-sided) with vehicle condition. Data shown as mean ± s.e.m.

Extended Data Table 2 | Correlations between NOR after 3 weeks and sleep parameters

	SWS	Spindles				Slow Oscillations			
	Duration	Number	Duration	Density	Power	Number	Duration	Density	Power
r	0.536	0.719	0.705	0.654	-0.192	0.259	-0.215	-0.132	-0.319
p	0.137	0.029*	0.034*	0.056	0.620	0.501	0.578	0.735	0.402

	REM Sleep	
	Duration	Theta power
r	0.251	0.081
p	0.514	0.836

Summary of correlations between NOR performance at the 3-week retrieval (1 min discrimination ratio) and sleep parameters during the 2-h post-encoding interval ($n = 9$ rats). Pearson's correlation coefficients and *P* values are indicated. $*P < 0.05$ level (uncorrected).

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Reporting Summary

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n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

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Software and code

Policy information about availability of computer code

Data collection	ANY-Maze software (Stoelting Europe, Dublin, Ireland) was used for collecting behavioral data. Electrophysiological data were acquired using Cheetah data acquisition software version 5 (Neuralynx, USA).
Data analysis	ANY-Maze software (Stoelting Europe, Dublin, Ireland) was used for tracking and analyzing animal behavior, i.e., for scoring exploratory behavior, tracking animal movements, measuring speed and distance travelled in the maze, and confirming behavioral sleep. Electrophysiological analyses were performed using custom-made scripts created in MATLAB 2015b. All software and data are available from the corresponding author upon request. SPSS 21.0 (IBM, Armonk,USA) was used for all statistical analyses.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We did not carry out formal power analyses. However, in all cases, we aimed to keep sample sizes similar to or larger than those used in previous rodent studies on the effects of sleep on memory consolidation, from our own (e.g., Oyanedel et al. 2014) or other labs (e.g., Oliveira et al. 2010).
Data exclusions	Regarding retrieval performance, we limited our analysis of discrimination ratios to the first and to the first 3 min of the Retrieval phase which lasted 5 min. The restriction to the overall first 3 min was done to take into account the typical dynamics of memory driven exploratory behavior on the NOR and OPR tasks (Dix and Aggleton, Behav Brain Res, 1999). The criterion is consistent with the procedures of most previous studies in the field. For the analysis of EEG recordings in rats with post-encoding intrahippocampal infusions, data from 3 post-encoding intervals were excluded because of technical problems causing recording artefacts. The artifacts prevented the classification of sleep stages according to standard criteria (Neckelmann et al., Sleep, 1994).
Replication	The central finding of the main experiments of a sleep-induced enhancement of remote NOR memory at the 3-week Retrieval test was successfully replicated in a different sample of rats undergoing intrahippocampal infusion of saline during post-encoding sleep. We also successfully replicated previous findings from our and other labs as to the effects of post-encoding sleep on recent NOR and OPR memory (Binder et al., 2012, Inostroza et al., 2013, Oyanedel et al., 2014).
Randomization	In all experiments, rats were randomly assigned to experimental groups and conditions before the experiment. In the experiments testing the effects of post-encoding sleep vs. wakefulness on the retention of NOR and OPR memories, with the retrieval test taking place either 2 hours, 1 week or 3 weeks after encoding, different groups of rats were used to test NOR and OPR memories. Different groups were also used for testing retrieval at the different time points after encoding. The comparison between the effects of post-encoding sleep vs. wakefulness was done in a within-subject design, i.e., each rat was tested on both Sleep and Wake conditions. For these within-subject comparisons the order of experimental conditions was counterbalanced across animals. Additionally, control ANOVA including an additional Order factor (Sleep-Wake vs. Wake-Sleep) were run which did not reveal any significant main or interaction effects for this factor (all $p > 0.172$), thus excluding any substantial effects of the order in which Sleep and Wake conditions were performed. Effects of muscimol vs. vehicle were compared using a between-groups design to assess NOR memory at the 3-week retrieval test, and using a within-subject design to assess the immediate effects of muscimol on hippocampal local field potential recordings.
Blinding	The experimenter was not blind to the experimental group or condition during data collection. However, all behavioral and electrophysiological recordings were analyzed offline, with the experimenter blind to the specific experimental groups and conditions.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Subjects were adult male Long Evans rats (Janvier, Le Genest-Saint-Isle, France, 260–310 g, 10-12 weeks). Rats were housed in groups of 2-4 rats per cage on a 12-h light/12-h dark cycle (lights on at 6:00 h), except during the post-surgery recovery period when they were kept individually. Animals had unrestricted access to water and food throughout the experiments. 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 Baden-Württemberg state authority.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.

2.2) Study 2: Deepened sleep makes hippocampal spatial memory more persistent

Anuck Sawangjit, Carlos Oyanedel, Niels Niethard, Jan Born and Marion Inostroza. Deepened sleep makes hippocampal spatial memory more persistent. *Under revision*.

Deepened sleep makes hippocampal spatial memory more persistent

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Conflict of interest: 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.

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Abstract

Ample evidence has indicated a beneficial role of sleep, and particularly of slow wave sleep (SWS) in memory consolidation. However, how basic features of sleep, its depth and duration, contribute to this process remained elusive. Here, we investigated spatial object-place recognition (OPR) memory in rats, to systematically dissociate effects of sleep depth and duration on the formation of recent and remote hippocampus-dependent memory. Encoding of the spatial configuration was followed by an experimental post-encoding period of either 2 or 4 hours, during which the rats had either “regular sleep”, “deeper sleep”, or were kept awake. A deeper sleep was achieved by an extended habituation of the rats to the sleep environment. Retrieval was tested either immediately after the 2-hour post-encoding period (recent memory test) or 1 week later (remote memory test). Deeper sleep expressed itself in a selective increase in the time spent in SWS, and in numbers of slow oscillations, spindles, and hippocampal ripples during SWS, whereas preREM and REM sleep were not affected. At the recent test, OPR memory was preserved only after sleep, but independent of its depth. At the remote test, however, OPR memory was preserved only after deeper sleep, whereas the wake and the regularly sleeping rats did not show remote OPR memory, even with the longer 4-h post-encoding period. Our results indicate that, rather than a longer duration, deeper sleep, i.e., a longer time in SWS together with enhanced oscillatory signatures of mnemonic processing during this sleep stage, occurring within a 2-hour window after encoding, is the factor that makes hippocampus-dependent memory more persistent.

Key words: spatial memory, remote memory, hippocampal memory, deeper sleep, slow wave sleep.

Significance statement

Comparing effects of the depth and duration of sleep after encoding spatial information, we show in a rat model that deeper sleep, i.e., a longer time spent specifically in slow wave sleep, is the essential factor mediating sleep-dependent long-term memory formation. Only with deeper sleep after encoding were remote spatial memories formed and successfully retrieved after one week. We identified a 2-hour window after encoding to be critical for sleep-dependent formation of remote memory.

Introduction

Sleep enhances the consolidation of memory (Stickgold, 2005; Rasch and Born, 2013; Tononi and Cirelli, 2014). This memory effect of sleep is thought to originate from a systems consolidation process which likewise captures hippocampus-dependent and non-hippocampus-dependent aspects of an episodic memory representation, and is essentially established during slow wave sleep (SWS) (Inostroza and Born, 2013; Sawangjit et al., 2018; Klinzing et al., 2019). Specifically it is assumed, that during SWS newly encoded episodic memory features are reactivated primarily in hippocampal networks which leads to the transmission of the reactivated memory information and, more gradually, to the redistribution of the representation such that extra-hippocampal connectivity is increasingly strengthened. The consolidation process is coordinated in time by the neocortical (~1 Hz) slow oscillation and thalamic (10-15 Hz) sleep spindles, both representing EEG oscillatory hallmarks of SWS. While the slow oscillations drive spindles; the spindles, in turn appear to synchronize ripples which enwrap neuronal reactivation in hippocampal networks. Thereby, reactivations occur during a window of increased excitability and plasticity allowing the redistribution of representations towards extra-hippocampal storage sites (Staresina et al., 2015; Latchoumane et al., 2017; Seibt et al., 2017; Niethard et al., 2018).

Although some knowledge about the detailed mechanism underlying memory consolidation during sleep has been elucidated, basic questions about how this memory process is linked to the sleep process itself, remained

unanswered. Does a longer duration of sleep itself produce better long-term memory? Or is the efficacy of long-term memory formation during sleep after encoding mainly dependent on the depth of sleep? The few human studies on this issue have produced mixed results. For example, extending sleep from 40 min to 90 min after encoding benefited memory performance on a hippocampus-dependent visuo-spatial task (Diekelmann et al., 2012). However, in other studies, a short period of sleep was found to be as effective in enhancing learning of texture discriminations and word-pair associations, respectively, as a whole night of sleep (Mednick et al., 2003; Tucker and Fishbein, 2009). Also, memory improvements over sleep have been found to correlate with both the time spent in sleep and SWS (Takashima et al., 2006) as well as with the average EEG slow wave activity during non-rapid eye movement (NonREM) sleep (Wilhelm et al., 2011; Wilhelm et al., 2013) suggesting that both duration and depth are relevant for consolidation. Here, we approached this issue using a rat model of hippocampus-dependent spatial memory formation on an object–place recognition (OPR) task. Rats encoded a spatial configuration and then slept (or remained wake) for either 2 or 4 hours, and retrieval was tested either immediately after or 1 week after the post-encoding sleep period. We experimentally deepened sleep by extending the duration of the habituation sessions of the rat to the sleep environment. This procedure is related to the well-known “first-night-effect” in humans and, here, was established in separate experiments in rats. Our findings identify the depth of sleep, i.e., a selectively increased time spent in SWS, rather than an overall increase in sleep duration as the primary factor supporting OPR long-term memory.

Methods

Animals

Seventy-five adult male Long Evans rats (Janvier, Le Genest-Saint-Isle, France, 250-350 g, 10-12 weeks) were used for the experiments. Rats were housed in groups of 2-4 rats per cage. They were kept on a 12-h light/ 12-h dark cycle (lights on at 6:00 h) and had unrestricted access to food and water throughout

the experiments. Rats were handled daily for 5-10 min for 5 days before starting an experiment. All experimental procedures were performed in accordance with the European animal protection laws and policies and were approved by the Baden-Württemberg state authority.

Experimental design

The main experiments examined the effect of sleep depth and duration on recent and remote object-place recognition (OPR) memory (Figure 1A). Each experimental condition comprised an encoding phase, during which the task stimuli were encoded, a subsequent 2-hour or 4-hour post-encoding period, and a retrieval phase that occurred either 2 hours (recent test) or 1 week (remote test) after the encoding phase. The groups differed according to the sleep depth during the post-encoding period ("regular" versus "deeper"), and the duration of the post-encoding period (2 versus 4 hours). In the groups with a 2-hour post-encoding period, OPR was tested either 2 hours or 1 week after encoding (recent versus remote). In the groups with a 4-hour post-encoding period, OPR was tested only 1 week after encoding. Thus, 6 experimental groups including a total of 64 rats resulted, i.e., 2 groups testing recent retrieval: "Regular sleep/ 2-hour period/recent" (n = 15), "Deeper sleep/ 2-hour period/recent" (n = 11), and 4 groups testing remote retrieval: "Regular sleep/ 2-hour period/remote" (n = 10), "Regular sleep/ 4-hour period/remote" (n = 8), "Deeper sleep/ 2-hour period/remote" (n = 11), and "Deeper sleep/ 4-hour period/remote" n = 9). Deeper (versus regular) sleep was induced by extending the animal's habituation to the sleep environment (resting cage) from 3 x 2 hours/day (regular sleep) to 3 x 4 hours/day (deeper sleep, see below). Each group of rats was tested on a sleep condition (regular or deeper) and a wake condition in which they stayed awake during the post-encoding period. The sleep and wake conditions were separated by a 2-weeks interval, with the order of conditions counterbalanced across animals in each group.

To characterize deeper versus regular sleep, in a further experiment in different groups of rats (n = 11) electrodes were implanted for recordings of the EEG and hippocampal local field potentials (LFP). The rats were assigned to

two experimental groups in which EEG and LFP signals were recorded during a 2-hour post-encoding period filled either with regular sleep (*n* = 5) or deeper sleep (*n* = 6). The experimental procedures were the same as described in the main experiments except that the rats did not perform the retrieval phase.

In all experiments, animals were randomly assigned to the experimental groups and conditions before the experiment. The experimenters were not blinded to the animal group or condition during data collection. However, all behavioral and electrophysiological recordings were analysed offline, with the experimenters blinded to the experimental groups and conditions.

Behavioral procedures

The behavioural procedures were the same as described in Sawangjit et al., 2018. In brief, animals were first habituated to the task and sleep environment before tested on the OPR task. For habituation, the rats were brought into a test room once a day on three consecutive days. The habituation session started with an object familiarization phase where the rats were placed into an empty cage with an object (not used for experiments) positioned in the center of the cage. They were allowed to explore the object for 10 min. Then, the rats were placed into the empty open field for 10 min to freely explore the open field and its distal cue contexts. Afterward they were left undisturbed in a plastic box (35 × 35 cm, height: 45 cm) serving as ‘resting-box’, for 2 hours in the regular sleep condition. In order to deepen sleep in the deeper sleep condition, the time the rat spent habituating to the resting box was increased to 4 hours.

Twenty-four hours after the last habituation session, the rats were again brought into the test room for the encoding phase of the OPR task. They were placed into the open field containing two identical objects and were allowed to explore the objects for 10 min. The rats were then placed into the ‘resting-box’ for the post-encoding period condition (sleep or wake) and the duration (2 hours or 4 hours) according to the assigned experimental groups. For retrieval testing on the OPR task (2 hours or 1 week after encoding), one of the two objects of the encoding phase was displaced to a different location. During the retrieval phase, the rats were allowed to explore the arena for 5 min.

Sleep was assessed using video recorded behavior according to standard procedures (see below). In the wake condition, wakefulness was enforced using gentle handling (Inostroza et al., 2013). This procedure minimizes stress and confounding influences of locomotion. It involved tapping on the 'resting-box' and, if necessary, gently shaking the box. No intense stimulation was used, and video records ensured that signs of startle or freezing behavior did not occur. The rats were brought to their home cages after the post-encoding period and kept under routine conditions until retrieval testing.

The OPR task was performed in a room with masking noise. The open field (80 cm × 80 cm, height of walls: 40 cm) was made of grey PVC. The rats could see the distal cues through the open upper side of the arena. Objects were made of glass, with different shapes and colors, and heavy enough not to be moved by the rat (height: 15–30 cm; base diameter: 7–12 cm). They were placed at least 10 cm equidistant from the walls. Pilot studies ensured that the rats could discriminate among the different objects and did not show any preference for one of the objects. The locations of objects during the encoding and retrieval phases were counterbalanced across the retention conditions. After each phase, the objects and arena were cleaned with water containing 70% ethanol. The exploratory behavior of rats was monitored by a video camera and analysed offline by an experienced researcher using ANY-maze software (Stoelting Europe, Dublin, Ireland). All experiments took place during the animal's rest phase (between 8:00 and 13:00 h).

Analysis of memory performance

Exploration behaviors were defined by the rat being within 2 cm of an object, directing its nose towards the object and engaging in active exploration behaviors such as sniffing. A discrimination ratio was calculated according to the general formula: (time spent at displaced object − time spent at stationary object)/(time spent at displaced object + time spent at stationary object). Preferential exploration of the displaced object, i.e., a positive value of the discrimination ratio, indicates memory for the familiar object configuration (of the encoding phase), whereas a value of zero indicates no exploration preference.

The total time of object exploration (across both objects), distance travelled and mean speed were also assessed as indicators of locomotion and motivation.

EEG and hippocampal LFP recordings, and histology

The surgical implantation of electrodes for EEG and LFP recordings was performed under general anaesthesia (induction: 1–2%, maintenance: 0.8–1.2% in 0.35 l/min O_2). Preoperatively, fentanyl (0.005 mg/kg), midazolam (2 mg/kg) and medetomidine (0.15 mg/kg) were administered intraperitoneally. Rats were placed in the stereotaxic frame and the skull was exposed. For EEG recordings, four screw electrodes were implanted: two frontal electrodes (AP: +2.6 mm, ML: ±1.8 mm, relative to bregma) and two occipital electrodes (AP: −10.0 mm, ML: ±1.8 mm), serving as reference and ground electrode, respectively. For additional LFP recordings in the dorsal hippocampi, two platinum electrodes were implanted (AP: −4.3 mm, ML: ±2.8 mm, DV: −2.3 mm, relative to bregma). For EMG recordings, two stainless steel wire electrodes were also implanted bilaterally in the neck muscles. All electrodes were connected to a Mill-Max pedestal (Mill-Max Mfg. Corp., New York, USA) and fixed to the skull with cold polymerizing dental resin. After the surgery, carprofen (5 mg/kg) was injected subcutaneously, and the rats were allowed to recover for at least 8 days. For recordings, the electrodes were connected through a preamplifier headstage (HS-18MM, Neuralynx, Dublin, Ireland) to a Digital Lynx SX acquisition system (Neuralynx), amplified, filtered (EEG: 0.1–50.0 Hz; EMG: 30.0–300.0 Hz), and sampled at a rate of 1,000 Hz.

After completion of the experiments, histological verification of the placement of hippocampal LFP electrodes was performed. The rats were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde (PFA). After decapitation, the brains were removed and immersed in the 4% PFA for at least two days. Coronal sections of 50–70 µm were cut on a vibratome, stained with toluidine blue and examined under a light microscope. For all rats (n=11), the tips of LFP electrodes were located in the dorsal hippocampus.

Analysis of sleep, EEG, and hippocampal LFP recordings

In the main experiment, sleep was assessed using video recordings according to standard visual procedures (Van Twyver et al., 1973; Pack et al., 2007; Sawangjit et al., 2017). In brief, sleep was scored whenever the rat showed a typical sleep posture and stayed immobile for at least 5 s. If brief movements interrupted sleep epochs by <5 s, continuous sleep was scored. The agreement of the procedure with EEG-based scoring of sleep in the present (see below) and previous studies was >92 % (Pack et al., 2007; Inostroza et al., 2013). In the experiments that aimed at characterizing deeper sleep, sleep was additionally assessed using EEG and EMG recordings. Sleep stage classification was performed offline by an experienced experimenter using 10-s epochs according to standard criteria (Neckelmann et al., 1994). The stages identified were SWS, pre-rapid eye movement (PreREM) sleep, REM sleep, and wakefulness. 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 (4.0–8.0 Hz), phasic muscle twitches and minimum EMG activity. PreREM sleep was identified by a decrease in delta activity, a progressive increase of theta activity and the presence of sleep spindles (10.0–16.0 Hz).

Procedures for identification of slow oscillations (SO), spindles, and hippocampal ripples during SWS were the same as described in Sawangjit et al., 2018. In brief, the EEG signal during all SWS epochs of an animal was filtered between 0.3 and 4.5 Hz. A slow oscillation event was selected 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, 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. The algorithm resulted in the identification of SOs with negative peak amplitudes exceeding −80 µV and peak-to-peak amplitudes exceeding 120 µV. For spindle detection, the Hilbert transform was calculated for the filtered EEG

signal (10.0−16.0 Hz). The signal was smoothed with a moving average (window size 200 ms). A spindle was identified when the absolute value of the transformed signal exceeded a threshold of 1.5 standard deviations of the mean signal during the animal's SWS epochs, for at least 0.4 s and for not more than 2.0 s. To identify ripples in the hippocampal LFP recordings, the Hilbert transform was calculated for the filtered EEG signal (150.0−250.0 Hz) and smoothed with a moving average (window size 200 ms). A ripple was identified when the transformed signal exceeded 2.5 standard deviations 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.

Statistical analyses

Statistical analyses were performed using SPSS 21.0. To investigate the effect of sleep manipulation on memory performance (discrimination ratios), we used Analyses of Variance (ANOVA) including group factors for the sleep depth (Deeper/Regular sleep) and the length of the post-encoding period (2 hours/4 hours), and a repeated-measures factor representing the post-encoding Sleep/Wake conditions. ANOVA were separately applied to the recent (2-hour) and remote (1 week) recall data. Discrimination ratios were also compared with chance level performance (zero) using one-sample *t*-tests. For analysis of sleep data, the sleep duration for subsequent 1-hour time bins was included as repeated-measures factor (Hour). To examine the relationship between post-encoding sleep and memory performance, Pearson product–moment correlation coefficients were calculated. In EEG and LFP recordings from the additional experiment performed to compare Deeper vs Regular sleep during a 2-hour post-encoding period were likewise analysed base on ANOVA including a Deeper/Regular sleep group factor and a repeated measures factor Hour (1^{st} vs. 2^{nd} hour). Generally, ANOVA indicating significance for main or interaction effects of interest were followed by post hoc *t*-tests (two-sided). A $P < 0.05$ was considered significant.

Results

Stronger remote but not recent OPR memory after "deeper" compared with "regular" sleep

Figure 1A summarizes the design of the experiments. At the recent recall test (following the 2-hour retention interval), OPR memory was enhanced in both the regular and deeper sleep conditions, in comparison with the wake condition ($F(1,24) = 15.932$, $p = 0.001$, for Sleep/Wake ANOVA main effect, see Figure 1B, for pairwise comparisons). In both sleep conditions exploration discrimination ratios significantly differed from chance level confirming OPR memory (regular sleep: $t(14) = 3.865$, $p = 0.002$; deeper sleep: $t(10) = 3.134$, $p = 0.011$, one-sample t test), with no difference between the conditions ($p = 0.214$. for pairwise comparison).

Strikingly, at the 1-week (remote) recall test, significant OPR memory was only found when the post-encoding sleep period was filled with deeper sleep, regardless of whether the post-encoding period lasted 4 hours (deeper sleep: $t(8) = 5.071$, $p = 0.001$, wake control: $t(8) = -1.361$, $p=0.211$, one-sample t test, $t(8) = 4.798$, $p = 0.001$, for pairwise comparison between conditions), or only 2 hours (deeper sleep: $t(10) = 6.993$, $p < 0.001$, wake control: $t(10) = -1.248$, $p = 0.240$, one-sample t test, $t(10) = 4.654$, $p = 0.001$, for pairwise comparison between conditions, $F(1,34) = 6.071$, $p = 0.019$, for Deeper/Regular x Sleep/Wake interaction in global ANOVA, Figure 1C). In fact, OPR memory at the 1 week test was closely comparable for the rats with deeper sleep covering a 2-hour and 4-hour post-encoding period ($p = 0.442$, for pairwise comparison between conditions). For the 2-hour period, OPR memory after deeper sleep at the 1-week retrieval test was also superior to that after regular sleep ($p = 0.025$ for pairwise comparison).

In contrast, in the regular sleep condition, like in the wake control condition, rats did not anymore show significant OPR memory at the 1-week (remote) retrieval test, i.e., discrimination ratios did not differ from chance level, regardless of whether the post-encoding interval covered a 2-hour period (regular sleep: $t(9) = 0.695$, $p = 0.505$, wake control: $t(9) = -0.065$, $p = 0.950$,

one-sample *t* test, $p = 0.591$ for pairwise comparison between conditions), or a 4-hour period (regular sleep: $t(7) = 1.040$, $p = 0.333$, wake control: $t(7) = -0.689$, $p = 0.513$, one-sample *t* test, $p = 0.206$, for pairwise comparison between conditions, Figure 1C). Taken together, these results indicate that the consolidation of both recent and remote OPR memory requires post-encoding sleep but, to form more persistent remote OPR memory post-encoding sleep needs to be deeper than for the formation of recent OPR memory.

Total object exploration, total distance travelled and mean speed at the encoding and retrieval phases were comparable between experimental groups (all $p > 0.177$, for relevant ANOVA main and interaction effects, Table 1), confirming that the differences in OPR memory were not influenced by nonspecific changes, for example, in locomotion or motivation.

Characteristics of "deeper sleep"

To probe the efficacy of our manipulation to selectively deepen sleep we recorded, in a separate group of rats, the EEG and hippocampal LFPs during a 2-hour post-encoding period filled either with deeper or regular sleep. Deeper sleep during the 2-hour period was associated with a selectively increased time spent in SWS, but not in preREM or REM sleep, when compared with the regular sleep group ($t(9) = 3.643$, $p = 0.005$, Figure 2). Also, the average duration of an SWS epoch was enhanced during deeper sleep ($t(9) = 2.762$, $p = 0.022$). Interestingly, the enhancement in SWS duration occurred exclusively in the second hour of post-encoding sleep (first hour: $t(9) = 1.027$, $p = 0.331$, second hour $t(9) = 5.579$, $p = 0.0003$, $F(1,9) = 20.187$, $p = 0.002$ for Deeper/Regular x Hour interaction; Figure 2D). There were also differences in SWS oscillations between the deeper and regular sleep condition that occurred exclusively in the second hour of the post-encoding period: Number of slow oscillations (SOs) and spindles, spindle duration, and the number of hippocampal ripples were all higher during deeper than regular sleep ($t(9) = 3.260, 2.967, 2.864, 7.436$, $p < 0.019$, $F(1,9) > 5.187$, $p < 0.049$ for respective Deeper/Regular x Hour interactions, Figure 2E-H).

Consonant with these findings, (video-based) analyses of sleep in our behavioural study revealed that sleep duration was increased in the deeper versus the regular sleep condition for the 2-hour post-encoding period (from 34.60 ± 3.00 min to 45.86 ± 3.81 min; $p = 0.034$, for pairwise comparison) as well as for the 4-hour post-encoding period (from 114.55 ± 2.78 min to 149.47 ± 12.81 min, $p = 0.024$, for pairwise comparison; Figure 3A). Interestingly, an analysis on subsequent 1-hour intervals indicated that the increase in sleep duration in the deeper sleep condition was focussed on the second hour of post-encoding sleep in both the 2-hour and 4-hour retention conditions ($p = 0.015$ and $p = 0.027$, for pairwise comparisons, $F(1,19) = 5.061$ and $F(1,15) = 6.544$, $p < 0.037$, for respective Deeper/Regular x Hour interactions; Figure 3B). Notably, correlation analyses revealed that in the deeper sleep condition with a 2-hour post-encoding period, remote OPR memory (at the 1-week recall test) was strongly correlated with the total sleep time ($r = 0.805$, $p = 0.003$, Pearson's correlation) as well as with the sleep duration during the second hour of post-encoding sleep ($r = 0.697$, $p = 0.017$, Figure 3C). Similar associations were not observed for the conditions with a 4-hour post-encoding period ($r = -0.097$, $p = 0.804$, and $r = -0.461$, $p = 0.212$), overall suggesting that sleep depth during the first 2 hours after encoding is crucial for the consolidation of remote OPR memory.

Discussion

Using a classical object-place recognition (OPR) task in rats, the present study confirms that sleep, in comparison with post-encoding wakefulness, enhances the consolidation of hippocampus-dependent memory. Previous studies demonstrated a beneficial effect of sleep for OPR memory tested up to 24 hours after learning (Ozawa et al., 2011; Binder et al., 2012; Bett et al., 2013; Inostroza et al., 2013; Ishikawa et al., 2014; Oyanedel et al., 2014; Howard and Hunter, 2019). By systematically varying the duration and depth of post-encoding sleep, the present study goes beyond those previous findings indicating that sleep can strengthen OPR memory such that it is even maintained over one week. However, such persisting long-term OPR memories

emerge only when the post-encoding sleep is of deeper quality. On the other side, we did not find evidence that the mere duration of sleep plays an essential role in producing persisting OPR memory, but the relevant consolidation processes appear to be associated with the first 2 hours – particularly with the second hour – after the encoding session.

Our findings seem to diverge from previous human study suggesting that enhanced time spent in sleep per se is linked to building stronger memories (e.g., Diekelmann et al., 2012). However, such study manipulated sleep duration within shorter intervals (e.g., between 40 and 90 min), whereas the present study compared a 2-hour and 4-hour post-encoding periods of sleep. Thus, the findings can be reconciled by assuming a minimum amount of sleep (of about 2 hours) that provides optimal consolidation and with no additional benefits when sleep duration is further enhanced. This view also concurs with the present evidence that the enhancing effects of deeper sleep results from sleep changes focussing on the second hour after encoding.

The central finding of this study is that deeper sleep – irrespectively of whether it extended over a 2-hour or 4-hour post-encoding period – strengthens OPR memory such that it can be retrieved 1 week later. Previous studies showed that OPR memory can be maintained up to 1 week (Hardt et al., 2010; Migues et al., 2016). However, those studies used a more intense training protocol for encoding the task. We show here that, without repeated exposures to the same learning context, a single 10-min exposure to the task configuration at encoding is sufficient for forming a long-term memory when the rat has subsequently deeper sleep. Moreover, deeper sleep seems to specifically support memory for the hippocampus-dependent spatial task aspects. In a previous study using a protocol identical with the “regular sleep” condition of the present study, we showed that, following a 2-hour post-encoding period of regular sleep non-hippocampus-dependent novel-object recognition memory is preserved for at least 3 weeks (Sawangjit et al., 2018). By contrast, in the regular sleep condition of the present study, OPR memory had already completely faded at the 1-week recall which, against this backdrop, reflects the

inability to specifically maintain spatial aspects of the encoded episode, rather than a failure to recognize the objects (Moscovitch et al., 2016).

What defines “deeper” sleep? Our comparisons of EEG recordings during regular and deeper sleep conditions revealed a selectively increased time in SWS, while time in preREM and REM sleep remained unchanged. In addition, the oscillatory hallmarks of SWS, i.e., number of slow oscillations, spindle number and duration as well as hippocampal ripples were increased. Altogether, these changes underline the importance of SWS for forming long-term hippocampus-dependent memory (Marshall and Born, 2007; Klinzing et al., 2019). The increase in ripples appeared to be particularly robust. Ripples in hippocampal networks typically accompany replay of newly encoded spatial memory, and the suppression of ripples impairs spatial memory formation (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010). Ripple as well as spindle activity is found to be enhanced during sleep after encoding of hippocampus-dependent memory (Eschenko et al., 2006, 2008). Against this backdrop, increases in ripple and spindle activity during deeper sleep after OPR encoding might partly reflect the increased processing of the newly encoded spatial information especially occurring in conditions of deeper sleep. Enhanced slow oscillatory and spindle activity during deeper sleep might prime ripple-coupled replay of the newly encoded spatial memory and the transmission of the replayed information to networks outside the hippocampus. Concurrently, these enhancements in slow oscillatory and spindle activity support synaptic plastic processes enabling the formation of long-term memory for the information in extra-hippocampal spatial networks (Maviel et al., 2004; Eichenbaum, 2017; Seibt et al., 2017; Niethard et al., 2018) and, consequently, the better retrievability of these memories at a remote recall.

Importantly, the changes in sleep and associated oscillatory signatures characterizing deeper sleep focussed on the second hour after encoding. Fittingly, only the increased time asleep in this second post-encoding hour was found to positively correlate with OPR performance at the remote 1-week recall test, and there was no similar correlation for time asleep in the later hours of the 4-hour post-encoding sleep period. These results suggest that SWS-rich sleep

within 2 hours after encoding effectively strengthens memory, with additional sleep providing no further benefit. Findings from other studies likewise point to a particular importance of SWS within the first two hours after encoding for memory formation: Reinforcing the coordination of spindle-ripple-SO events by ripple-triggered cortical stimulation applied approximately within this post-encoding time interval, distinctly improved OPR memory performance tested on the next day (Maingret et al., 2016). Moreover, sleep spindle and hippocampal sharp-wave ripple activity after encoding of a hippocampus-dependent odor-place association task were elevated for up to 2 hours after post-encoding sleep onset (Eschenko et al., 2006, 2008). Also, the hippocampal replay of newly encoded memory appears to occur most frequently during this early post-encoding period (Kudrimoti et al., 1999; O'Neill et al., 2010; Giri et al., 2019). Sleep deprivation in mice limited to a 3-hour window after learning impaired long-term potentiation (LTP) as well as OPR memory tested 24 hours later (Prince et al., 2014). Indeed, within hippocampal networks, protein synthesis is required within 2 hours after encoding for consolidating OPR memory (Ozawa et al., 2017), and also hippocampal NMDA receptors have been shown to be specifically involved at this early stage of consolidation (Shimizu et al., 2000; Yamada et al., 2017). Deeper, i.e., SWS-rich sleep in the 2-hour post-encoding period might primarily support synaptic consolidation and a temporary stabilization of hippocampal OPR memory in this early phase, thereby setting the stage for an enhanced hippocampo-neocortical coordination underlying the more gradual emergence of long-term OPR memory in neocortical networks (Lesburguères et al., 2011; Kitamura et al., 2017).

Our experiments established the extended habituation to the sleep environment as an effective experimental procedure to deepen sleep and to specifically enhance SWS in rats. This habituation effect has been the focus of numerous studies in humans and, in this context, is explained by reduced activity of brainstem arousal systems that counteract sleep-promoting systems (e.g., Toussaint et al., 1997; Newell et al., 2012). The human studies confirm our findings in rats in consistently showing that increased habituation to the sleep lab over consecutive nights increases sleep efficiency and reduces time

awake. However, in humans habituation also increased REM sleep, which we did not observe in our rats, possibly reflecting that the changes in sleep architecture vary depending on the actual degree of habituation achieved (Toussaint et al., 1997). If so, graded sleep habituation might be a promising tool for the study of effects of sleep depth with strong relevance for comparisons between species and for clinical populations. Whatever the case, here, we successfully used this procedure to show in rats that the effective formation of long-term OPR memory depends on the depth of post-encoding sleep, rather than on its duration.

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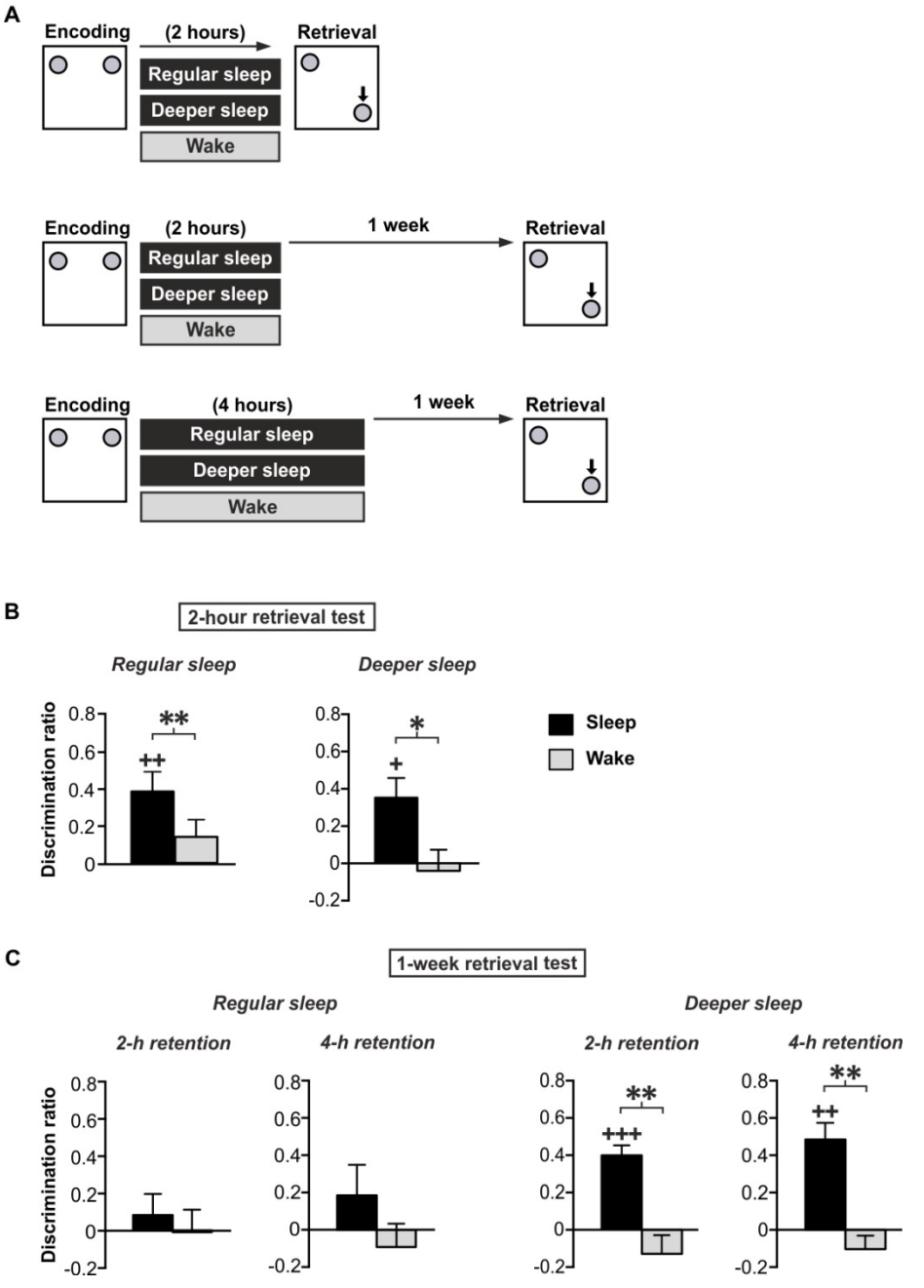

Figure 1. Deeper sleep after encoding enhances consolidation of remote OPR memory. (A) Study design: During the Encoding phase of the OPR task, the rats explored two identical objects in an open field for 10 min. Then, an experimental period of either 2 hours (upper panels) or 4 hours (lower panel) followed, during which the rats slept in an “deeper sleep” or “regular sleep” condition, or the rats remained awake (“wake” control condition). To generally deepen sleep in the “deeper sleep” condition, rats in this condition underwent

an extended prior habituation to the sleep environment, 3 x 4 hours/day compared to 3 x 2 hours/day in the rats of the “regular sleep” conditions. The retrieval was tested either 2 hours after encoding (recent test) or 1 week later (remote test). In the retrieval test, the rats explored the same objects in the arena for 5 min. One of the two objects was displaced (relative to its location at the encoding phase, arrow). The preferential exploration of the displaced object as compared to the stationary object (i.e., the discrimination ratio) represents memory for the place. (B) At the (recent) 2-hour retrieval test, OPR memory significantly benefited from both regular (n = 15 rats) and deeper sleep (n = 11 rats), in comparison with the respective wake control conditions, but was comparable between the regular sleep and deeper sleep conditions (p = 0.214). (C) OPR performance at the remote 1-week retrieval test did not reveal any significant memory in the regular sleep or wake conditions, independently of whether the post-encoding period covered a 2-hour or 4-hour interval (left panels). By contrast, remote OPR memory was distinctly enhanced after deeper sleep, in comparison with the wake control condition (right panels), with the enhancement being comparable for the 2-hour and 4-hour retention intervals (p = 0.442), overall indicating that sleep depth rather than duration benefits the formation of long-term OPR memory. +++p < 0.001, ++p < 0.01, +p < 0.05 for one-sample t tests against chance level; **p < 0.01, *p < 0.05 for pairwise t tests (two-sided) between sleep (black bars) and wake (white bars) conditions.

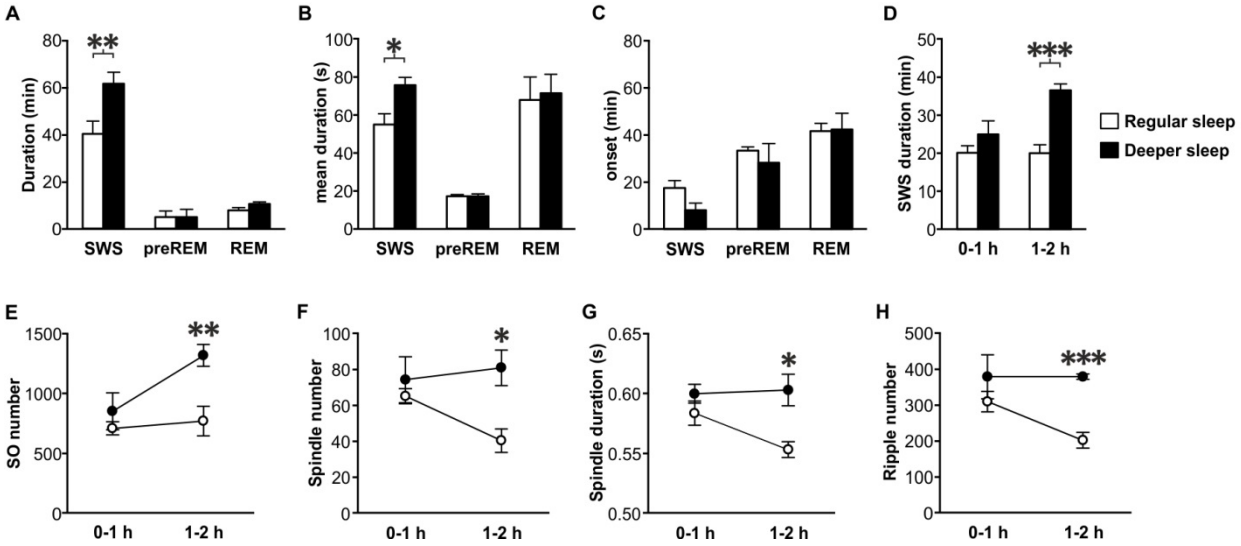

Figure 2. "Deeper sleep" is characterized by a selectively enhanced SWS-related activity. EEGs and intrahippocampal LFPs were recorded in two additional groups of rats to characterize deeper sleep (*n* = 6 rats, black bars and circles) in comparison with regular sleep (*n* = 5 rats, open bars and circles) during a 2-hour interval following encoding of the OPR task. Experimental procedures were the same as described in Figure 1A except that the rats did not perform the retrieval test. Compared with regular sleep, deeper sleep was associated with an increased (A) total SWS duration, and (B) mean duration of SWS epochs. (C) Sleep stage onsets were comparable in both conditions. (D) Enhanced SWS duration during the deeper sleep condition was observed exclusively in the second hour of post-encoding sleep. Numbers of slow oscillations (E), and spindles (F), spindle duration (G), and the number of hippocampal ripples (H) were selectively increased in the second hour of post-encoding sleep. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ for t-tests (two-sided) between deeper and regular sleep.

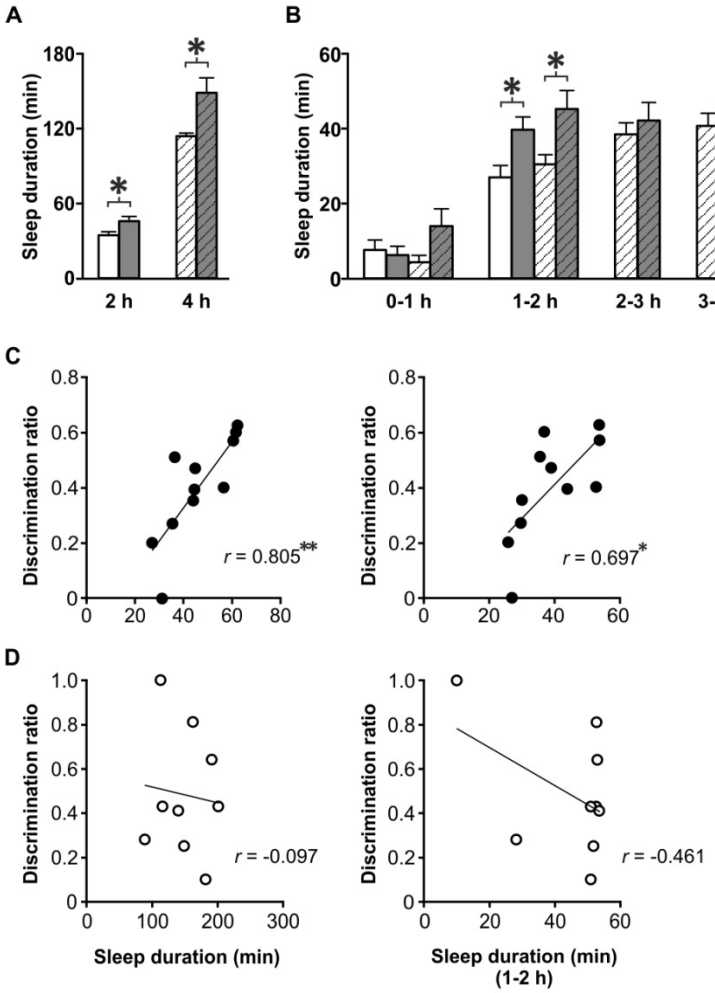

Figure 3. "Deeper sleep" is characterized by enhanced sleep duration during the second hour of post-encoding sleep. (A) Total time spent asleep in the conditions of "deeper sleep" (grey bars,) and "regular sleep" (white bars) during post-encoding periods of either 2 hours (plain) or 4 hours (hatched). (B) Sleep duration for consecutive 1-hour bins in the same conditions (Deeper sleep/ 2-hour retention: n = 11; Deeper sleep/4-hour retention, n = 9; Regular sleep/2-hour retention, n = 10; Regular sleep/4-hour retention, n = 8). Note, increase in sleep duration during deeper sleep in the second hour of both 2-hour and 4-hour intervals. *$p < 0.05$ for *t* test between deeper vs regular sleep. (C) Correlation between OPR performance at the 1-week (remote) retrieval test and (left) the total sleep duration during a 2-hour post-encoding period of deeper sleep, and (right) sleep duration during only the second hour of this post-encoding period of deeper sleep (*n* = 11 rats, black circles). (D) The same

as in C for deeper sleep during a 4-hour post-encoding period (n = 9 rats). Note, significant positive correlations in the 2-hour, but not in the 4-hour post-encoding period. $^{**}p < 0.01$, $^{*}p < 0.05$ for Pearson's correlations.

Table 1. Total exploration time, distance travelled, and mean speed during the Encoding and Retrieval phases.

	2-hour OPR test				1-week OPR test			
Encoding phase	2-hour retention interval				2-hour retention interval			
	Regular sleep	Wake control	Deeper sleep	Wake control	Regular sleep	Wake control	Deeper sleep	Wake control
Total exploration (s)	8.70±1.30	8.34±0.94	9.65±3.15	7.16±0.87	13.61±3.01	13.56±3.48	11.55±1.94	9.14±0.99
Distance travelled (m)	53.84±2.96	52.53±4.15	48.15±3.09	47.37±2.43	42.03±2.73	37.70±3.24	60.46±2.48	60.17±3.59
Mean speed (m/s)	.091±.005	.088±.007	.080±.005	.079±.004	.070±.005	.076±.012	.101±.004	.100±.006

	1-week OPR test				2-hour OPR test			
	4-hour retention interval				2-hour retention interval			
Retrieval phase	Regular sleep	Wake control	Deeper sleep	Wake control	Regular sleep	Wake control	Deeper sleep	Wake control
Total exploration (s)	9.48±2.20	8.03±1.30	9.38±2.47	11.49±1.71	5.59±0.81	5.33±0.72	3.22±0.52	3.56±0.75
Distance travelled (m)	60.45±7.38	57.36±7.00	50.38±3.44	52.92±4.02	24.36±2.38	26.29±2.67	26.37±1.93	27.26±1.93
Mean speed (m/s)	.101±.012	.096±.012	.084±.006	.088±.007	.081±.008	.088±.009	.088±.006	.091±.006

	1-week OPR test							
	4-hour retention interval				2-hour retention interval			
	Regular sleep	Wake control	Deeper sleep	Wake control	Regular sleep	Wake control	Deeper sleep	Wake control
Total exploration (s)	6.16±0.70	7.25±1.96	8.74±2.77	8.57±1.04	8.89±2.14	8.71±1.28	6.15±0.77	4.64±0.70
Distance travelled (m)	33.38±3.48	37.58±3.61	31.86±2.28	32.04±2.68	27.09±2.31	27.12±2.06	38.22±1.59	37.91±2.25
Mean speed (m/s)	.113±.011	.125±.012	.107±.008	.108±.009	.090±.008	.090±.007	.128±.005	.126±.007

Values represent mean ± s.e.m. Result are from the main experiment as illustrated in Figure 1.

2.3) Study 3: Sleep enhances recognition memory for conspecifics as bound into spatial context

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Sleep Enhances Recognition Memory for Conspecifics as Bound into Spatial Context

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Social memory refers to the fundamental ability of social species to recognize their conspecifics in quite different contexts. Sleep has been shown to benefit consolidation, especially of hippocampus-dependent episodic memory whereas effects of sleep on social memory are less well studied. Here, we examined the effect of sleep on memory for conspecifics in rats. To discriminate interactions between the consolidation of social memory and of spatial context during sleep, adult Long Evans rats performed on a social discrimination task in a radial arm maze. The Learning phase comprised three 10-min sampling sessions in which the rats explored a juvenile rat presented at a different arm of the maze in each session. Then the rats were allowed to sleep (n = 18) or stayed awake (n = 18) for 120 min. During the following 10-min Test phase, the familiar juvenile rat (of the Learning phase) was presented along with a novel juvenile rat, each rat at an opposite arm of the maze. Significant social recognition memory, as indicated by preferential exploration of the novel over the familiar conspecific, occurred only after post-learning sleep, but not after wakefulness. Sleep, compared with wakefulness, significantly enhanced social recognition during the first minute of the Test phase. However, memory expression depended on the spatial configuration: Significant social recognition memory emerged only after sleep when the rat encountered the novel conspecific at a place different from that of the familiar juvenile in the last sampling session before sleep. Though unspecific retrieval-related effects cannot entirely be excluded, our findings suggest that sleep, rather than independently enhancing social and spatial aspects of memory, consolidates social memory by acting on an episodic representation that binds the memory of the conspecific together with the spatial context in which it was recently encountered.

Keywords: sleep, memory consolidation, social recognition, spatial context, episodic memory

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INTRODUCTION

Recognition of conspecifics is a fundamental cognitive ability in social species that combines innate and learning components. For example in humans, encoding and discrimination of faces relies on stimulus processing in predisposed cortical networks. However, new faces are learned and stored together with social and spatial features of the context in which they are encountered,

and accordingly recognition of faces strongly depends on such context features (Dudas et al., 2005; Ison et al., 2015; Viskontas et al., 2016), and such binding of social experience in spatial context similarly occurs in rodents (e.g., Fellini and Morellini, 2013). In rodents, social recognition memory involves spontaneous exploratory behavior which makes the animal to explore a novel, unfamiliar conspecific longer than a familiar one. In fact, taking advantage of this innate behavioral preference for unfamiliar conspecifics, the relatively longer social investigation towards an unfamiliar vs. a familiar conspecific has been established as a standard measurement of memory for a previously encountered animal (Thor and Holloway, 1982; Engelmann et al., 1995; van der Kooij and Sandi, 2012; Lukas et al., 2013). The procedure requires a direct encounter between conspecifics which allows the animal to investigate both volatile and non-volatile fractions of an individual's olfactory signature which is important because accessing only the volatile fraction is not sufficient to either encode or retrieve social recognition memory in rats (Noack et al., 2010; Engelmann et al., 2011).

Sleep has been shown to support consolidation of various types of memory in both humans and animals (Rasch and Born, 2013). However, little is known about the effect of sleep on the formation of social memory, which is typically assessed in recognition tasks. Two human studies reported an enhancing effect of post-learning sleep on the recognition of previously encountered face stimuli (Clemens et al., 2005; Wagner et al., 2007). Recognition performance was positively correlated with the amount of non-rapid eye movement (NonREM) sleep, consistent with the notion that NonREM sleep specifically benefits memories involving the hippocampal memory system (Diekelmann and Born, 2010). One study in rats revealed influences of the circadian rhythm on social learning. An increase in exploration time toward a novel juvenile conspecific was greater when the rats were tested during the rest phase than when tested during the active phase, and when the retention interval was extended from 30 min to 60 min (Moura et al., 2009), suggesting consolidation processes taking place during sleep might facilitate social recognition performance. However, to the best of our knowledge, the role of sleep in social recognition memory has not directly been examined in a rodent model, so far.

The present study aimed to test the effects of post-learning sleep on consolidation of social recognition memory in rats. We hypothesized that sleep enhances memory formation for conspecifics. A second goal was to explore whether the presumed consolidation of social memory interacts with the simultaneous consolidation of spatial memory. Indeed, in natural conditions social learning typically occurs embedded in a spatial context, and spatial memory is well-known to benefit from sleep (Inostroza et al., 2013; Oyanedel et al., 2014; Maingret et al., 2016). Moreover, memory formation in both domains shares common hippocampal circuitry, specifically in CA2 (Hitti and Siegelbaum, 2014; Schwarb et al., 2015; Alexander et al., 2016; Smith et al., 2016), rendering the possibility of interactions between the two domains occurring also during sleep-dependent consolidation. To simultaneously measure the effects of sleep on social memory and to dissociate contributions of spatial memory, we combined a standard social discrimination task with a modified radial arm maze in which the rat had direct access to both volatile and non-volatile fractions of each conspecific's olfactory signature.

MATERIALS AND METHODS

Animals

Thirty-nine 9–10 weeks old male Long-Evans rats (Janvier, Le Genest-Saint-Isle, France, 280–340 g) were used in this study. Eight 4–5 weeks old male juvenile rats of the same strain (Janvier, Le Genest-Saint-Isle, France, 85–125 g) served as social stimuli. Rats were housed in pairs and kept on a 12 h/12 h light/dark cycle (lights on at 6:00 h). They had free access to food and water 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 Baden-Württemberg state authority (MPV 1/16).

Experimental Task and Procedures

The social recognition task was performed in a modified radial arm maze (**Figure 1A**) consisting of eight arms (14.5 cm wide, 35 cm long) placed radially around a central circular platform (11 cm wide, outer diameter 77 cm). Only four opened arms were used throughout the experiments (arm #1, #3, #5 and #7). The maze was made of wood and the outer part of the circular platform was surrounded by clear plexiglass acrylic sheets (18 cm height). At the end of each arm was a small platform (20 × 20 cm, not elevated) for placing the juvenile rat. The entire maze was lifted up 70 cm from the ground and surrounded by a white curtain. To allow navigation, distal cues were provided at the ceiling and attached at the arms of the maze, and the circular maze platform was surrounded by clear plexiglass such that the rat during navigation could see the whole maze including further proximal cues. During the sleep and wake retention intervals between the Learning and Test phase the animal was kept in another "retention box" (35 × 35 cm wide, 45 cm height) which was made of plastic and provided some bedding materials.

The experiments were performed during the light phase of the day (7:00–14:00 h). After 7 days of animal handling, habituation sessions were performed for five consecutive days (1 session per day). An opaque plastic basket with a lid was used to transport the adult rats to the experimental room and to the maze. The adult rats were allowed to explore freely in both the maze and retention box for 10 and 120 min, respectively. The experimenter left the room right after the rat was introduced to the maze, like in the experiment proper. To restrict movement of the juvenile rats to the square platform (further referred to as "juvenile zone"), they wore a harness made of latex, with a leash (30 cm) that was fastened to the end of the radial arm (Supplementary Figure 1). The juvenile was able to freely move within the juvenile zone but could not leave it. The juvenile rats were habituated to wearing

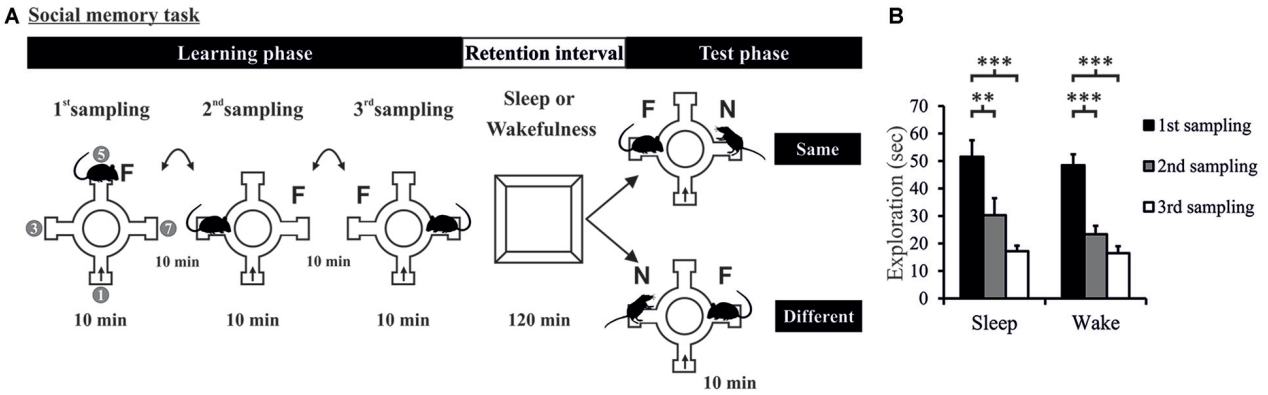

FIGURE 1 | (A) Task and procedure. Social recognition memory was tested in a radial arm maze (four arms used). The Learning phase comprised three 10-min sampling sessions (separated by 10-min intervals) during which the experimental rat was allowed to explore a juvenile conspecific (i.e., familiar rat: F). The rat entered the maze always at the same arm (arrow). In each sampling session, the juvenile conspecific was placed at a different of the three remaining arms. The Learning phase was followed by a 120-min retention interval (spent in the retention box) in which the rat was allowed to sleep (Sleep group) or deprived of sleep (Wake group). Then, the 10-min Test phase took place during which the familiar rat (F) was presented along with a novel conspecific (N) at the opposite arm. For some rats, during the Test phase, the novel consepecific was located in the same arm as that of the familiar rat on the last sampling session ("Same"—upper panel). For other rats, the novel conspecific was placed in an arm different from that of the famliar rat during the last sampling session ("Different"—lower panel). "1", "3", "5", "7" in left most maze denote the arms of the 8-arm radial maze used. **(B)** Exploration time during the Learning phase decreased across the three sampling sessions and did not differ between Sleep and Wake groups. ***$p < 0.001$, **$p < 0.01$.

the harness (also in the presence of an adult conspecific) and to stay in the juvenile zone over five consecutive days (10 min/day), whereby the arm used was changed across sessions. The habituation sessions for the adult and juvenile rats were performed separately.

One day after the habituation sessions, the Learning phase started with three 10-min sampling sessions. In each sampling session the adult rat was allowed to explore the juvenile rat which was presented at three different locations in the maze (**Figure 1A**). The starting point of the adult rat remained the same throughout the experiment. During the 10-min intervals between sampling sessions, the adult rats were kept in new cages with fresh bedding materials in a separate room. They were also habituated for this procedure during the habituation sessions. The Learning phase was followed by a 120-min retention period of Sleep ($n = 18$) or wakefulness (Wake, $n = 18$). During the sleep retention interval, the rat was left undisturbed in the retention box. The animal's behavior was video-recorded, and sleep was assessed offline. In the wake retention interval the animal was deprived from sleep by gently handling (tapping on the retention box, or if necessary shaking the cage; Colavito et al., 2013). No intense stimulation was used to minimize stress. Arousal-interventions were introduced whenever the animal closed their eyes (with or without sleep posture) and was immobile for more than 5 s. In general, the rate of arousal-intervention to prevent the animal from sleeping during the 120-min sleep deprivation interval was <10 per hour, the overall number of arousals introduced per animal was <20. In fact, several previous studies showed that sleep deprivation with this procedure for a short period does not promote stress or anxiety, nor does it alter spontaneous motor activity or recognition memory, compared to undisturbed control animals (Kopp et al., 2006; Palchykova et al., 2006; Vecsey et al., 2009; Hagewoud et al., 2010a,b; Binder et al., 2012; Inostroza et al., 2013; Melo and Ehrlich, 2016).

During the 10-min Test phase following the retention period, a novel juvenile rat was presented along with the familiar rat, both at two opposing arms. The sequence of the juvenile rat's locations in the Learning phase and the locations of the novel and familiar juveniles in the Test phase were randomized across rats but, kept balanced between Sleep and Wake conditions (Supplementary Tables 1, 2). The juvenile rats were littermates and they were housed in pairs. One hour before the Learning phase the juvenile rats were kept individually in new cages with fresh bedding materials outside the experimental room to avoid any contaminating odors. After the Learning and Test phase the maze and juveniles' harness were cleaned with water containing 70% ethanol. The exploratory behavior of the adult rats was recorded by two video cameras placed on the two closed arms adjacent to the juvenile rat's zone. Another camera was attached to a ceiling at the center of the maze to record the rats' navigation through the maze. The recorded behavior was analyzed offline by an experienced researcher blind to the experimental condition using the ANY-Maze tracking software (Stoelting Europe, Dublin, Ireland).

Data Analysis

Sleep during the retention interval was assessed using standard visual procedures (Kelemen et al., 2014). 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. The validity of this visual scoring procedure was demonstrated in previous studies in rats and mice, as well as in our own lab, consistently providing an agreement with conventional EEG/EMG based

scoring of sleep of greater 92% (Van Twyver et al., 1973; Pack et al., 2007; Borquez et al., 2014). In animals of the Sleep group, the average sleep duration during the 120-min sleep retention interval was 49.22 ± 4.40 min (sleep-onset latency: 40.17 ± 3.86 min). Exploratory analyses did not reveal any significant correlation between sleep parameters (sleep latency, duration) and any of the performance scores during the Test phase.

In the *social recognition* task, exploratory activity was defined by the adult rat approaching the juvenile rat (to <2 cm) and sniffing the juvenile's body surface irrespective of body area (Popik et al., 1991; Noack et al., 2010). During the Learning phase, the decrease in time spent exploring the (same) juvenile across the three sampling sessions served to confirm that the adult rat learned to discriminate the conspecific. Learning was defined by the decrease in exploration time from the first to the third session (Time exploring the juvenile rat during the 1st Sampling − Time exploring the juvenile rat during the 3rd Sampling) × 100/Time exploring the juvenile rat during the 1st Sampling]. Only animals reaching a learning criterion of >33% were included for further analyses. The learning criterion was introduced to reduce variance in the behavioral expression of memory.

In the Test phase, *social recognition memory* was measured by the animal's preference to explore the novel rat, i.e., [(Time exploring the novel rat/Time exploring both rats) × 100]. Total exploration time spent with the juvenile rats was also analyzed. Additionally, preference to spend time in the novel rat's zone was calculated by the formula: [(Time spent in the novel rat's zone/Time spent in both rats'zone) × 100]. Statistical comparisons concentrated on: (i) cumulative scores for 10-s intervals across the initial 3-min interval of the Test phase and, to assess slower dynamics; on (ii) cumulative scores for 1-min intervals across the entire 10-min Test phase (Note, for calculating cumulative scores, rather than the preference scores *per se*, only the time the animal spent with the novel and familiar juveniles, respectively, was cumulated for a given time interval). Latency to explore the novel and familiar rat was analyzed with reference to the start of the Test session.

To assess *spatial memory*, the exploration preference for the novel rat during the Test phase was calculated separately for four spatial sub-conditions depending on the retention conditions (Sleep and Wake) and the location of the novel juvenile rat with reference to the location of the familiar juvenile on the last (i.e., 3rd) session of the Learning phase: (i) Sleep rats which were exposed to the novel juvenile at the same location as that where they encountered the familiar juvenile on the last sampling session ("Sleep-Same"); (ii) Wake rats which were exposed to the novel juvenile at the same location as that where they encountered the familiar juvenile on the last sampling session ("Wake-Same"); (iii) Sleep rats which were exposed to the novel juvenile at a location different from that where they encountered the familiar juvenile on the last sampling session ("Sleep-Different"); (iv) Wake rats which were exposed to the novel juvenile at a location different from that where they encountered the familiar juvenile in the last sampling session ("Wake-Different"). Please, note the terms "Same" and "Different" always refer to the novel juvenile's location (during the Test phase) with reference to the familiar juvenile's position on the last sampling session.

Statistical comparisons of exploration preferences for the novel rat relied on analyses of variance (ANOVA) with a group factor Sleep/Wake and a repeated measures factor Time interval. To dissociate effects on spatial and social memory in the spatial sub-conditions, ANOVA were run on the exploration preferences containing the group factors "Sleep/Wake" and "Spatial location", the latter reflecting whether the juvenile rat was located at the same or a different location as the location of the familiar rat in the last sampling session. *Post hoc* *t*-tests were used to specify significant main and interaction effects. To test whether the exploration preference for the novel rat was above chance level (50%), one-sampled *t*-tests (two-tailed) were used. To reduce Type I error probability, the latter tests were only calculated after ANOVA indicated a significant Time main effect or Sleep/Wake × Time interaction, and significance is reported only when a p-value <0.05 was revealed for clusters of at least three neighboring time points. All statistical analyses were performed using SPSS 21. Results are reported as the mean ± SEM. Estimates of effect size, i.e., Cohen's d and partial eta squared (η^2), respectively) were also provided for the significant terms.

RESULTS

Performance during the Learning Phase

Analysis of exploration times during the Learning phase showed that the rats in both Sleep and Wake groups learned to recognize the same juvenile rat across the three sampling sessions ($F_{(2,68)}$ = 57.231 and 25.451, $p < 0.001$, partial η^2 = 0.386, for ANOVA Session main effect, **Figure 1B** and Supplementary Figure 2), with no difference between Sleep and Wake groups ($F_{(1,34)}$ = 0.784, p = 0.382, for ANOVA Group main effect, and $F_{(2,68)}$ = 0.462, p = 0.632, for ANOVA Group × Session interaction effect). Exploration time also significantly decreased from the first to the second and to the third sampling session ($p < 0.006$, for all pairwise comparisons). Two rats showed learning performance below the criterion (of a 33% decrease in exploration time from the 1st to the 3rd sampling session) and were therefore excluded from further analyses; decreases in these rats were 1.27% and 7.65%. Another rat was excluded due to technical problem during the Test phase. A total number of 36 rats (Sleep: n = 18, Wake: n = 18) was included in the analyses of the Test phase.

Social Recognition Memory

In the Test phase, only rats of the Sleep group, but not of the Wake group, displayed significant social recognition memory as shown by preferential exploration of the novel juvenile conspecific. Analysis of 10-s intervals revealed that sleep most profoundly affected exploration in the beginning of the Test

phase. Exploration preference scores in the Sleep group were already above chance level within the first 30-s interval, and in this interval were also significantly different from exploration scores in the Wake group ($t_{(32)}$ = 2.328, p = 0.026, d = 0.799; **Figure 2A**). To assess slower changes, we cumulated exploration preference scores across subsequent 1-min intervals. In the Sleep rats these scores reached significance from the 3rd min onwards ($p < 0.05$, one-sampled t-test), whereas in the Wake group scores remained at chance level (**Figure 2B**). Essentially the same results were obtained when exploration preference was scored based on the time the rat spent in each juvenile rat's zone during the Test phase (see Supplementary Figure 3).

Latency to exploration of the familiar rat was significantly longer in the Sleep (53.67 ± 9.14 s) than Wake group (28.47 ± 6.92 s; $t_{(34)}$ = 2.198, p = 0.035, d = 0.732), both groups did not differ in latency to explore the novel conspecific (Sleep = 25.90 ± 8.51 s, Wake = 31.66 ± 6.77 s; $t_{(34)}$ = −0.529, p = 0.60, **Figure 2C**). Total exploration time spent with the juvenile rats (novel and familiar) was comparable between groups (t-test: $p > 0.20$ and 0.35 for the relevant 10-s and 1-min intervals, respectively, **Figures 2D,E**), indicating that the lack of novelty preference in the Wake rats was not a consequence of a non-specific reduction in exploration drive.

Interaction between Sleep Effects on Social Recognition Memory and Spatial Memory

To discriminate interactions between the effects of sleep on social recognition and on spatial learning, exploration preferences in the Sleep and Wake groups were compared depending on whether the juvenile rat was located on the same spatial location (Sleep-Same, Wake-Same groups) or on a location different from that where the familiar rat was encountered in the last sampling session (Sleep-Different, Wake-Different groups). ANOVA on exploration scores cumulated across 10-s intervals confirmed the higher exploration preference scores in the Sleep than Wake group during the initial 30-s interval, as reported above (p = 0.026), and a main effect of Spatial location with a later onset, i.e., after 60 s, indicating preferential exploration toward the novel juvenile when it was placed on a location different from that of the familiar juvenile on the last sampling session ($F_{(1,32)}$ = 4.265, $p < 0.047$, partial η^2 = 0.118, for Spatial location main effect between 60–90 s).

Separate analyses of the two spatial conditions revealed exploration scores that were above chance level, only in the Sleep-Different group, whereas in the other three conditions, i.e., the Sleep-Same, the Wake-Different and the Wake-Same rats, exploration scores failed to exceed chance level. In the Sleep-Different rats cumulated exploration scores reached significance during the first 70-s interval and again towards the end of the 3-min period of analysis. Considering that exploration scores remained at chance level in three of the four conditions, we used planned contrasts to directly test the hypothesis that sleep specifically enhances social recognition depending on where the novel juvenile was placed. Indeed, this analysis revealed that in the Sleep-Different group, exploration preference towards the novel juvenile across the first 60-s interval was significantly higher than in the Sleep-Same and Wake-Same groups ($t_{(16)}$ = 2.270, p = 0.037, d = 1.070 and $t_{(16)}$ = 2.659, p = 0.017, d = 1.253, **Figures 3A,B**) and, across the first 30-s interval, it was also significantly higher than that of the Wake-Different rats ($t_{(15)}$ = 2.434, p = 0.028, d = 1.167). Moreover, in the Sleep-Different group latency to explore the novel juvenile the first time was significantly shorter than that for the familiar juvenile (10.38 ± 4.74 vs. 63.88 ± 12.82 s; $t_{(8)}$ = 3.180, p = 0.013, d = 1.060) and was significantly shorter than the exploration latency for the novel rat in the Wake-Different group (34.23 ± 7.91 s; $t_{(16)}$ = 2.438, p = 0.027, d = 1.149, **Figure 3D**). For all other groups exploration latencies were comparable for the familiar and novel juvenile (all $p > 0.7$). Overall, these data indicate that sleep robustly enhances exploration towards the novel conspecific depending on where this novel rat is encountered, i.e., only if this novel conspecific is encountered at a place different from that of the familiar rat in the last sampling session.

Notably, during the last 3 min of the 10-min test interval the sleep effect appeared to turn such that here exploration preference towards the novel juvenile was significantly greater in the Sleep-Same than in the Sleep-Different rats ($t_{(15)}$ = 2.668, p = 0.018, d = 1.301, **Figure 3C**). Also, during these last 3 min, only the Sleep-Same rats showed above chance level exploration scores ($t_{(7)}$ = 2.625, p = 0.034, d = 0.928, one-sampled t-test, $p > 0.233$ for the other 3 groups).

DISCUSSION

We examined the effect of post-learning sleep on consolidation of social recognition memory in rats. We found that sleep enhanced recognition of a conspecific as indicated by preferential exploration of the novel as compared to the familiar conspecific during the first min of the 10-min Test phase. We also found clues that enhanced consolidation of social recognition memory during sleep depends on spatial context features of the social episode encoded before sleep: only the rats of the Sleep group which encountered the novel conspecific at a place different from that of the familiar rat during the last sampling phase, i.e., the Sleep-Different group, but not the Sleep-Same group, expressed significant social memory during the first minute of the Test phase, and only this Sleep-Different group showed significantly enhanced social recognition memory during this 1-min period in comparison to all other groups, including the Sleep-Same group. This pattern of findings argues against a direct enhancing effect of sleep on social memory representations but is rather consistent with the notion that the consolidation effects of sleep on social and spatial aspects of a memory representation interact.

While there is evidence that sleep in rats can facilitate intermediate and long-term memory for social information like the transmission of food preference (Wooden et al., 2014), social recognition memory for conspecifics, has so far only been observed in adult rats with retention intervals of

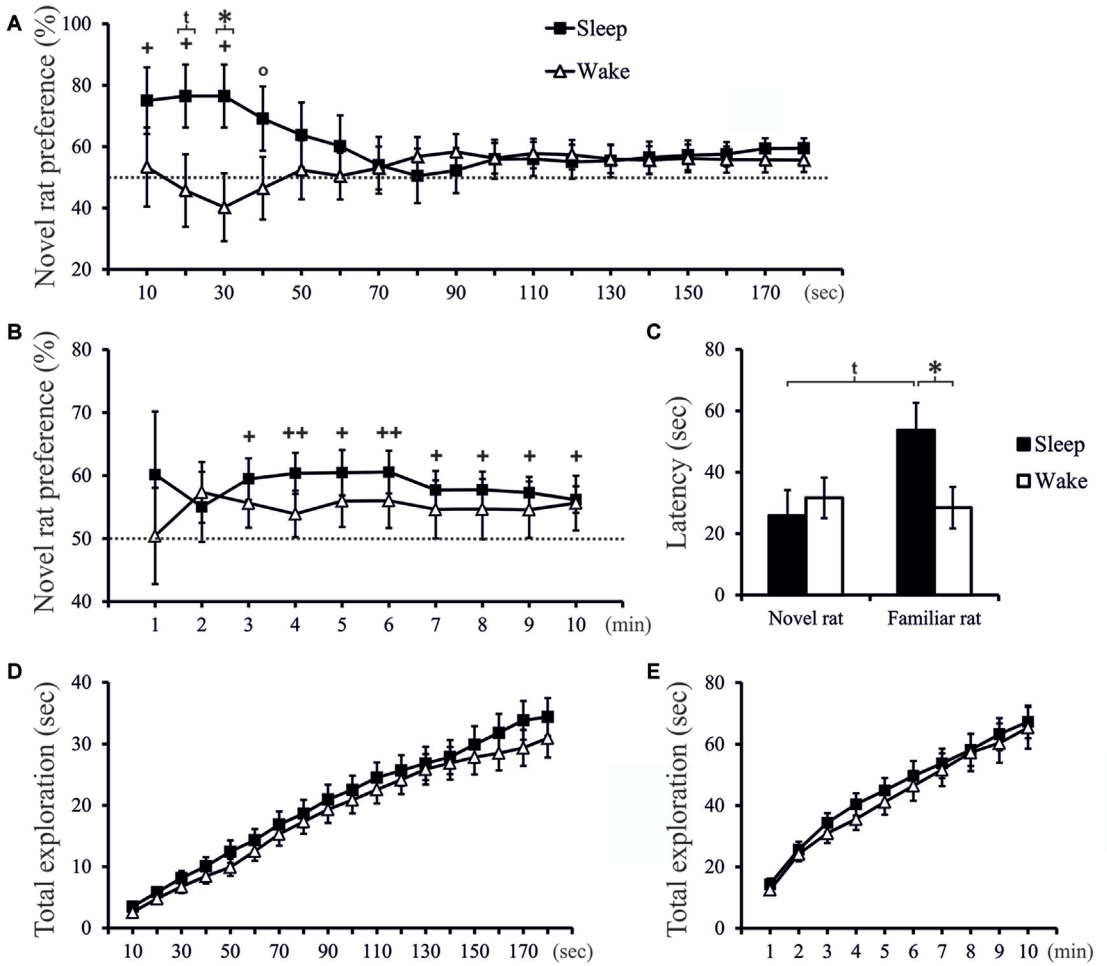

FIGURE 2 | (A) Exploration preference for novel rat (in % of total exploration time) cumulated across 10-s intervals during the first 3 min of the Test phase, and **(B)** exploration preference cumulated across 1-min intervals across the total 10-min period of the Test phase, separately for the Sleep group (n = 18) and Wake group (n = 18). $^{++}p < 0.01$, $^{+}p < 0.05$, $^{\circ}p < 0.1$, for tests against chance level (50%); $^{*}p < 0.05$, $^{t}p < 0.1$, for difference between Sleep and Wake groups. **(C)** Latency (first approach) to explore the novel rat and the familiar rat. $^{*}p < 0.05$, $^{t}p < 0.1$. **(D)** Total time spent exploring the juvenile rats (novel and familiar) cumulated across the first 3 min **(E)** and across the entire10-min Test phase. There was no significant difference in total exploration time between groups.

shorter duration, i.e., no longer than 30 min (Sekiguchi et al., 1991; Engelmann et al., 1995; Squires et al., 2006; Noack et al., 2010). Against this backdrop the present study provides first time evidence that the memory of conspecifics can last distinctly longer after sleep, i.e., a 2-h duration that pertains to so-called intermediate-term memory (Kesner and Hunsaker, 2010). The sleep effect expressed itself in the beginning of the Test phase within the first 30 s. In this interval, the Sleep group displayed exploration preference of the novel juvenile significantly above chance level, and the exploration preference for the novel conspecific was also significantly stronger if compared to those of the Wake group. Fittingly, in the Sleep rats latency to explore the novel conspecifics was shorter than to explore the familiar one, whereas such difference was not observed in the Wake rats. The latency of exploration might be related to the rat's ability to detect the novelty of the conspecific based on its olfactory signature which seemed to be facilitated after sleep (Popik et al., 1991; Noack et al., 2010).

It might be argued that rather than effects of sleep, the observed differences in exploration preference between the Sleep and Wake animals during the Test phase reflect non-specific effects on memory retrieval, due to the fact that the Wake animals were tested immediately after the 120-min period of enforced wakefulness. However, in the Test phase, total exploration time spent with both novel and familiar juvenile rats, total time spent in the juvenile rat's zone as well as the total number of entries into this zone (data not shown) were closely comparable between the Sleep and Wake animals. This makes it highly unlikely that the lack of social recognition memory in the Wake rats was a mere consequence of, e.g., a generally reduced motor activity or a decline in explorative drive, due to increased fatigue or a lack of attention in these animals after the extended wake period (Palchykova et al.,

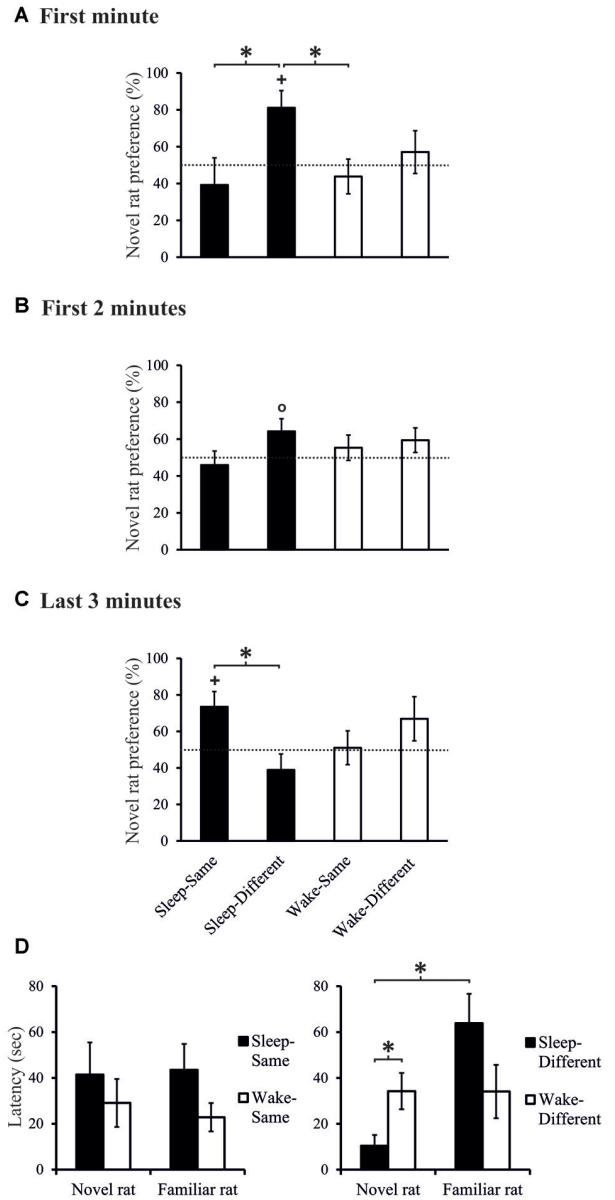

FIGURE 3 | Exploration preference for novel rat (in % of total exploration time) during (A) the first minute, **(B)** the first 2-min interval, and **(C)** during the last 3-min interval of the 10-min Test phase, separately for the Sleep-Same, Sleep-Different, Wake-Same and Wake-Different groups. $^{+}p < 0.05$, $^{\circ}p < 0.1$, for tests against chance level (50%); $^{*}p < 0.05$, for difference between spatial sub-conditions. Note, only the Sleep-Different group shows above chance exploration preference in the first minute of the Test phase which also significantly differs from that of the Sleep-Same and Wake-Same groups (and from that of the Wake-Different group during the first 30-s interval, not shown). At the end of the Test phase, the exploration pattern in the Sleep groups reverses with higher exploration scores in the Sleep-Same than Sleep-Different group. $n = 9$ for all spatial sub-conditions during **(A)** the first minute and **(B)** the first 2-min interval of the Test phases, $n = 8$, 9, 9 and 8, for Sleep-Same, Sleep-Different, Wake-Same and Wake-Different groups, respectively during **(C)** the last 3-min interval of the Test phase. **(D)** Latency to explore (first approach) the novel and the familiar rat, separately for the Sleep-Same, Sleep-Different, Wake-Same and Wake-Different groups. $^{*}p < 0.05$. Note, only the Sleep-Different group shows shorter latency to explore the novel rat in comparison to the familiar rat.

2009; Cho et al., 2010; Colavito et al., 2013). Likewise, stress as a consequence of depriving the rats from sleep, can be ruled out as a factor that substantially affected exploration behavior in the Wake group animals because we chose a rather short period of sleep deprivation (120 min) and sleep deprivation was established by the gentle handling procedure. These conditions are well-known to keep potential stress at a minimum and not to induce substantial increases in blood levels of the stress hormone corticosterone (Kopp et al., 2006; Palchykova et al., 2006; Vecsey et al., 2009; Hagewoud et al., 2010a,b; Melo and Ehrlich, 2016). Moreover, previous experiments of ours (Inostroza et al., 2013; Borquez et al., 2014) comparing effects of a slightly shorter 80-min retention interval of sleep deprivation with effects of a 80-min interval of spontaneous wakefulness (in the animals' active period) did not reveal any difference in subsequent retrieval of hippocampus-dependent memories between these conditions, thus further excluding the possibility that impaired social recognition in our Wake animals would reflect adverse side effects of having the animals deprived from sleep before the Test phase. Also, both adult and juvenile rats were extensively habituated to the experimental setting which included habituation of the juvenile rats to being explored by an adult conspecifics. Thus, stress-related ultrasound vocalizations by the juvenile should not have substantially affected the adult rat's exploratory behavior. Even if there was some vocalization by the juvenile rats, it should have equally affected performance of the Sleep and Wake group animals. Finally, both Sleep and Wake rats displayed comparable learning of the familiar conspecific as indicated by the decrease in exploration time spent with the juvenile rat across the three consecutive sampling sessions of the Learning phase. This assured that Sleep and Wake rats equally well learned to discriminate the familiar juvenile. Thus, the overall pattern of findings justifies to conclude that processes presumably taking place during sleep—rather than at learning or at retrieval—enhanced formation of social recognition memory in the Sleep rats. Nevertheless, this conclusion needs to be further scrutinized, e.g., by experiments directly controlling for possible differential effects on retrieval of sleep and wakefulness immediately preceding the retrieval test.

Our paradigm allowed us to test possible interactions between social recognition and spatial memory formation. During the three consecutive sampling sessions the adult rat was exposed to the juvenile rat presented at three different locations in the maze, and the decrease in exploration time across sampling sessions suggested that in both the Sleep and Wake group the rats were able to recognize the juvenile regardless of the location where it was located. Shifting the conspecific's location across sessions is expected to foster the formation of a social representation that is quite independent of the places where the experimental rat encountered the juvenile during the Learning phase. Nevertheless, our results suggest that the formation of social recognition memory during sleep is modulated by spatial information, and depends on where the familiar juvenile was located on the last sampling session before sleep. After sleep, the adult rat showed a significant preference to explore the novel juvenile rat only when this

novel juvenile was placed at a location different from that of the familiar juvenile during the last sampling session. Moreover, these Sleep-Different rats were not only the only group that displayed significantly shorter latencies to explore the novel than the familiar conspecifics, but their latency to explore the novel conspecific was also significantly shorter than that in the respective Wake-Different control group. Considering the distance between the novel and familiar rat's zones, the latency of exploration is likely related to the rat's ability to detect the novelty of the conspecific based on the non-volatile fraction of the juvenile rat's olfactory signature. Overall, these effects of sleep revealed exclusively in the Sleep-Different group support the view that consolidation processes during sleep do not enhance the social representation of the conspecific *per se*, but that these processes act primarily on episodic-like representations (Kart-Teke et al., 2006; Inostroza et al., 2013) binding the social event into concurrent spatial contexts.

Interestingly, the Sleep-Same rats which encountered the novel juvenile at the same place as that of the familiar rat in the last sampling session, also formed significant recognition memory for the juvenile conspecific. However, this memory expressed only after a substantial delay in the last 3 min of the Test phase. This late onset of preferential exploration of the novel conspecific is difficult to explain, though it corroborates the view that social and spatial memory formation during sleep interact. It might be explained by a competing influence of spatial memory for the familiar conspecific's location at the last sampling session which prevented an earlier expression of recognition memory for the juvenile. A previous study provided evidence for competition between two memory domains (item vs. space) in recognition memory (Haettig et al., 2011). In that study, mice failed to show preference for a novel object when the location of a familiar object was changed between learning and testing. Reversible inactivation of the dorsal hippocampus revealed that object recognition memory *per se* remained intact in this modified test context, suggesting that such competition affects primarily the expression of memory rather than the memory itself. Accordingly, it is possible that in the present study sleep independently enhanced spatial and social aspects of the sampling sessions experienced before, and that the different dynamics in the expression of exploratory preferences between the Sleep-Different and Sleep-Same group were merely due to the fact that in the latter group, placing the novel juvenile at the same location as that of the familiar juvenile during the last sampling epoch induced competition. However, this explanation is unlikely for the following reasons: rats of the Sleep-Different group expressed most pronounced exploratory preference in the very beginning, i.e., within the first minute of the Test phase and, thereafter quickly ceased to show preference behavior which is a typical dynamics for memory-driven exploratory behavior (e.g., Dellu et al., 1992; Dix and Aggleton, 1999; Chambon et al., 2011). By contrast, in the Sleep-Same group a significant exploratory preference for the novel juvenile emerged not until the seventh minute of the Test phase, i.e., long after any competing spatial exploration behavior should have ceased. Moreover, analysis of the Sleep-Same rats did not provide any evidence that these rats during the first minute of the Test phase, more strongly engaged in exploring the novel spatial aspects (i.e., the spatially displaced familiar juvenile).

We did not include a "non-social" control condition in our study (with object presented instead of juvenile conspecifics) which limits the interpretation of our findings as to whether they are specific to social experience. However, previous studies have revealed that the concurrent displacement of a familiar object can prevent expression of novel object preference (Haettig et al., 2011), very much agreeing with the present experiments using juvenile conspecifics instead of objects, and there is likewise evidence that sleep enhances the binding of an experienced object into its spatial context (Binder et al., 2012; Oyanedel et al., 2014). Also, performance during the Learning phase suggested that memory formation as tested in our task does not differently operate depending on whether social or non-social stimuli were employed. Rats of all groups learned to discriminate the (familiar) juvenile rat across the three sampling sessions as indicated by a most robust decrease in exploration time in the first minute of the 10-min sessions, similar to what is typically seen with non-social objects (e.g., Antunes and Biala, 2012). Interestingly, this decrease in exploration time during the Learning phase indicated that the rats learned to recognize the juvenile conspecific independent of its spatial location (which changed across sampling sessions) which is in stark contrast to the performance of the Sleep-Same animals which during the Test phase showed a distinctly delayed expression of social memory (i.e., not until the last 3 min of the Test phase). Thus, it appears that spatial context binding does not influence the formation of social recognition memory on the short-term (i.e., across the sampling sessions spaced 10 min apart) but rather emerges as an aspect of intermediate term consolidation during sleep.

It might also be argued that sleep promoted memory for a rule the rats learned across the three sampling sessions of the Learning phase, i.e., there is juvenile rat in the apparatus and it is always placed at a location different from that during the preceding trial. This view assumes that the rat learns to avoid arms of the maze that have been visited most recently. However, this explanation is very unlikely for two reasons: first, rats typically need much more trials than just the three sampling trials of the Learning phase in the present experiments to learn an alternation-like rule where rats requires to avoid the most recent response (e.g., Aggleton et al., 1986; Dudchenko, 2001). Second, if sleep had enhanced such learned rule—or if sleep had just enhanced spatial learning *per se*-, the Sleep-Same rats in the Test phase should have shown shorter latency to explore the displaced familiar juvenile and/or increased exploration time toward the displaced familiar conspecific in the beginning of the Test phase. However, exploration latencies and times in the Sleep-Same rats were very similar for the familiar and novel juveniles.

Our study aimed at establishing behavioral evidence that sleep affects the formation of social recognition memory and that this effect might interact with spatial episodic aspects of memory formation. Against this backdrop our study is limited

as it does not provide any direct insight into the underlying neurophysiological mechanism of the effect of sleep. Recent studies have implicated a role of the hippocampal subfield CA2 in social recognition and contextual memory (Wintzer et al., 2014; Alexander et al., 2016), allowing for direct interactions between these aspects of memory to occur during sleep-dependent consolidation (Inostroza et al., 2013). Exposure to a conspecific induced remapping of CA2 place fields indicating how CA2 encodes social stimuli by modifying existing spatial representations. The CA2 region was found to be most sensitive in response to subtle changes of familiar spatial context, and remapping of CA2 ensembles for such spatial context also occurred when a familiar or novel conspecific was added to this context (Alexander et al., 2016). Together, this evidence suggests that during encoding the hippocampus integrates both social and spatial information into a single episode. Consequently, sleep might benefit social recognition memory by strengthening the integrated neuronal representation of the episode thereby enhancing social information as it is embedded in the spatial context most recently experienced. Our results support this view by demonstrating that after sleep the rats rapidly discriminated the familiar and novel conspecifics only in conditions most similar to the recently experienced episode (last sampling session), whereas displacing the familiar rat to a novel place attenuated social recognition. In this way the present findings might also be relevant for the understanding of social recognition in healthy humans as well as in patients with social-deficit disorders (like autism-spectrum disorders) that go along with specific alteration of sleep (e.g., Hirata et al., 2016; Mutluer et al., 2016).

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AUTHOR CONTRIBUTIONS

EK, JB and MI designed the study. AS collected the data. AS and EK analyzed the data. AS, JB and MI wrote the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnbeh.2017.00028/full#supplementary-material

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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.

Supplementary Material

Sleep Enhances Recognition Memory for Conspecifics as Bound into Spatial Context

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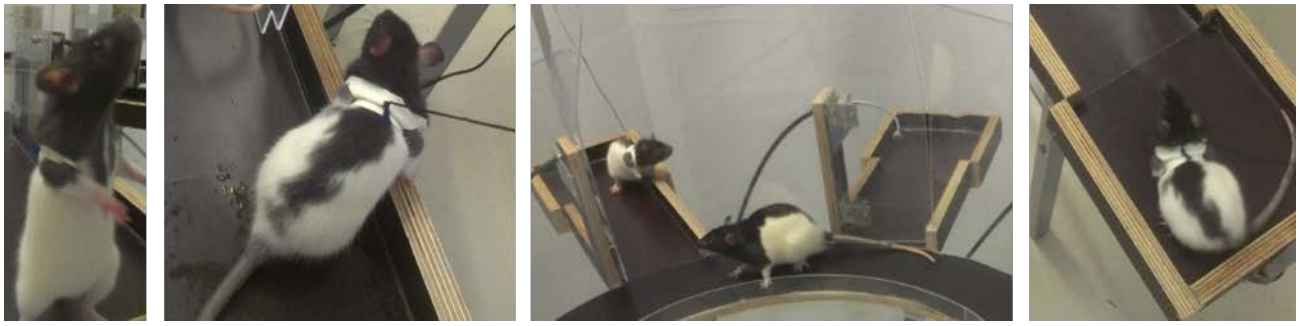
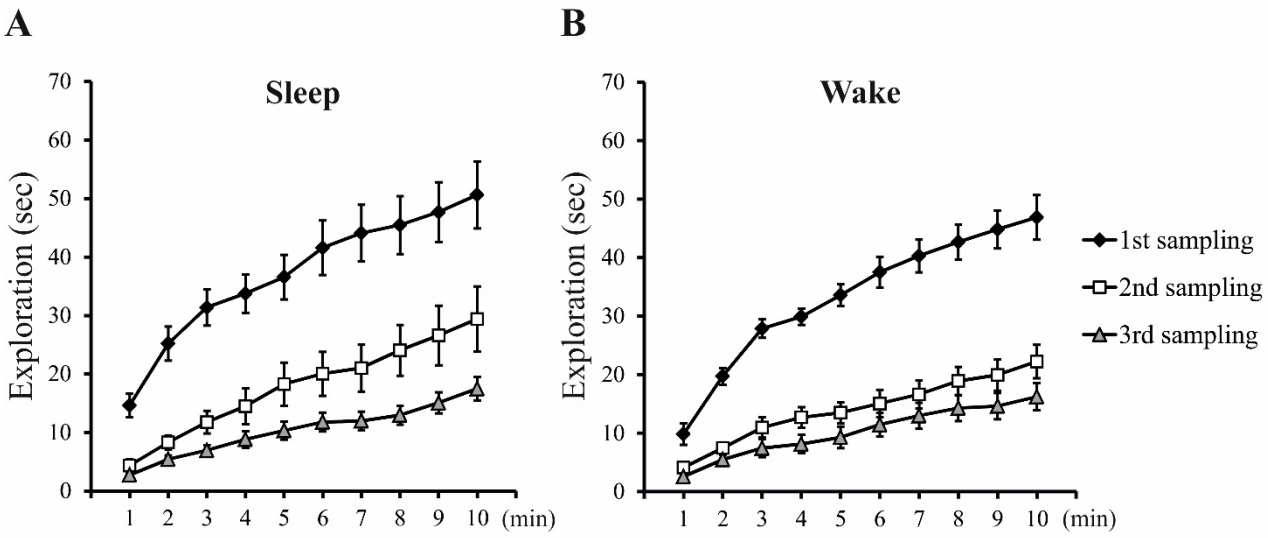
1 Supplementary Figures and Tables

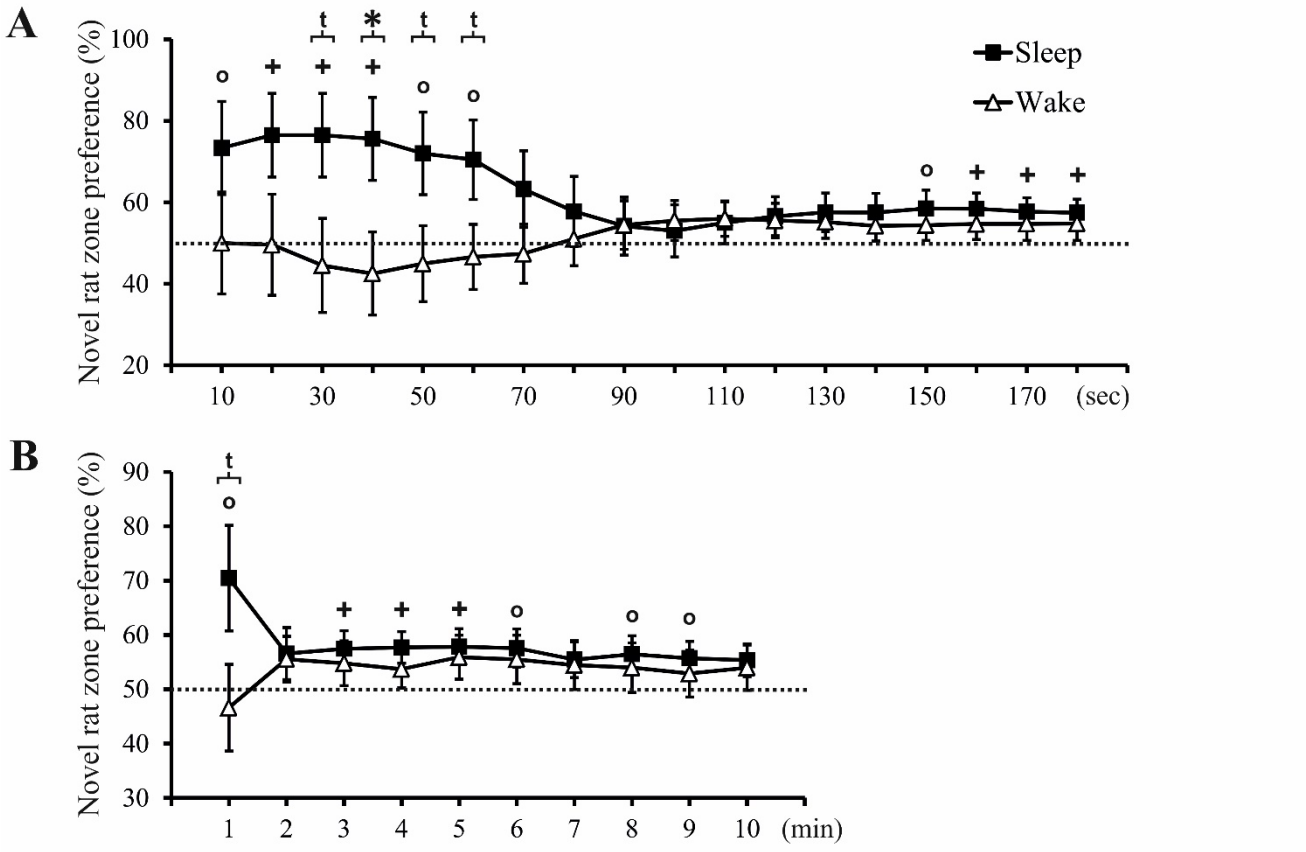
1.1 Supplementary Figures



Supplementary Figure 1. Illustration of a juvenile rat wearing a harness in the juvenile's zone. A harness was used to allow the adult rat to have direct interactions with the juvenile rat and to concurrently control the spatial context of the social stimulus. The juvenile rat was able to freely move within the juvenile zone but could not leave the zone. The procedure allowed the adult rat to perceive both volatile and non-volatile fractions of the individual's olfactory signature.



Supplementary Figure 2. Exploration time during the Learning phase cumulated across 1-min intervals, separately for (A) the Sleep group and (B) Wake group (n=18 for each sampling session in both groups). Note, significant decrease in exploration time from the first to the second and to the third sampling session was observed in both Sleep and Wake groups across 1-min intervals of the Sampling phase ($p<0.006$).There were no significant differences in exploration time between the second and the third sampling session, or between Sleep and Wake groups.



Supplementary Figure 3. Exploration preference assessed by the time spent in the novel rat's zone (in % of total time spent in the zones of the novel and familiar rat) cumulated (A) across 10-sec intervals during the first 3 min of the Test phase, and (B) across 1-min intervals during the total 10-min Test phase, separately for the Sleep group (n = 18) and Wake group (n = 18). +: $p<0.05$, o: $p<0.1$, for tests against chance level (50 %); *: $p<0.05$, t: $p<0.1$, for difference between Sleep and Wake groups.

1.2 Supplementary Tables

Positions of the Juvenile Rat during the 3 Sampling Sessions of the Learning Phase

Position of juvenile rat - arm number - in session 1-2-3	Number of juvenile rats			
	Sleep-Same	Sleep-Different	Wake-Same	Wake-Different
3-5-7	3	3	3	3
7-5-3	4	1	3	1
5-3-7	1	2	2	1
5-7-3	1	3	1	4
Total	9	9	9	9

Supplementary Table 2. For placing the juvenile rat during the Learning phase 4 possible sequences of sampling locations were used, randomized across groups. One juvenile rat served as familiar or (in the Test phase) novel conspecific for 8-12 different adult rats, with a minimum of 24 hours between uses.

Positions of Novel and Familiar Juvenile Rat during the Test Phase

Position of juvenile rat in the Test phase	Numbers of juvenile rats			
	Sleep-Same	Sleep-Different	Wake-Same	Wake-Different
3 (Familiar) – 7 (Novel)	4	4	6	5
7 (Familiar) – 3 (Novel)	5	5	3	4
Total	9	9	9	9

Supplementary Table 2. During the Test Phase the novel and familiar juvenile rats were presented in two possible sequences of locations.

3. Discussion

3.1) Temporal development of sleep's effects on consolidation of hippocampus-dependent and non-hippocampus-dependent memory

The "Study 1" and "Study 2" investigated the effects of post-encoding sleep compared to wakefulness on consolidation of hippocampus-dependent and non-hippocampus-dependent forms of memory in an object-place recognition (OPR) or novel-object recognition (NOR) task, respectively, in rats as well as described the distinctive features of sleep underlying the formation of long-term object recognition memory.

The first aim of the "Study 1" was to investigate the temporal development of the sleep's effects on the formation of recent and remote memories for the two different types of object recognition memory. Rats were allowed to freely explore two identical objects in a familiar open field surrounding by several distinct visual contextual cues. The encoding phase was followed by a 2-hour period during which the rats either slept in a sleeping box or remained awake. Memory retrieval was tested either immediately after the 2-hour post-encoding interval (recent test) or 1 week or 3 weeks later (remote test) (Study 1, Figure 1a). At the retrieval phase, the rats were re-exposed to the familiar experimental context and were allowed to explore two objects. For testing hippocampus-dependent OPR memory, one of the two familiar objects of the encoding phase was moved to a different location in the open field. Exploration preference to the displaced object indicates memory for spatial location of the objects. In contrast, for testing non-hippocampus-dependent NOR memory, one of the two familiar objects was replaced by a novel object, while locations of both objects remained the same as those at the encoding phase. In this task, exploration preference to the novel object indicates memory for the familiar object of the encoding phase.

In the recent OPR testing, only rats that had slept after encoding, compared to being awake, could discriminate a displaced object from an object remained at the same location as during the encoding phase (Study 1, Figure 1b; Study 2, Figure 1b). In contrast, rats could discriminate a novel object from

a familiar object in the recent NOR testing regardless of whether or not the rats had slept after encoding (Study 1, Figure 1b). These findings confirm previous studies from our lab and others using the same behavioral tasks in rats (Binder et al. 2012, Inostroza et al. 2013, Ishikawa et. 2014, Oyanedel et al. 2014) and have been taken as evidence to support that sleep preferentially benefits hippocampus-dependent memory. Preliminary results indicated that rats which had been awake after encoding object information could perform the OPR task when the retrieval was tested 10 minutes later (unpublished data), whereas without sleep after encoding the rats could no longer discriminate the object which had been moved when the interval between the encoding and testing was extended to 80 minutes (Inostroza et al. 2013). This evidence suggests that hippocampal memory traces decay quickly and sleep is required for preserving such traces. Without sleep after learning hippocampal memory traces could not be maintained for a period longer than 80 minutes.

Interestingly, post-encoding sleep benefited non-hippocampus-dependent NOR memory only when the interval between the encoding and testing was extended more than 1 week. Rats in both conditions (i.e. post-encoding sleep and wakefulness) showed exploration preference to the novel object at the 1-week test indicating that the NOR memory was still preserved, and sleep did not benefit memory performance at this time point. Remarkably, at the 3-weeks NOR testing, only rats in the post-encoding sleep condition could discriminate a novel object, whereas rats which remained awake during the 2-hour post-encoding interval showed no exploration preference (Study 1, Figure 1b). Remote NOR memory in the wake condition had faded already at the 2-week retrieval test (Study 1, Extended data Figure 2c), suggesting that without sleep immediately after encoding the rats are not capable of forming long-lasting (> 1 week) remote NOR memory. The results from “Study 1” argue against the notion suggested by previous works that only the consolidation of hippocampus-dependent memory critically relies on sleep whereas consolidation of non-hippocampus-dependent memory does not (Graves et al. 2003, Cai et al. 2009, Inostroza et al. 2013, Ishikawa et al. 2014, Oyanedel et al. 2014).

It is important to note that although these two types of object recognition task (i.e. OPR and NOR tasks) were used to assess different components of recognition memory, the encoding phase of both tasks and the experimental contexts were the same. The objects used in both “Study 1” and “Study 2” as well as in within-study group comparisons were the same set of objects and were also counterbalanced between tasks. Therefore, the findings from “Study 1” suggest that memory for objects can be maintained for relatively longer period than memory for their spatial locations. Accordingly, in both “Study 1” and “Study 2” OPR memory deficits in the wake condition for both recent and remote testing were not caused by failure to recognize the object identity, but rather by failure to detect change of the object’s placement location. Control parameters such as total exploration time of both objects at encoding and retrieval testing as well as locomotor activity (e.g. average speed and distance travelled in the open field) did not differ between sleep and wake conditions in all experiments. These control results provide further support that memory deficits observed in the wake conditions were not influenced by non-specific changes in motivation or alertness, but rather by memory-driven exploratory behaviors.

In summary, the “Study 1”, to my knowledge, provides the first evidence that sleep also supports consolidation of non-hippocampus-dependent object memory. However, the beneficial effects of post-learning sleep only emerged 2 weeks after the encoding, when the object memory in the wake condition had decayed. Since most of sleep studies focus on short-term effects of sleep on memory performance, it is tempting to speculate that other types of non-hippocampus-dependent memory (e.g. procedural memory) might also profit from sleep when the memory performance is tested after a prolonged delay (Stickgold et al. 2000, Schönauer et al. 2015).

3.2) The hippocampus is crucial for forming non-hippocampal long-term memory during sleep

As previously mentioned, the perirhinal cortex is critical for processing object information in mammalian brain. Distinct stages of processing object recognition memory (i.e. encoding, consolidation, and retrieval) rely on functional integrity of the perirhinal cortex (Brown and Aggleton 2001, Winters et al. 2004, Winters and Bussey 2005b, Winters et al. 2008). In contrast, the hippocampus is essential for object recognition memory only when the behavioral task requires the animals to remember an individual object in a particular location or a particular context (Mumby et al. 2002, Barker and Warburton 2011), or when spatial, contextual, and/or temporal aspects of the task are constituted as episodes (Eacott and Norman 2004, Dere et al. 2005, Kart-Teke et al. 2006, Langston and Wood 2010).

In the “Study 1”, preserved NOR memory in the sleep condition which was revealed only after a long delay (2 weeks) suggests that the formation of long-term non-hippocampus-dependent object memory is not only a sleep-dependent process, but also a time-dependent process. The benefits of sleep might involve large-scale changes in structural neural networks serving as long-term storage for object information. According to the standard consolidation theory, repeated hippocampal activation promotes the gradual transformation of initially encoded hippocampal memory traces to neocortical long-term storage site. This systems-level memory consolidation process takes time and sleep has been proposed to facilitate this process (McClelland et al. 1995, Frankland and Bontempi 2005, Diekelmann and Born 2010, Inostroza and Born 2013). This theory has been primarily conceptualized regarding to the hippocampus-dependent memory system. However, it is not clear about how non-hippocampus-dependent forms of memory are consolidated and whether the hippocampus also involves in this process. Therefore, the second aim of “Study 1” was to investigate whether the hippocampus also critically contribute to the sleep-dependent consolidation of remote NOR memory. To determine the role of the hippocampus during sleep on the formation of long-term NOR memory, the $GABA_A$ receptor agonist muscimol was used to temporary inactivate the

hippocampus during post-encoding sleep, while leaving hippocampal function undisturbed during the encoding and retrieval of NOR memory. After encoding the NOR task, either muscimol or saline (control condition) was infused bilaterally into the dorsal hippocampus when the rat showed continuous SWS for at least 10s. This infusion procedure did not disturb the rat's ongoing sleep and the onset of substance administration did not differ between muscimol and control groups.

The "Study 1" indicates that the hippocampus is crucial for forming long-term non-hippocampal NOR memory during sleep. Rats that had received saline infusion into the dorsal hippocampus during sleep after learning showed exploration preference to the novel object at the 3-weeks remote NOR testing (Study 1, Figure 2a). This finding was consistent with the previous behavioral result (Study 1, Figure 1b). The rats from both behavioral and hippocampal saline infusion groups showed similar memory performance on the NOR task, suggesting that the surgical procedures, the substance delivery method, and the control substance used for intrahippocampal infusion did not influence recognition memory performance in the hippocampal infusion groups. Remarkably, rats with intrahippocampal infusion of muscimol during post-encoding sleep showed NOR memory deficits at the 3-weeks remote testing (Study 1, Figure 2a). In this hippocampal inactivation group, rats could not discriminate between the novel and familiar objects. Importantly, effects of hippocampal inactivation were restricted to the consolidation phase (i.e. lasted around 2 hours after infusion) (Martin 1991), suggesting that long-term consolidation of object memory relies on hippocampal mechanisms during sleep. To further investigate the involvement of the hippocampus in distinct processes of object recognition memory, in separate groups of rats, muscimol was infused into the hippocampus either 1) before encoding phase of the recent (30 min) NOR task (Study 1, Figure 2c), or 2) before retrieval phase of the recent (30 min) (Study 1, Figure 2c), or the remote (3 weeks) NOR task (Study 1, Figure 2a). The results demonstrated that none of the hippocampal inactivation groups showed NOR memory deficits. These findings confirm previous studies suggesting that the hippocampus is not necessary for

encoding and retrieval of NOR memory (Winters et al. 2004, Winters et al. 2008, Barker and Warburton 2011). Previous studies further suggest that performance on NOR task might be impaired after hippocampal lesions when the task involves spatial-contextual features (e.g. Winter et al. 2004). This study argues against their notion, but further extends their findings indicating that rats with hippocampal inactivation can also form memory for objects, although possible interferences from hippocampus-dependent processing of spatial-contextual information are not minimized. Together, the "Study 1" indicates that the hippocampus has an essential role in formation of long-term memory during sleep even for the NOR memory which has previously been classified as non-hippocampus-dependent memory.

It should be noted that the remote 1-week NOR memory was preserved not only in the rats which had slept after encoding, but also in the rats which remained awake during the 2-hour post-encoding interval (Study 1, Figure 1b). Therefore, hippocampal activity during wakefulness might also involve in the consolidation of long-term (1-week) NOR memory as well. To further examine this possibility, intrahippocampal infusion of muscimol was conducted during post-encoding wakefulness and the NOR memory was tested 1 week later. Interestingly, suppressing hippocampal activity during wake consolidation did not disturb NOR memory at the remote 1-week testing. There was even a tendency towards enhanced NOR memory when compared with a wake control condition (Study 1, Figure 2b), which, in this case, represents the strictest control condition because the rats did not undergo surgery and the hippocampus was assuredly intact. This enhancement of NOR memory at the remote 1-week test might reflect interactive multiple memory systems in the brain (Poldrack and Packard 2003). This hypothesis suggests that multiple memory systems are activated in parallel and can interact with each other, raising the possibility that interference may occur. Previous studies in rodents reported the competitive interaction of the hippocampus in the NOR task. Infusion of muscimol into the dorsal hippocampus after learning the NOR task enhanced object recognition memory tested 24 hours later (Oliveira et al. 2010), while post-learning intrahippocampal infusion of $GABA_A$ receptor antagonist

drug, bicuculline, impaired object recognition memory (Kim et al. 2014). Interestingly, in these two studies the modulation of hippocampal activity after encoding had no effect on object recognition memory when the animals were repeatedly exposed to the learning context. These findings suggest that hippocampal activity interferes with the consolidation of object memory when object information is encoded in an unfamiliar context. However, in the “Study 1” rats were allowed to freely explore the learning context for 3 consecutive days (10 min per day) before the encoding phase, and preliminary results revealed that rats’ locomotor activity at the habituation phase decreased after second exposure to the open field and did not decrease further in the last habituation session (i.e. the third habituation session) (unpublished data), suggesting that the rats were habituated to the learning context. Therefore, it is unlikely that enhanced NOR memory after hippocampal inactivation in the wake condition can be fully explained by decreased interference from the concurrent consolidation of contextual information in the hippocampus. The enhancement of NOR memory in this condition might rather reflect compensatory plasticity in extra-hippocampal regions upon hippocampal suppression (Goshen et al. 2011). The state dependency of the hippocampus for forming long-term memory might suggest distinct consolidation processes between sleep and wake conditions. Whatever the case, the findings of “Study 1” indicate that formation of long-lasting (3-weeks) non-hippocampus-dependent object memory relies on a hippocampal mechanism which is specifically active during sleep, whereas extra-hippocampal mechanisms during post-encoding wakefulness maintain object recognition memory over 1-week period.

3.3) How does the hippocampus contribute to long-term memory consolidation during sleep in a task which the hippocampus is not required for initial learning?

Based on a traditional classification of the memory systems, the hippocampus is required for encoding and retrieval of hippocampus-dependent memory. This is not the case for non-hippocampus-dependent memory which normal hippocampal function is not required for encoding and retrieval. An intriguing question arises from “Study 1” “how does the hippocampus contribute to consolidation of non-hippocampus-dependent object memory during sleep?”.

It should be noted that the NOR memory which is classified as non-hippocampus-dependent does not exclude any susceptibility of the task performance to hippocampal manipulations, for instance, the degree of contextual habituation (e.g. Oliveira et al. 2010, Kim et al. 2014), numbers of encoding sessions (Broadbent et al. 2010, Iwamura et al. 2016), or availability of spatial and contextual cues (Winters et al. 2004). The variability of NOR task protocols across studies drives a long-standing debate about the role of hippocampus in object recognition memory in rodents. A qualitative model was proposed in attempt to reconcile the discrepancies between findings (Cohen and Stackman 2015). This model suggests that strength of acquired object information is a decisive factor determining when the hippocampus and perirhinal cortex are contributing to object recognition memory. Cohen and Stackman (2015) proposed that the perirhinal cortex initially encodes object information. As the exploration continues and when a threshold amount of the acquired information is reached, object information begins to “transfer” to the hippocampus and will become hippocampus-dependent memory. Weakly encoded object memory is stored in the perirhinal cortex. The findings of “Study 1” suggest that, instead of “transferring” object information from the perirhinal cortex to the hippocampus, both structures in the medial temporal lobe might interact to form episodic representations of object memory. In the “Study 1” and “Study 2”, an open field was used as an experimental apparatus. Object recognition test in an open field is known to involve spatial and contextual cues and exploratory behavior can be influenced by these spatial-contextual

information (Winters et al. 2004). In both studies, the open field was surrounded by several distinct distal cues which were visible to the rats. Importantly, rats were introduced into the open field in different directions (e.g. facing different arena's wall) across 4 experimental sessions (3 sessions at the habituation phase and 1 session at the encoding phase). This way of introducing the rat into the experimental context facilitates the rat to utilize the distal cues for navigation, thereby facilitating allocentric navigation strategy which requires functional hippocampus (Langston and Wood 2010, Schöberl et al. 2019). Furthermore, although both "Study 1" and "Study 2" did not apply any exploration criteria in the encoding phase (i.e. the fixed amount of time the rat spent exploring objects), the duration of the encoding phase was relatively long (i.e.10 min) which should be sufficient for the rats to acquire object information. Indeed, additional analyses from both studies revealed that object exploration time highly decreased after the third minute of the encoding phase (unpublished data), suggesting that rats sufficiently encode object information. This decrease in object exploration time would therefore allow the rats to acquire more contextual information surrounding the objects. Accordingly, rats could form episodic representations of the event apart from object memory.

Episodic memory composes of three fundamental components: "What", "Where", and "When/Which". In this process, the hippocampus has been proposed to bind together items and contextual information. The binding of items and context (BIC) model (Eichenbaum et al. 2007, Ranganath 2010) has suggested that item information ("What" component) is processed by perirhinal cortex which projects to lateral entorhinal cortex, while spatial-contextual information is processed by parahippocampal cortex (the homologue of postrhinal cortex in rodents) which projects to medial entorhinal cortex. These two information streams are converged in the hippocampus for associations between items and their contextual information. Correspondingly, object recognition tests in rodents support this hypothesis showing that perirhinal cortex-lesioned rats were impaired on memory for objects, while postrhinal cortex-lesioned rats were impaired on memory for object in context (Norman and Eacott 2005). Furthermore, the hippocampus has been shown to be

involved in processing memory for an object presented in a particular place and/or a particular context (Langston and Wood 2010, Barker and Warburton 2011). This engagement of hippocampus in object recognition memory requires a coordination between the hippocampus and the perirhinal, or medial prefrontal cortex (Barker and Warburton 2011). In the “Study 1”, inactivation of the hippocampus during post-learning sleep impaired non-hippocampal long-term object recognition memory. Notably, muscimol infusion was likely restricted to a small area of CA1 region of the dorsal hippocampus (Study 1, extended data figure 5). Given that a small volume of drug (0.5 µl/ hemisphere) was infused, it is likely that some parts of the dorsal hippocampus and most ventral parts of the hippocampus remain functional after muscimol infusion (Martin 1991, Corcoran et al. 2005). In preliminary experiments, with the same infusion procedures as the “Study 1”, this relatively small area of dorsal hippocampal inactivation caused impairment of hippocampus-dependent OPR memory in rats with pre-encoding intrahippocampal infusion of muscimol (unpublished data). Strikingly, the discrete inactivation of hippocampal CA1 during sleep yielded a significant effect on remote (3-weeks) NOR memory (Study1, Figure 2a), suggesting a critical role of this hippocampal region for forming long-lasting object recognition memory during sleep. The CA1 region of the hippocampus receives spatial information inputs directly from CA3 region and medial entorhinal cortex, together with direct non-spatial inputs from perirhinal cortex and lateral entorhinal cortex (Kealy and Commins 2011), making this region suitable for integrating information across different memory domains. Accordingly, recent evidence revealed a critical role of hippocampal CA1 region in episodic-like memory in rats. Muscimol infusion into the dorsal CA1, before exposure to objects, impaired both spatial (“What” and “Where”) and temporal (“What” and “When”) components of the episodic-like memory task tested 24 hours after encoding (Barbosa et al. 2012). Moreover, recent study indicated that the integration aspect of this task (“What”, “Where”, and “When”) was also impaired after dorsal CA1 inactivation (Drieskens et al. 2017). Interestingly, mapping neuronal activation by detecting immediate-early gene *Arc* after mice performed hippocampus-dependent OPR or non-hippocampal NOR memory test revealed

that both tasks induced a comparable level of *Arc* expression within the hippocampal CA1 region, postrhinal cortex, and entorhinal cortex, whereas the expression within the perirhinal cortex was tuned to the stimulus type, independent of the spatial demand of the tasks (Beer et al. 2013). This evidence suggests that although the behavioral task is not specifically design for testing episodic-like memory in rodents, retrieving object information can also trigger recollection of episodic representations of the event. Altogether, previous findings suggest that hippocampal CA1 region plays an essential role in episodic-like memory in rodents. In the "Study 1" inactivation of the hippocampal CA1 region during sleep might disrupt integrative functions of the hippocampus to forming episodic representations of the event which are required for organizing long-term consolidation across different memory domains.

The "Study 1" indicated that sleep spindles during SWS contribute to consolidation of long-term memory. Retrieval of remote (3-weeks) NOR memory was correlated with spindle activity during post-encoding SWS (Study1, Figure 3a). Spindles have been implicated to facilitate synaptic plasticity changes in both hippocampal and neocortical networks (Seibt et al. 2017). Spindle-like firing patterns induced both short-term potentiation (STP) and long-term potentiation (LTP) in neocortical synapses (Rosanova and Ulrich 2005). Previous studies indicate that synaptic plasticity in both the hippocampus and perirhinal cortex is necessary to modulate object recognition performance in rodents. Exposure to objects increased hippocampal synaptic plasticity in mice (Clarke et al. 2000). Increased synaptic potentiation in the hippocampal CA1 region after overexpression of NMDA receptor 2B subunit (NR2B) in transgenic mice leaded to superior performance on the NOR task, relative to control mice (Tang et al. 1999). In contrast, NMDA receptor dysfunction in the hippocampal CA1 region impaired hippocampal LTP and caused NOR memory deficits in rodents (Rampon et al. 2000, Kersten et al. 2019). Furthermore, pre-encoding infusion of NMDA receptor antagonist, that blocks LTP, in the hippocampus or perirhinal cortex impaired NOR memory tested after a long (3- or 24- hours) post-encoding delay but not after a short (5- or 20-min) delay (Baker and Kim

2002, Winters and Bussey 2005a, Barker et al. 2006). Consistent with these findings, the “Study 1” suggests that spindle oscillations during post-encoding SWS might promote long-term synaptic plasticity in both hippocampal and extra-hippocampal regions for the formation of long-lasting object recognition memory.

Interestingly, “Study 1” showed that the correlation with spindle activity was strongest in the first 30 min of post-encoding sleep (Study1, Figure 3a, low panel), corresponding to the time in which neuronal reactivation in the hippocampus occurs most robustly (Kudrimoti et al. 1999, O’Neill et al. 2010, Giri et al. 2019). Hippocampal ripples accompany neuronal ensembles reactivation which mostly occurs during SWS. In separate experiments, intrahippocampal LFP recordings revealed that muscimol infusion during sleep reduced hippocampal ripple activity during SWS without affecting cortical EEG activity (Study1, Figure 3b and Extended data table1). These results suggest that the formation of long-term NOR memory requires spindles and ripples-associated neuronal reactivation in the hippocampus during SWS. The temporal coupling of spindle-ripple events has been identified as a candidate mechanism for forming long-term memory during sleep. Sleep spindles are thought to promote the transmission of ripples-associated memory reactivation from the hippocampus to extra-hippocampal networks (Latchoumane et al. 2017, Klinzing et al. 2019), together with inducing synaptic plasticity changes (Rosanova and Ulrich 2005, Seibt et al. 2017), thereby strengthening memory representations in these extra-hippocampal regions for long-term memory storage. According to a functional double dissociation between the perirhinal cortex and hippocampus in object recognition memory, one might assume that reactivation of the perirhinal cortex, which is anatomically connected with the hippocampus (Kealy and Commins 2011) and is critical for encoding and storing object memory, should facilitate the formation of long-lasting NOR memory, whereas reactivation of newly encoded hippocampal neuronal ensembles should not critically contribute to this process. Although, no evidence has emerged to date that neuronal ensembles representing newly encoded object information in perirhinal cortex are reactivated during subsequent sleep,

cumulative evidence has suggested that reactivation of learning-related neuronal firing patterns in the hippocampus guides the concurrent reactivation in extra-hippocampal regions. Indeed, the reactivation in extra-hippocampal area occurs slightly after the hippocampal reactivation. Ji and Wilson (2007) trained rats to run an alternation running task on a figure 8-shaped maze. The reactivation of temporally structured firing patterns was observed in both the hippocampus and visual cortex during SWS. However, the reactivation of awake experience in the hippocampus tended to lead those in the visual cortex. Furthermore, Peyrache et al (2009) employed an attentional set shift task, which performance in this task relies mainly on medial prefrontal cortex, to investigate memory reactivation in the hippocampal-prefrontal network in rats. They showed that after learning a new rule, the reactivation of learning-related neuronal firing patterns in the medial prefrontal cortex during sleep slightly followed (~40 ms) hippocampal sharp-wave ripples, corresponding to the duration of synaptic transmission between the hippocampal neurons and pyramidal cells of the prefrontal cortex (Dégenètais et al. 2003). In addition to the hippocampus and neocortex, previous studies revealed that the memory reactivation was also observed in a subcortical structure. Reactivation in the ventral striatum after learning a reward-searching task also predominantly occurred during post-learning SWS and was modulated by hippocampal sharp-wave ripples (Pennartz et al. 2004, Lansink et al. 2009). Importantly, this hippocampal reactivation of spatial information leaded the reactivation of reward-associated neuronal firing in the ventral striatum (Lansink et al. 2009). During learning the task, the sequence of firing patterns in the associated hippocampal-striatal neuronal pairs were bidirectional (for instance, the neuronal pairs fired in the direction from the hippocampus to the ventral striatum and also in the opposite direction from the ventral striatum to the hippocampus). However, during subsequent sleep the sequential firing pattern from the hippocampus to the ventral striatum was predominantly observed. Overall, these studies suggest an influence of the hippocampus in triggering experience-dependent reactivation in extra-hippocampal area, even for the behavioral task which does not rely primarily on the hippocampus during the initial learning.

This coordinated reactivation of learning experience in both structures is exclusively observed during post-learning SWS with a close temporal relationship with hippocampal sharp-wave ripples.

Taken together, the findings of “Study 1” suggest that memories for items (i.e. non-hippocampus-dependent memory) are encoded together with their contextual information (i.e. hippocampus-dependent memory). Accordingly, spindle-ripple events during SWS promote reactivation of learning-associated neuronal ensembles from both hippocampus-dependent and non-hippocampus-dependent components of the encoded episode. The coordinated reactivation of episodic memory representations is required for the formation of long-term memory during sleep even for the memories which are previously believed to be hippocampus-independent. Recent human study provides direct support for this suggestion, showing that hippocampal damaged patients performed equally well relative to control participants in learning a motor sequence task. However, only control participants showed performance improvement after an overnight sleep (Schapiro et al. 2019).

3.4) Sleep longer? or sleep deeper? Which one is better for forming long-term memory?

Based upon the findings of “Study 1” that sleep after learning enhances long-term memory formation, the “Study 2” asked more basic questions about which basic sleep feature is the most important factor for forming long-term memory. Does memory formation benefit more from a longer sleep duration? Or is the depth of sleep more essential for long-term memory consolidation?

The aim of “Study 2” was to dissociate effects of duration and depth of sleep on the formation of recent and remote hippocampus-dependent object-place recognition (OPR) memory. In this study, the depth of sleep was manipulated by extending the time rats spent in sleeping environment. In the habituation phase, one group of rats spent 2 hours in the sleeping box for 3 consecutive days (6 hours in total), while another group spent 4 hours in the sleeping box during these habituation sessions (12 hours in total). Importantly, other behavioral procedures of the OPR task, including the total duration were

the rat exposed to the open field in the learning context, were the same as described in the “Study 1”. This sleep manipulation procedure differentiated the two groups of rats based on their sleep profiles: “regular sleep” and “deeper sleep”. The deeper sleep was characterized by a selective increase in the time spent in SWS and in the SWS-related oscillatory activities. Characteristics of the deep sleep will be discussed further below. In the “Study 2”, rats were assigned to six groups to investigate the effects of duration and depth of sleep on the formation of recent (2 hours) and remote (1 week) OPR memory. Different groups of rats differed in the depth of sleep during the post-encoding period (regular sleep vs. deeper sleep), and the duration of the post-encoding period (2 hours vs. 4 hours). Two groups tested recent OPR memory, i.e., “regular sleep/ 2-hour post-encoding period”, and “deeper sleep/ 2-hour post-encoding period”, and four groups tested remote 1-week OPR memory, i.e., “regular sleep/ 2-hour post-encoding period”, “regular sleep/ 4-hour post-encoding period”, “deeper sleep/ 2-hour post-encoding period”, and “deeper sleep/ 4-hour post-encoding period” (Study 2, Figure 1a).

The “Study 2” showed that the depth of post-encoding sleep influences the formation of long-term hippocampus-dependent OPR memory. Recent OPR memory (2-hours) was preserved only after sleep in both regular and deeper sleep conditions. The memory performance did not differ between the two sleep conditions (Study 2, Figure 1b). In all groups for both recent and remote testing, rats which had been awake after encoding showed no exploration preference to the objects, indicating spatial memory deficits in both 2-hour and 1-week retention intervals. Sleep is required for the consolidation of OPR memory confirms several previous findings in rats which OPR memory was tested up to 24 hours after learning (Ozawa et al. 2011, Binder et al. 2012, Bett et al. 2013, Inostroza et al. 2013, Ishikawa et al. 2014, Oyanedel et al. 2014, Howard and Hunter 2019). However, at the 1-week remote OPR testing, rats in the regular sleep condition could not discriminate the object which had been moved (relative to its location at the encoding phase) from the object which was placed at the same location as the encoding phase, even when the duration of post-encoding sleep was extended to 4 hours (instead of 2 hours) (Study 2, Figure

1c left). Extending duration of post-encoding sleep was shown to benefit a hippocampus-dependent visuo-spatial task in humans (Diekelmann et al. 2012). Remarkably, the “Study 2” showed that sleep benefits the formation of long-lasting OPR memory only when the post-encoding sleep is of deeper quality. In the deeper sleep condition, rats could discriminate the displaced object from the stationary object at the 1-week remote memory test and the memory performance was superior to that of the wake condition (Study 2, Figure 1c, right). Interestingly, performance on the OPR task did not differ between the two groups of deeper sleep condition (i.e., deeper sleep during the post-encoding period of either 2 hours or 4 hours), although one group had longer post-encoding period. This evidence suggests that an increase in sleep duration does not proportionally enhance long-term hippocampus-dependent memory. Previous studies showed that OPR memory can be maintained up to 1 week when the rats re-encoded object information and its spatial configuration for several consecutive days (Hardt et al. 2010, Migues et al. 2016). However, the “Study 2” indicates that, without repeated exposures to the same learning context, a single 10-min encounter to the object configuration is sufficient for forming long-term OPR memory, but only when the rat has deeper sleep after learning.

One possible explanation for the present findings could be that, in the deeper sleep condition, extending sleep duration in the sleep habituation sessions, following habituation to the open field, might facilitate sleep-dependent consolidation of memory for the learning context (i.e., the open field and its surrounding visual cues). Consequently, this enhanced contextual memory might boost the formation of object location memory at the encoding phase. However, it is unlikely to be a major factor influencing long-term memory formation in the present study for following reasons. First, control parameters, for instance, object exploration time, mean speed, and distance travelled in the open field during the encoding and retrieval phases did not differ between rats in the regular sleep and deeper sleep conditions. If contextual memory was enhanced in rats of the deeper sleep condition, then the rats should have changed exploratory behaviors in the open filed. Previous studies consistently

showed that enhanced contextual memory by repeated exposures to a learning context decreased the animal's locomotor activity at the subsequent exposure (e.g. Oliveira et al. 2010, Kim et al. 2014). Second, memory performance of the deeper sleep condition was closely comparable to that of the regular sleep condition at the recent OPR testing. It is therefore unlikely that the difference in remote memory performance could be attributed to the difference in memory strength between regular and deeper sleep conditions. Impaired long-term spatial memory in the regular sleep condition rather reflects distinct consolidation processes during sleep between both sleep conditions.

To probe the efficacy of the sleep manipulation procedure, additional experiments were conducted in different groups of rats. Simultaneous EEG and hippocampal LFPs were recorded during a 2-hour post-encoding period in both regular and deeper sleep conditions. The results indicated that sleep characteristics differed between the two sleep conditions with particular focus on SWS. Rats in deeper sleep condition showed a significant increase in duration of post-encoding SWS, but not pre-REM and REM sleep, compared to the regular sleep condition (Study 2, Figure 2a). Moreover, oscillatory hallmarks of SWS, for instance, number of SOs, spindles, and hippocampal ripples were also enhanced during deeper sleep after encoding. Interestingly, the enhanced duration of SWS as well as its oscillatory activities after encoding were observed exclusively in the second hour of post-encoding sleep (Study 2, Figure 2d-h). The visual sleep scoring results from the rats in the main experiment showed a similar pattern of selectively enhanced sleep duration in the second hour of post-encoding period (Study 2, Figure 3b).

It should be considered that distinct mechanisms underlying memory consolidation during sleep might be employed for different memory systems across different temporal development. In contrast to remote OPR memory, the present results indicated that deeper sleep after encoding did not further enhance OPR memory at the recent memory test. The memory performance did not differ between the regular and deeper sleep conditions when OPR memory was tested 2 hours after encoding. It is tempting to speculate that the higher amount of hippocampal ripple and spindle activities in the deeper sleep

condition might reflect more reactivation of hippocampal memory representations, together with an increase in hippocampal-cortical dialog during SWS, thereby facilitating the gradual redistribution of the memory representations toward cortical networks that serve as long-term storages. Hippocampal memory traces might be preserved in a shorter period without the need of increased hippocampal reactivation during sleep. Conversely, repeated reactivation more frequently in the hippocampus might be required to promote the time-dependent reorganization of the hippocampal-cortical networks for forming more persistent OPR memory. In contrast to long-term hippocampus-dependent memory for object location, deeper sleep after learning is not critical for forming long-term non-hippocampal memory for object identity. Using the same behavioral procedures without manipulating the depth of sleep (i.e. regular sleep condition), the "Study 1" showed that novel-object recognition (NOR) memory was preserved for at least 3 weeks, even when encoding object information was followed by regular sleep (Study 1, Figure 1b). It should also be noted that in the "Study 1" memory for objects can be maintained for up to 1 week without sleep after learning. Rats which remained awake after encoding the NOR task could discriminate the novel object from the familiar object at the 1-week remote memory test (Study 1, Figure 1b). Given that the encoding phase is identical for both NOR and OPR tasks (i.e. the rat explored two identical objects in the same context, Study 1, Figure 1a and Study 2, Figure 1a), impaired long-term OPR memory in the regular sleep condition is not attributed to the failure to recognize object identity, but rather reflects the inability to maintain spatial details that comprise the encoded episode (Moscovitch et al. 2016).

Overall, these findings indicate that the depth of sleep, rather than its duration, is more essential for forming long-lasting hippocampus-dependent memory. Deeper sleep (i.e. a selective increase in the time spent in SWS) during the 2-hour post-encoding period might enhance the temporal interaction between cortical SOs, thalamic spindles, and hippocampal ripples, thereby strengthening memory for object location such that it can be maintained over a long period. However, the consolidation of non-hippocampus-dependent

components of an episode does not require SWS-rich sleep after encoding, suggesting that different components of the episodic memory system might rely on distinct mechanisms during sleep. Benefits of sleep on non-hippocampus-dependent memory is less robust and inconsistent (Rasch and Born 2013). Also, the influences of sleep depth on performance of such task have not yet been investigated. Further studies are needed to examine whether other heterogeneous types of non-hippocampus-dependent memory (e.g. procedural memory) profit from deeper sleep. Such studies can provide valuable insight into the imbalanced effects of sleep on different memory systems.

3.5) Critical time window for sleep to facilitate long-term memory formation

What all three studies in this dissertation have in common is that sleep shortly after learning benefits memory consolidation. These studies showed beneficial effects of sleep on the formation of both recent and remote recognition memory. Notably, although the experimental designs for testing remote memory in the "Study 1" and "Study 2" involve the comparisons between post-encoding conditions (i.e. sleep vs. wakefulness, or regular sleep vs. deeper sleep), a time window for sleep manipulation was limited only to a short period after encoding. In the wake condition of both "Study 1" and "Study 2", sleep was restricted only during the first 2 hours after the rat learned the task. After this experimental time window, the rat was brought back to the home cage and kept under routine conditions until the remote memory testing. It is very likely that rats slept soon after they had returned to their familiar condition. In all three studies, impaired recognition performance in the rats which had the delayed onset of post-encoding sleep suggests a critical time window for sleep to facilitate memory consolidation in both hippocampus-dependent and non-hippocampus-dependent forms of memory.

The present findings support several evidence showing that sleep after learning benefits memory across different behavioral domains (Palchykova et al. 2006, Talamini et al. 2008, Inostroza et al. 2013, Ishikawa et al. 2014). The "Study 2" underlines the importance of the first 2 hours after encoding by

showing that, enhanced duration of sleep, in particular SWS, within this time window strengthened OPR memory (Study 2, Figure 1c, right). Interestingly, extending post-encoding period to 4 hours did not benefit OPR memory performance tested at 1 week (Study 2, Figure 1c, left). Correlation analyses revealed that sleep and particular oscillations in SWS during the first 2 hours after encoding predicted recognition memory performance at the remote testing. In the “Study 2”, OPR memory performance at the remote (1-week) retrieval test positively correlated with the duration of sleep during the 2 hours after encoding (Study 2, Figure 3c, left). There was no similar correlation with the time spent asleep in the 4-hour post-encoding sleep period (Study 2, Figure 3d, left), even when the data were pooled from both the regular sleep and deeper sleep condition (unpublished data). Interestingly, in the deeper sleep condition, an increase in the duration of sleep was evident only in the second hour of post-encoding sleep, but not in the third or the fourth hour after encoding (Study 2, Figure 3b). Importantly, the enhanced sleep duration in the second hour after encoding correlated with the better performance on the OPR task at the 1-week retrieval test (Study 2, Figure 3c, right). A study in mice supports the importance of this time window showing that sleep deprivation beginning 1 hour after learning impaired hippocampal long-term potentiation (LTP) as well as OPR memory tested 24 hours later (Prince et al. 2014). These results suggest that additional sleep periods beyond the 2 hours after encoding provide no further benefit to remote hippocampus-dependent memory. Furthermore, the “Study 1” emphasizes the contribution of spindles and hippocampal ripples within this post-encoding time window to the formation of long-term non-hippocampus-dependent memory. Spindle activity during SWS was correlated with NOR memory performance at the remote (3-week) retrieval test (Study 1, Figure 3a). The total time the rats spent in SWS, however, could not predict remote NOR memory performance. Additionally, temporary inactivation of the hippocampus during post-encoding sleep, for approximately 2 hours by using muscimol (Martin 1991), suppressed hippocampal ripples, without affecting the duration of SWS, and impaired long-term NOR memory (Study 1, Figure 2a and 3b). The present findings suggest that the 2-hour period after encoding provides an

optimal time window for SWS, in particular spindle and ripple activities, to facilitate long-term memory consolidation.

Two hours after learning might represent a critical time window for long-term memory formation during sleep. Reactivation of newly encoded memory in hippocampal networks occurs most frequently during this early post-encoding period (Kudrimoti et al. 1999, O'Neill et al. 2010, Giri et al. 2019). Hippocampal ripples-associated reactivation of neuronal ensembles together with thalamic spindles time locked to the up state of SOs are thought to orchestrate systems-level memory consolidation during SWS. Reinforcing the coordination between SO-spindle-ripple events by ripple-triggered cortical stimulation for 1 hour during post-encoding SWS improved OPR memory performance tested on the next day (Maingret et al. 2016). It was also shown that enhanced sleep spindle and hippocampal sharp-wave ripple activity after learning hippocampus-dependent memory task were maintained up to 2 hours after sleep onset (Eschenko et al. 2006 and 2008). The proposed time window for SWS to facilitate long-term memory consolidation is also consistent with the molecular basis of the consolidation of object recognition memory. Recent study showed that hippocampal protein synthesis is required within 2 hours after learning for the consolidation of OPR memory. Hippocampal administration of protein synthesis inhibitors immediately but not 2 hours after leaning impaired memory performance on the OPR task tested 6 hours later (Ozawa et al. 2017). Also, hippocampal NMDA receptors have been shown to be involved in the early-stage, but not the late-stage, of hippocampus-dependent memory consolidation (Shimizu et al. 2000; Yamada et al. 2017). Furthermore, the consolidation of non-hippocampus-dependent NOR memory also requires protein synthesis in the hippocampus within this time window. Infusion of protein synthesis inhibitor, anisomycin, in the rat's hippocampal CA1 region immediately or 3 hours, but not 6 hours, after encoding object information impaired NOR memory performance tested 24 hours later (Rossato et al. 2007).

Altogether, these results suggest that the early stage of consolidation provides a critical time window for SWS to promote long-term memory formation. The hippocampal ripples during SWS are associated with memory

reactivation and coincide with thalamic sleep spindles. Spindles have been implicated to promote long-term synaptic plasticity changes (Rosanova and Ulrich 2005, Seibt et al. 2017). Moreover, it was recently shown that long-term memory representations can be initially generated during the early stage of consolidation (Lesburguères et al. 2011, Kitamura et al. 2017, Brodt et al. 2018). In the present findings, spindle and ripple activity in SWS during the 2-hour post-encoding period might support long-term memory formation as follows: Spindle promotes transmission of ripple-associated memory reactivation from the hippocampus to extra-hippocampal regions. Concurrently, spindle induces synaptic plasticity changes in both hippocampal and extra-hippocampal memory representations and strengthens hippocampal-neocortical connectivity through synaptic consolidation during this time window of increased excitability and plasticity (i.e. 2-hour post-encoding period). Subsequent sleep periods might utilize this established hippocampal-neocortical coordination to gradually form more stable and long-lasting memory representations.

3.6) Interaction of different components of episodic memory representations during sleep

The findings of the “Study 1” that the hippocampus is crucial for forming long-term non-hippocampus-dependent memory during sleep lead to the suggestion that hippocampus-dependent episodic memory system orchestrates the formation of long-term memory across different memory domains. In the “Study 1”, the contribution of the hippocampus during sleep to remote NOR memory might be related to the reactivation of spatial-contextual aspects of the encoded episode, rendering the possibility of the hippocampal memory to interact with other memory domains and to organize long-term memory formation during sleep. However, one open question arising from the findings of “Study 1” is that if the hippocampus is involved in the formation of long-term non-hippocampus-dependent memory during sleep by interacting on episodic features of the encoded episode, then consolidation during sleep would possibly lead to long-term memory which remains associated with its spatial-contextual features. The question about the quality of memory after sleep cannot be directly answered by

the “Study 1” and “Study 2” because both studies aimed at investigating a single component of the episodic memory system, namely “what” and “where” component, respectively, which limits the possibility to directly test whether the memory for a single item is retrieved with its spatial-contextual features. The “Study 3”, however, aimed at investigating the contribution of spatial memory (“where” component) to recognition memory (“what” component), and provided evidence for possible interactions between these two memory domains during sleep.

The “Study 3” investigated the effects of sleep on recognition memory for the rat’s conspecific (i.e. social recognition memory). Social recognition memory in rodents mainly relies on chemosensory stimuli. The CA2 region of the dorsal hippocampus as well as the ventral hippocampus are necessary for encoding, consolidation, and recall of social information (Hitti and Siegelbaum 2014, Okuyama et al. 2016, Meira et al. 2018). Although these two brain regions have direct anatomical projections to other regions of the hippocampus (e.g. CA1, CA3, dentate gyrus), their functions are distinct and both structures are less involved in spatial memory processing (Kjelstrup et al. 2002, Kesner et al. 2011, Mankin et al. 2015, Alexander et al. 2016). Because the two memory domains (i.e. social memory and spatial memory) represent different components of episodic memory but share common anatomical connections, interactions between both memory domains might also occur during sleep. Therefore, the “Study 3” aimed at dissociating interactions between the consolidation of “non-spatial” social information and of spatial memory during sleep. In this study, social recognition memory was tested using a radial arm maze, in which a juvenile rat, serving as social stimuli, was presented in a different arm of the maze in each encoding session (Study 3, Figure 1a). The adult rat of the same strain explored the juvenile conspecific in the maze for 3 sessions, followed by a 2-hour post-encoding period of sleep or wakefulness. Social interaction with the juvenile conspecific during the encoding phase induced the formation of social recognition memory, demonstrating by decreased time the adult rat spent exploring the same juvenile across the 3 encoding sessions (Study 3, Figure 1b). At the retrieval test, the familiar juvenile (from the encoding sessions) was

presented along with a novel conspecific in the opposite arm. To dissociate the effects of spatial memory on social recognition memory, the locations of the novel and familiar juveniles at the retrieval test differed in two spatial configurations: 1) the novel juvenile was presented in the same location as that of the familiar juvenile at the last encoding session ("Same" condition), 2) the novel juvenile was presented in a location different from that of the familiar juvenile at the last encoding session ("Different" condition).

The "Study 3" indicated that sleep does not only enhance recognition memory for the conspecific, but also interacts with its episodic representations. Rats which had slept after learning showed exploration preference to the novel conspecific, relative to the familiar one, and the recognition performance was superior than that of rats which remained awake after encoding (Study 3, Figure 2a and 2b). However, the recognition performance of rats in the sleep condition differed depending on spatial configurations of the conspecifics at the retrieval test. Remarkably, rats in the sleep condition showed exploration preference to the novel juvenile conspecific only when this novel conspecific was presented in a location different from that of the familiar conspecific at the last sampling session ("Sleep-Different" condition: Study 3, Figure 3a). In other words, after sleep rats recognized the familiar conspecific together with the spatial location in which it was recently encountered. Moreover, in the "Sleep-Different" condition, rats showed shorter latency to explore the novel than familiar conspecific, and this latency to explore the novel conspecific was also shorter than that in the "Wake-Different" condition (Study 3, Figure 3d, right). In contrast, rats in the "Sleep-Same" condition could not discriminate the juvenile conspecifics at the beginning of the retrieval test. These findings suggest that sleep does not only enhance memory for conspecific, but also binds contextual details into the social information.

Although sleep is known to preferentially support the formation of hippocampus-dependent memory, the present results did not reveal that spatial memory per se is robustly enhanced after sleep. Rats in the "Sleep-Same" condition did not show exploration preference to the familiar juvenile conspecific which had been moved to a location different than that of the recently

encountered episode (Study 3, Figure 3a). Moreover, latency to explore the spatially displaced familiar conspecific did not differ from that of the novel conspecific, or that in the respective “Wake-Same” condition (Study 3, Figure 3d, left). In contrast, using the same 2-hour retention interval, the “Study 2” showed that at the recent OPR memory test rats in the sleep condition could discriminate the displaced object from the object which remained at the same location as during encoding. This evidence supports the notion that social recognition performance of the rats in the “Sleep-Same” condition was not predominantly dominated by spatial memory. Interestingly, rats of the “Sleep-Same” condition showed a delayed expression of the social recognition memory (Study 3, Figure 3a), which might be explained by an influence of the spatial memory on the expression of novelty preference at the beginning of the social recognition test. A previous study using a modified NOR task with similar spatial configurations as the “Sleep-Same” condition supports this explanation. Haettig et al. (2011) indicated that mice could not discriminate the novel object when the familiar object was moved to a location different from that of the encoding phase. However, inactivation of CA1 region of the dorsal hippocampus during the retrieval test unmasked the exploration preference to the novel object, indicating that spatial memory influenced the expression of memory for objects.

Interestingly, in both sleep and wake conditions, social recognition memory at the encoding phase was not influenced by the spatial configurations of the familiar conspecific. Rats could recognize the familiar conspecific independent of its spatial context. Time exploring the same juvenile conspecific, which was moved to a different location in each encoding session, decreased across the sessions in both sleep and wake conditions (Study 3, Figure 1b), and the strongest decrease in exploration time was observed at the first minute of the encoding phase (unpublished data). These findings suggest that before consolidation during sleep has taken place recognition memory for the conspecific is context independent. During sleep memory for the conspecific might interact with the spatial context in which it was recently encountered, thereby strengthening coordination between different memory domains and integrating spatial information into the social recognition memory. Previous

studies indicate that rodents can recall memory for items together with the associated spatial-contextual information (Eacott and Norman 2004, Dere et al. 2005, Kart-Teke et al. 2006) and sleep benefits the formation of this episodic-like memory (Inostroza et al. 2013, Oyanedel et al. 2014, Oyanedel et al. 2019). Social recognition memory can be considered as a “Who” component of the episodic memory system (Okuyama 2018). Item information (“What” component) and social information (“Who” component) constitute an identity of the event, whereas spatial-contextual features (“Where” component) and temporal information (“When” or “Which” component) separate that unique identity from those of other experienced episodes. Recent study suggests interactions between the “Who” (e.g. social information) and “Where” (e.g. spatial memory) components of the episodic memory system. Alexander et al. (2016) showed that social interaction did not change activity levels of both CA1 and CA2 neurons in rats. However, exposure to the conspecific induced global remapping of the CA2 place fields, but not the CA1 place fields. Interestingly, the CA2 place fields did not change back to their original maps when the rats were re-exposed to the familiar arena after social interaction, suggesting that hippocampal CA2 stores another distinct spatial representations of the context in which the social stimuli had recently been encoded.

Taken together, the findings of “Study 3” that sleep enhances memory for conspecifics as bound into the spatial context suggest the roles of sleep in consolidation of different components of episodic memory representations. Sleep does not preferentially consolidate a single component of an event but rather converges these distinct memory representations into a unique episodic memory for the event. Indeed, the associations between the item (“What”/ “Who”) and distinctive features of the event (“Where” and “Which”) were formed only after post-encoding sleep. The “Study 2” provides some hints that these associations might also be preserved after a long delay, showing that post-encoding sleep promotes the formation of long-term OPR memory which could be considered to comprise hippocampus-dependent memory for object location (“Where”) integrated into non-hippocampal object memory (“What”). Accordingly, the present studies suggest that memory consolidation during

sleep typically relies on the hippocampal mechanisms to integrate spatial-contextual information into the recently encoded item memory. However, future studies are needed to investigate how this integrated memory representations after sleep develop across time. Is the memory still integrated with the contextual information at the remote recall? or does it become more hippocampus-independent and contain less contextual features? This knowledge will further expand our understanding about sleep's roles in systems memory consolidation.

4. Summary

Memory is not a simple unitary system. A long-standing division between hippocampus-dependent and non-hippocampus-dependent memory has been widely accepted, based on numerous studies, in both humans and animal models, showing that functional integrity of the hippocampus is not required for encoding and retrieving non-hippocampus-dependent memories. Sleep is known to support memory consolidation, in particular hippocampus-dependent memory. The “active systems consolidation” has been proposed to explain how sleep contributes to long-term memory formation. During slow-wave sleep (SWS) repeated reactivation of hippocampal neuronal ensembles that were active during encoding promotes gradual transformation of hippocampal memory representations towards extra-hippocampal long-term storage sites. The proposed mechanism has been primarily conceptualized regarding to the hippocampus-dependent memory system. Although sleep also benefits non-hippocampus dependent forms of memory, the mechanism underlying consolidation of such memories during sleep is poorly understood. This dissertation consists of three studies focusing on revealing this unknown function of sleep in rats, using behavioral tasks closely comparable to human recognition memory.

The first study aimed at investigating temporal development of sleep’s effects and the contribution of the hippocampus on consolidation of both hippocampus-dependent and non-hippocampus-dependent memories using object-place recognition (OPR) and novel-object recognition (NOR) tasks, respectively. Whereas sleep enhanced hippocampus-dependent OPR memory at both recent and remote testing, non-hippocampus-dependent NOR memory was preserved at the remote testing only after sleep followed learning. Notably, temporary inhibition of the hippocampus during post-learning sleep by $GABA_A$ receptor agonist muscimol abolished sleep-dependent consolidation of NOR memory. These results indicate a critical role of the hippocampus in forming long-term memory during sleep even for memories that have previously been considered hippocampus-independent. The first study suggests that the

hippocampus is a key structure to promote long-term memory formation during sleep across different memory domains.

Based upon the findings of the first study that sleep after learning enhances long-term memory formation, the aim of the second study was to investigate which basic sleep feature (sleep duration or sleep depth) is the most important factor for long-term memory formation. Rats were assigned to different groups according to the depth of sleep after encoding (“regular” versus “deeper”), and the duration of post-encoding period (2 versus 4 hours). Deeper sleep expressed itself in a selective increase in the time spent in SWS, and in numbers of slow oscillations, spindles, and hippocampal ripples during SWS, whereas rapid eye movement (REM) sleep and preREM sleep were not affected. The second study showed that recent OPR memory was preserved only after sleep, but independent of its depth. However, remote OPR memory was preserved only after deeper sleep, whereas rats in the regular sleep condition did not show remote OPR memory, even with the longer 4-h post-encoding period. These results elaborate previous findings of the first study and suggest a critical time window (i.e. within 2 hours after encoding) for SWS and its oscillatory signatures to promote consolidation of hippocampus-dependent memory.

The last study investigated the effects of sleep on memory for the rat’s conspecific (i.e. social recognition memory). This study aimed at dissociating interactions between the consolidation of “non-spatial” social information and of spatial memory during sleep. Social recognition memory was tested in a radial arm maze, in which a juvenile rat (social stimuli) was presented in a different arm of the maze in each encoding session. Memory performance at the encoding phase was not influenced by the spatial configurations of the familiar conspecific. Notably, after sleep rats showed social recognition memory only when the familiar conspecific was presented in the same location as the last encoding session, suggesting that sleep does not only enhance memory for conspecific, but also binds social information into its spatial-contextual features.

Taken together, these studies suggest a common hippocampal mechanism for memory consolidation during sleep in both memory domains.

The associations between items (i.e. non-hippocampus-dependent component) and the spatial-contextual features (i.e. hippocampus-dependent component) of the encoded event are represented in hippocampal networks. Spindle-ripple events in SWS during a window of increased excitability and plasticity (i.e. 2-hour post-encoding period) might promote reactivation of hippocampal memory representations, which in turn co-activates the associated neocortical networks and strengthens hippocampal-neocortical connectivity. Such coordinated reactivation in hippocampal-neocortical networks could serve as a mechanism underlying the gradual formation of long-lasting memory representations during subsequent sleep. The interactions between the hippocampal and non-hippocampal components of the encoded event during sleep might also lead to the integration of item memory into its episodic memory representations. Accordingly, the present findings underline the roles of sleep and the hippocampus in organizing long-term memory formation in the episodic memory system.

4.1 German summary (Zusammenfassung)

Das Gedächtnis stellt kein einheitliches System dar. Die weitgehend akzeptierte Unterscheidung zwischen Hippocampus-abhängigem und Hippocampus-unabhängigem Gedächtnis hat ihren Ursprung in zahlreichen Human- und Tierexperimentellen Studien, die zeigen, dass die funktionale Integrität des Hippocampus weder für das Enkodieren, noch für das Abrufen des Hippocampus-unabhängigem Gedächtnis erforderlich ist. Schlaf spielt eine entscheidende Rolle für die Gedächtniskonsolidierung, insbesondere die des Hippocampus-abhängigem Gedächtnisses. Ein möglicher Mechanismus wie Schlaf zur der Bildung von Langzeitgedächtnis beiträgt ist die „aktiven Systemkonsolidierung". Dabei wird angenommen, dass während des langsamwelligen Schlafes (slow-wave-sleep; SWS) diejenigen neuronalen Ensembles im Hippocampus wiederholt reaktiviert werden, die bereits während des Enkodierens aktiv waren, und dadurch Gedächtnisinhalte, die zunächst im Hippocampus repräsentiert sind, graduell in Langzeitspeicherorte außerhalb des Hippocampus überführt werden. Diese mechanistische Erklärung wurde hauptsächlich im Bezug auf das Hippocampus-abhängige Gedächtnis konzipiert. Obwohl auch Formen des Hippocampus-unabhängigen Gedächtnisses von Schlaf profitieren, ist der Mechanismus, der der Konsolidierung solcher Hippocampus-unabhängigen Gedächtnisinhalte zugrunde liegt, kaum verstanden. Die vorliegende Dissertation setzt sich aus drei Studien zusammen, die sich unter Verwendung verschiedener Verhaltensaufgaben mit der Aufdeckung dieser unbekannten Funktion des Schlafen bei Ratten befassen.

In der ersten Studie wurden erwachsenen Ratten Aufgaben der Objekt-Ort-Erkennung (object place recognition; OPR) bzw. der Erkennung neuartiger Objekte (novel object recognition; NOR) gestellt, um die zeitliche Entwicklung der Effekte von Schlaf auf und den Beitrag des Hippocampus zur Konsolidierung von Hippocampus-abhängigen Gedächtnis zu untersuchen. Während Schlaf einen positiven Effekt auf die Gedächtnisleistung in der Hippocampus-abhängigen OPR-Aufgabe sowohl bei Testung kurz nach der Lernphase, als auch in der Testung zu einem späteren Zeitpunkt, zeigte, blieb das Gedächtnis in der Hippocampus-unabhängigen NOR-Aufgabe nur dann zum späteren Zeitpunkt des Tests erhalten, wenn Schlaf unmittelbar auf die Lernphase folgte. Nennenswerterweise führte die vorübergehende Hemmung des Hippocampus während des ersten Schlafes nach der Lernphase durch den GABAA-Rezeptor Agonisten Muscimol zu einer Aufhebung des positiven Effekts des Schlafes auf die Gedächtniskonsolidierung in der NOR-Aufgabe. Diese Ergebnisse weisen auf eine

entscheidende Rolle des Hippocampus für die Bildung von Langzeitgedächtnis im Schlaf hin, selbst bei Gedächtnisformen die zuvor als Hippocampus-unabhängig galten. Die erste Studie legt daher nahe, dass der Hippocampus eine Schlüsselstruktur zur Förderung der Langzeitgedächtnisbildung während des Schlafes in verschiedenen Gedächtnisdomänen darstellt.

Basierend auf den Ergebnissen der ersten Studie, dass Schlaf unmittelbar nach dem Lernen die Bildung von Langzeitgedächtnis fördert, war das Ziel der zweiten Studie grundlegende Schlafmerkmale (Schlafdauer und Schlaftiefe) auf deren Bedeutung für die Bildung von Langzeitgedächtnis zu untersuchen. Dazu wurden Ratten abhängig von der Tiefe in der die Tiere nach der Lernphase schliefen („regulär“ versus „tiefer“) und der Dauer der Schlafphase nach der Lernphase (2 versus 4 Stunden) verschiedenen Gruppen zugeteilt. Tieferer Schlaf äußerte sich dabei in einer Zunahme der Zeit, welche das Tier im SWS verbrachte, und der Anzahl der langsamen Oszillationen (slow oscillations), Schlafspindeln und hippocampalen Ripples während des SWS, ohne das PreREM- oder REM-Schlafphasen eine signifikante Veränderung aufwiesen. Bei Testung der Gedächtnisleistung in der OPR-Aufgabe kurz nach der Lernphase zeigte sich ein positiver Effekt von Schlaf auf die Gedächtnisbildung, jedoch unabhängig von dessen Tiefe. Im Gegensatz dazu zeigte sich in der Testung zu einem späteren Zeitpunkt, dass nur diejenigen Ratten die tiefer geschlafen hatten ein Gedächtnis im OPR-Test aufwiesen, wohingegen Ratten mit regulärem Schlaf, selbst nach der längeren 4-stündigen Schlafphase, kein OPR-Gedächtnis mehr zeigten. Diese Ergebnisse stützen die Befunde der ersten Studie und legen ein kritisches Zeitfenster (innerhalb der ersten 2 Stunden nach der Lernphase) nahe, in welchem SWS und dessen charakteristische oszillatorische Aktivität die Konsolidierung des Hippocampus-abhängigen Gedächtnisses begünstigt.

Die letzte Studie untersuchte die Auswirkungen von Schlaf auf das Gedächtnis von Ratten für deren Artgenossen (soziales Erkennungsgedächtnis). Ziel dieser Studie war es, die Wechselwirkungen zwischen der Konsolidierung von“nicht-räumlichen“ sozialen Informationen und des räumlichen Gedächtnisses im Schlaf aufzudecken. Dazu wurde das soziale Erkennungsgedächtnis der Ratten in einem Radial-Arm-Labyrinth getestet, in dem eine jugendliche Ratte (= sozialer Reiz) in einem für jede Lernphase unterschiedlichen Arm des Labyrinths positioniert wurde. Die Ergebnisse dieser Studie zeigten, dass die Gedächtnisleistung unabhängig war von der Position, in welcher der bekannte Artgenosse präsentiert wurde. Bemerkenswerterweise zeigten Ratten, nachdem sie geschlafen hatten, nur dann ein soziales Erkennungsgedächtnis,

wenn der bekannte Artgenosse an der selben Position präsentiert wurde, wie in der letzten Lernphase. Dies deutet darauf hin, dass Schlaf nicht nur das Gedächtnis für Artgenossen verbessert, sondern auch eine Rolle in der Verbindung sozialer Informationen mit deren räumlich-kontextuellen Merkmalen spielt.

Zusammengenommen legen diese Studien einen gemeinsamen hippocampalen Mechanismus für die Gedächtniskonsolidierung während des Schlafes in beiden Gedächtnisdomänen nahe. Die Assoziation zwischen Objekten oder Gegenständen (Hippocampus-unabhängige Komponente) und räumlich-kontextuellen Merkmalen (Hippocampus-abhängige Komponente) des enkodierten Ereignisses werden in hippocampalen Netzwerken repräsentiert. Spindel-Ripple-Ereignisse im SWS könnten während eines Fensters erhöhter Erregbarkeit und Plastizität (2 Stunden nach der Enkodierung) die Reaktivierung von Repräsentationen der im Hippocampus repräsentierten Gedächtnisinhalte fördern, und auf diese Weise die Koaktivierung von assoziierten neocorticale Netzwerken bewirken, welche wiederum die Konnektivität von Hippocampus und Neocortex stärkt. Eine solch koordinierte Reaktivierung in hippocampalen-neocorticalen Netzwerken könnte als Mechanismus dienen, der der allmählichen Bildung von lang anhaltenden Gedächtnisrepräsentationen während des auf das Lernen folgenden Schlafes zugrunde liegt. Die Wechselwirkungen während des Schlafes zwischen hippocampalen und extra-hippocampalen Komponenten des enkodierte Ereignisses könnten ebenfalls zur Integration des Objektgedächtnisses in die zugehörige episodische Gedächtnisrepräsentation führen. Zusammenfassend unterstreichen die vorliegenden Ergebnisse die Rolle von Schlaf und des Hippocampus für die Organisation und Bildung von Langzeitgedächtnis im episodischem Gedächtnissystem

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6. Declaration of Contributions

I hereby declare that I essentially contributed to all three publications included in this cumulative dissertation. The works were carried out at the Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen under the supervision of Prof. Jan Born.

The “Study 1” and “2” were designed by Prof. Jan Born, Dr. Marion Inostroza and myself. The “Study 3” was designed in collaboration with Dr. Eduard Kelemen. After receiving technical advice from Dr. Marion Inostroza, I carried out all behavioral experiments independently. The experiments with intrahippocampal infusion and histology were carried out by myself with the assistance of Carolina Salazar. I carried out surgery in experiments with EEG, hippocampal LFP recordings, and intrahippocampal infusion with the assistance of Carlos Oyanedel, Niels Niethard, and Carolina Salazar. The experiments with fluorophore-conjugated muscimol were carried out by myself and Carlos Oyanedel. I received technical advice from Dr. Eduard Kelemen for conducting the “Study 3”.

Behavioural data analyses were carried out by myself. Electrophysiological data analyses were carried out in cooperation with Carlos Oyanedel and Niels Niethard. Statistical analyses were carried out by myself under the supervision of Prof. Jan Born and Dr. Marion Inostroza. Behavioral data of the “Study 3” were analyzed in collaboration with Dr. Eduard Kelemen.
I wrote all the manuscripts together with Prof. Jan Born and Dr. Marion Inostroza.

I declare that I wrote the dissertation myself and all the sources are duly acknowledged.

Anuck Sawangjit

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