Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats

Sonja Binder\textsuperscript{a,b}, Paul Christian Baier\textsuperscript{c}, Matthias Mölle\textsuperscript{a}, Marion Inostroza\textsuperscript{a,b}, Jan Born\textsuperscript{a,b,d}, Lisa Marshall\textsuperscript{a,d,\textsuperscript{⇑}}

\textsuperscript{a}University of Lübeck, Department of Neuroendocrinology, Haus 50.1, Ratzeburger Allee 160, 23538 Lübeck, Germany
\textsuperscript{b}University of Tübingen, Institute of Medical Psychology and Behavioral Neurobiology, Gartenstraße 29, 74072 Tübingen, Germany
\textsuperscript{c}Christian-Albrechts-University, Department of Psychiatry and Psychotherapy, Niemannsweg 147, 24105 Kiel, Germany
\textsuperscript{d}Graduate School for Computing in Medicine and Life Sciences, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany

\textsuperscript{⇑}Corresponding author at: University of Lübeck, Department of Neuroendocrinology, Haus 50.1, Ratzeburger Allee 160, 23538 Lübeck, Germany. Fax: +49 451 500 3640. E-mail address: marshall@uni-luebeck.de (L. Marshall).

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\begin{abstract}
The positive impact of sleep on memory consolidation has been shown for human subjects in numerous studies, but there is still sparse knowledge on this topic in rats, one of the most prominent model species in neuroscience research. Here, we examined the role of sleep in the object-place recognition task, a task closely comparable to tasks typically applied for testing human declarative memory: It is a one-trial task, hippocampus-dependent, not stressful and can be repeated within the same animal. A test session consisted of the Sample trial, followed by a 2-h retention interval and a Test trial, the latter examining the memory the rat had for the places of two objects presented at the Sample trial. In Experiment 1, each rat was tested twice, with the retention interval taking place either in the morning or evening, i.e., in the inactive or active phase, respectively. Rats showed significantly ($p<0.01$) better memory for object place after the Morning session. To control for confounding circadian factors, in Experiment 2 rats were tested four times, i.e., in the morning or in the evening while sleep was or was not deprived. Sleep during the retention interval was recorded polysomnographically. Rats only showed significant memory for the target object place in the Test trial after the Morning retention interval in the absence of sleep deprivation, and recognition performance in this condition was significantly superior to that in the three other conditions ($p<0.05$). EEG recordings during spontaneous morning sleep revealed increased slow oscillation (0.85–2.0 Hz) and upper delta (2.0–4.0 Hz), but reduced spindle band (10.5–13.5 Hz) activity, as compared to evening sleep. However, spindle band power was increased in the Morning retention interval in comparison to a Morning Baseline period ($p<0.05$). We conclude that consolidation of object-place memory depends on sleep, and presumably requires NonREM sleep rich in both slow wave and spindle activity.

\end{abstract}

1. Introduction

Studies, both in humans and animals indicate that sleep can promote memory for several different memory systems with specific contribution of different sleep stages, brain electric oscillations and/or brain structures (reviewed in Ambrosini & Giuditta, 2001; Dieckmann & Born, 2010; Hennevin, Huetz, & Edeline, 2007; Smith, 2011; Stickgold, 2005). Compared to human studies most mnemonic tests for long-term memory assessment in rodents, e.g., maze learning and conditioning experiments, employ relatively extensive training sessions and stressful procedures; and in part massive sleep deprivation protocols are involved (Fishbein & Gutwein, 1977; Rabat, Bouyer, George, Le, & Mayo, 2006; Smith, Conway, & Rose, 1998; Youngblood, Zhou, Smagin, Ryan, & Harris, 1997). Also, in animal research a vast body of literature has developed on post-learning modifications in brain electric activity ranging from the EEG/local field potential to the cellular level (reviewed in Girardeau & Zugaro, 2011; Hennevin et al., 2007; Smith, 2011). In light of this increasingly detailed assessment of underlying neurophysiological processes, essentially from animal research and acknowledgment of the highly task-specific nature of sleep-dependent mnemonic processes in animals and humans (Dieckmann, Wilhelm, & Born, 2009), a convergence of animal and human research is becoming a more pressing task.

To establish an animal model more closely comparable to human experiments on hippocampus-dependent episodic encoding in the declarative memory system we selected an object-place...
recognition task (OPR-task), i.e., a task which is feasible for one-trial learning, does not involve stressful procedures or previous treatments like food deprivation, is based on the rodents natural behavior (novelty preference) and is impaired by hippocampal lesions (Bussey, Duck, Muir, & Aggleton, 2000; Mumby, Gaskin, Glenn, Schrake, & Lehmann, 2002; Oliveira, Hawk, Abel, & Havekes, 2010). Another advantage of the OPR task is that it allows for repetitive testing in the same animal by using different objects and positions; that is, each animal can be used as its own control.

In Experiment 1, to rule out stress-induced effects of sleep on memory performance, we did not rely on sleep deprivation procedures, but took advantage of the rodents’ natural activity cycle with increased sleep during the day, particularly in the early morning hours after lights-on, and increased wakefulness during the evening and at night (Borbely & Neubauer, 1977; Rosenzweig, Bergmann, & Rechtschaffen, 1976). Thus Experiment 1 compared object-place retention performance of animals between the inactive ‘Morning’ phase and the active ‘Evening’ phase. In Experiment 2, to control for circadian factors, retention performance was examined in the Morning and Evening with or without additionally depriving the animals of sleep, and sleep was monitored electrophysiologically.

2. Methods

2.1. Experiment 1

2.1.1. Animals

Two- to three-month-old male Long Evans rats (Janvier, Le Genest-Saint-Isle, France) were used. Animals were housed individually in standard type IV Macrolon cages with ad libitum access to food and water under a 12 h light–dark cycle (lights-on 06.00 A.M.). Before starting behavioral testing animals were handled daily for 10 min on seven consecutive days. Ten animals already took part in a pilot study to find optimal exploration times for the Sample trial of the object-place recognition task, and underwent one to six trials in the same set up, but with different objects. All experimental procedures were performed in accordance with the European animal protection laws and policies (directive 86/609, 1986, European Community) and were approved by the Schleswig-Holstein state authority.

2.1.2. Apparatus and objects

Object-place recognition testing took place in a quadratic dark gray open field (80 × 80 cm W. 40 cm H, PVC), dimly lit with 12 lux. A camera (model DFK1BU03, The Imaging Source, Bremen, Germany) was mounted above the open-field. The arm and foot of the camera as well as surrounding furniture and posters affixed to the walls represented potential extra maze cues.

Objects were glass bottles of different shape, texture, size (height 17–26 cm, bottom diameter 6–9 cm), each type filled with sand of a different color. They had sufficient weight to ensure the rats could not displace them. Objects and open field were cleaned thoroughly between trials with 60% ethanol solution.

2.1.3. General procedure and design

On three consecutive days prior to the first object-place recognition session animals were habituated to the empty open-field for 5 min per day. Habituation trials took place during lights-on between 11.00 A.M. and 05.00 P.M.

Each session consisted of a Sample trial, followed by a 2-h retention interval, and a Test trial. During the Sample trial two identical objects were positioned in two far corners of the open field (Fig. 1A). The rat was put in the center of the open field and explorative behavior in reference to the objects was measured.

Touching the object with vibrissae, nose and/or forelegs was counted as ‘object exploration’, merely close proximity to the object or contact to it while passing were not counted. After 60 s of exploration time across both objects or after reaching the cut-off criterion of 10 min of the Sample trial the animal was brought back to a transient housing room using a transportation box where it spent the 2-h retention interval in its home cage. In the Test trial, the open field contained the same objects as before, but one object was now displaced to another corner. Preference for the displaced object indicates memory for the position of the stationary object. Test trial duration was 2 min.

Six to thirteen hours prior to each testing session animals were brought from their initial housing room to a transient housing room next to the experimental room to reduce possible stress effects due to transportation and, in case of early morning tests, to prevent exposure to light during the dark phase. Animals were already habituated to this room during the open-field habituation sessions. Each rat was tested on two conditions according to a within-subject crossover design, a Morning session at the beginning of the inactive phase (between 06.00 and 07.00 A.M.), and an Evening session at the beginning of the active phase (between 06.00 and 07.00 P.M.). Order of sessions was balanced. Sessions were separated by 5–6 days, and different objects were used in each session. Positions of objects in Sample and Test trials and type of object were counterbalanced between the two retention interval conditions.

2.1.4. Data reduction and statistical analyses

Scoring of explorative behavior was conducted semi-manually using tracking software (ANY-maze, Stoelting Europe, Dublin, Ireland) by an experienced observer according to the above mentioned criteria. A preference-index (P-Index) for object exploration within the Test trials was calculated as the quotient of exploration time of the displaced object and total exploration time (i.e., exploration of displaced object/[exploration displaced object + exploration stationary object]). Thus, if exploration of both objects is equal, the P-Index would be 0.5 (chance level). A P-Index >0.5 indicates a preference for the displaced, an index <0.5 a preference for the stationary object. Because the preference for the displaced object tends to fade with elapsed time (Dix & Aggleton, 1999) the P-Index was computed separately for the first minute and for the total Test trial duration of 2 min.

Student’s one-sample t-tests investigated whether the P-Index differed from chance level. Additionally, t-tests for dependent samples were used to compare the P-Index of Morning and Evening condition for the first and the total 2 min of the Test trial.

Total exploration time across both objects for each Sample and Test trial were compared between conditions using Student’s t-test. Additionally, for the Sample trials Morning and Evening trial durations were compared.
2.2. Experiment 2

2.2.1. Animals

Twenty-two male Long Evans rats (Janvier, Le Genest-Saint-Isle, France), 9–10 weeks old by start of the experiments, were used. Prior to start of the long-time recordings, they were housed in Standard type IV Macrolon cages with ad libitum access to food and water under a 12 h/12 h light–dark cycle (lights on 06.00 A.M.), first in groups of four, after surgery individually to prevent damage to the electrode montage. Starting 7 days prior to surgery they were handled for 10 min daily. Seven days after surgery animals were moved to the recording boxes (see Section 2.2.3) within the testing room and were housed there until the end of the experiments.

2.2.2. Surgery

Animals were anesthetized with ketamine (i.p., 75 mg/kg initial dose, 35 mg/kg for maintenance) and xylazine (i.p., 5 mg/kg). Four screw-electrodes (Plastics One, USA) were used for EEG-recordings, two frontal (AP: ±2.6 mm, L: ±1.5 mm) and two occipital reference electrodes (AP: −10.0 mm; L: ±1.5 mm). For EMG-recordings, two insulated stainless steel wire electrodes (Plastics One, USA) were implanted bilaterally in the neck muscles. All electrodes were connected to a plastic pedestal (Plastics One, USA) and fixed with cold polymerizing dental resin (Palapress, Heraeus Kulzer GmbH, Germany). Following surgery, rats were given 5 ml 0.9% NaCl-solution s.c. for fluid substitution. Animals had at least 7 days for recovery from surgery.

2.2.3. Sleep recordings

Four recording boxes (35 cm × 35 cm × 46 cm), made of dark-gray PVC and containing plexiglass-windows on two opposite sides for visual contact to the neighboring box were placed in a light-proof chamber within the behavioral testing room with a consistent 12 h light–dark-cycle (lights on at 06.00 A.M.). The electrodes were connected through a swiveling commutator (Plastics One, USA), allowing free movement inside the box, to a Grass Model 15A54 amplifier (Grass Technologies, AstroMed GmbH, Germany) in an adjacent room. EEG and EMG signals were amplified, filtered (EEG: high pass 0.01 Hz, low pass 300 Hz; EMG: high pass 30 Hz, low pass 300 Hz, −6 dB cutoff frequency and at least −12 dB per octave roll-off), subsequently digitized at a sampling rate of 1000 Hz (CED 1401, Cambridge Electronics, UK), recorded using Spike2 software (Cambridge Electronics, UK) and stored on hard disk. The animals could be visually monitored on a PC-monitor in an adjacent room via cameras mounted above the recording boxes.

Days 1–3 served to adapt animals to the environment. On day four, a 24 h baseline recording was taken, starting at 05.00 P.M. Twenty-four hour recordings were taken throughout the complete experimental period, interrupted only during behavioral testing.

2.2.4. General procedure and design

The procedure of object-place recognition testing was the same as in Experiment 1. However, each animal underwent four sessions, with Sample and Test trials separated again always by a 2-h retention interval, according to a within-subject design with the order of conditions balanced across animals. For each animal, two of the sessions started at the beginning of the inactive phase (between 06.00 and 07.00 A.M.), the other two at the beginning of the active phase (between 06.00 and 07.00 P.M.). Animals were deprived of sleep (SD) in the 2-h retention interval between Sample and Test trial during one of the morning (Morning SD) and evening sessions (Evening SD), whereas during the respective other session, they were not deprived (Morning S, Evening S).

To achieve shorter delays between the sessions, two different open-fields were used (the gray one used in Experiment 1, see Section 2.1.2, and a white one of the same dimensions). On three consecutive days, following the 24-h baseline recording and prior to the first experimental session animals were habituated to both empty open-fields for 10 min daily. A pulley-system mounted above the open field served to affix the commutator-end of the recording cable and thus minimize stress before placing the animal in the open field. Habituation trials took place during the inactive phase between 08.00 A.M. and 05.00 P.M. and were separated by at least 4 h. The following testing sessions were separated by at least 36 h, using the different open-fields alternately. Sleep deprivation was achieved by “gentle handling”: At the first sign of falling asleep (adopting a sleep posture) the experimenter tapped at the box, shuffled the bedding or, if necessary, gently touched the animal.

2.2.5. Data reduction and statistical analyses

Data analyses of behavioral measures were essentially the same as in Experiment 1. Sleep (EEG and EMG) recordings during the retention intervals between Sample and Test trial as well as a corresponding morning time interval of the baseline recording were scored using 10-s epochs according to standard criteria (Neckelmann, Olsen, Fagerland, & Ursin, 1994) with the software SleepSign for Animal (Kissei Comtec, Japan). In short, ‘waking’ was identified by sustained EMG activity and mixed-frequency EEG, ‘NREM sleep’ by low EMG, high-amplitude low-frequency EEG with a high proportion of delta activity, ‘Pre-rapid eye movement sleep’ (PreREM; in the literature also referred to as ‘transition sleep’, e.g. in Ambrosini & Giuditta, 2001) by low EMG and high-amplitude EEG spindle activity, and ‘REM sleep’ by a further reduced EMG-signal and low-amplitude EEG with high theta (5–10 Hz) activity. Regarding sleep architecture, the following measures were computed: total sleep time (TST), duration of the different stages in min (Wake, NonREM, REM, PreREM).

Further analysis of the sessions without SD involved 35 Hz lowpass filtering of the EEG signal and two subsequent EEG power spectral analyses for NREM and REM sleep epochs within a frequency range of 0.85–35 Hz (bin size of 0.061 Hz) calculated via Fast Fourier Transformation (FFT). Mean power was determined for the following bands: slow oscillations (SO) 0.85–2.0 Hz, upper delta 2.0–4.0 Hz, theta 5.0–10.0 Hz, and spindle 10.5–13.5 Hz. The spindle band was chosen after identifying the peak frequency of spindle activity in individual rats within a broader (10–15 Hz) frequency range. The SO and upper delta band together represent slow wave activity (SWA). We split the latter to enable a more detailed analysis.

Data from animals showing a disturbed circadian sleep–wake pattern in sessions without sleep deprivation, i.e., a TST in the Morning retention interval lower than the mean TST of all animals for the Evening condition and/or a TST in the Evening retention interval higher than the mean TST for the Morning interval, were excluded. This was the case for four animals. Data from three further rats were discarded due to technical problems (control of ambient temperature during the session), thus resulting in a final n = 15. FFT analysis were conducted for n = 14, due to artifacts in one recording. Statistical analyses relied on ANOVAs with a repeated measures factor for “Condition” (Morning S, Morning SD, Evening S, Evening SD). For behavioral data, directed Helmert contrasts were conducted, and on FFT data Fisher’s LSD tests were used as post hoc contrasts.

3. Results

3.1. Experiment 1

The two conditions did not differ regarding total object exploration time across both objects (Morning: 49.24 ± 3.03 s, Evening:
43.81 ± 3.32 s, \( p = 0.256 \) nor trial duration (Morning: 522.43 ± 23.41 s, Evening: 547.05 ± 22.44 s, \( p = 0.429 \)) during the Sample trials.

Fig. 2 reveals that the \( r \)-Index measured against chance was significantly only in the Morning condition (1st min: \( p < 0.01 \), total 2 min: \( p < 0.05 \)), but not in the Evening condition (1st min: \( p = .43 \), total 2 min: \( p = .86 \)). More importantly, results reveal a higher \( r \)-Index for Morning than Evening sessions (main effect for the factor “Time of Day” (\( F(1,20) = 11.42, p < .01 \)). Neither the factor “Minute” nor the interaction reached significance (Minute (1,20) = 2.09, \( p = .164 \), “Time of Day” × “Minute”: \( F(1,20) = 3.23, p = .087 \)). Altogether, these results indicate that the animals were able to discriminate between displaced and stationary object only in the Morning, but not in the Evening condition. There was no difference in general exploratory behavior between the two conditions (total object exploration: Morning: 141 ± 1.7 s, Evening: 145.7 ± 1.5 s, \( p = 0.818 \)).

3.2. Experiment 2

3.2.1. Sample trials

The four conditions did not differ statistically regarding total object exploration (Morning S: 56.67 ± 2.56 s, Evening S: 54.19 ± 2.88 s, Morning SD: 55.49 ± 2.64 s, Evening SD: 52.96 ± 3.26 s, \( F(3,42) = .31, p = 0.806 \)) nor regarding trial duration (Morning: 417.9 ± 42.53 s, Evening: 420.99 ± 42.53 s, Morning SD: 417.9 ± 42.53 s, Evening SD: 420.99 ± 42.53 s, \( F(3,42) = .29, p = 0.833 \)).

3.2.2. Test trials

In agreement with the findings of Experiment 1, the \( r \)-Index was significantly increased above chance only in the Morning condition with undisturbed sleep (1st min: \( p < .001 \), total 2 min: \( p = 0.001 \)), but not in the three other conditions (Evening S: 1st min: \( p = .193 \), total 2 min: \( p = 0.344 \), Morning SD: 1st min: \( p = 0.147 \), total 2 min: \( p = 0.508 \), Evening SD: 1st min: \( p = 0.439 \), total 2 min: \( p = 0.859 \), 1st min: \( F(3,42) = 2.92, p < 0.05 \), total 2 min: \( F(3,42) = 3.22, p < 0.05 \), Fig. 2). The significant difference between the Morning condition and the others was confirmed by Helmert contrasts (1st min: \( p < 0.05 \), total 2 min: \( p < 0.05 \)) indicating that the animals were only able to solve the task after the Morning retention interval with undisturbed sleep. Again, there was no difference regarding general exploratory behavior between the conditions (total object exploration: Morning S: 32.0 ± 2.66 s, Evening S: 35.6 ± 3.19 s, Morning SD: 35.6 ± 3.19 s, Evening SD: 36.5 ± 3.43 s, \( F(3,42) = 3.22, p = .007, \text{total 2 min: } F(3,42) = 3.05, p = .011 \).). Altogether, these results indicate that the animals were only able to discriminate between displaced and stationary object only in the Morning condition (1st min: \( p < 0.05 \), total 2 min: \( p = 0.07 \)).

3.2.3. Sleep parameters and EEG power

Sleep architecture for each condition is given in Table 1 and Table 2. As expected, animals overall slept more during the Morning S than Evening S session and SD effectively hindered rats from falling asleep. Furthermore, the composition of sleep differed between conditions regarding NonREM and REM sleep (\( F(2,28) = 14.28, p < 0.001 \), and \( F(2,28) = 29.59, p < 0.001 \), respectively), whereas the proportion spent in PreREM sleep did not differ significantly (see Table 2 for detailed results). Generally, rats spent in all of these sleep stages more time during the Morning than Evening sleep session. The EEG power spectra of NonREM sleep epochs during the retention interval differed in the Evening, Morning and at the corresponding Morning Baseline interval. Fig. 3 reveals for the NonREM sleep epochs that the EEG slow oscillation (0.85–2.0 Hz) and upper delta (2.0–4.0 Hz) bands contain less power during the Evening compared to the Morning and Baseline condition (\( F(2,26) = 9.08, p = 0.001 \), and \( F(2,26) = 4.81, p < 0.05 \), respectively, Fig. 3). In contrast, power in the spindle frequency band (10.5–13.5 Hz) was higher in the Evening S compared to the Morning S condition and also higher in the Morning S compared to the respective Morning Baseline interval (\( F(2,26) = 23.67, p < 0.001 \), Fig. 3). Regarding REM sleep, EEG power of Morning S did not differ from the Baseline condition for SO (power in \( \mu V^2/Hz \): Morning S:356.29 ± 12.27, Baseline: 348.6 ± 13.46, \( p = .62 \)), upper delta (power in \( \mu V^2/Hz \): Morning S:234.48 ± 13.3, Baseline: 233.07 ± 9.92, \( p = .87 \)) and spindle band (power in \( \mu V^2/Hz \): Morning S:127.96 ± 7.59, Baseline: 140.58 ± 7.23, \( p = .24 \)), but there was a trend towards decreased theta power in the Morning condition (power in \( \mu V^2/Hz \): Morning S:853.7 ± 48.07, Baseline: 902.38 ± 43.77, \( p = .09 \)). A comparison to Evening S could not be made, since half of the animals (N = 7) showed no REM sleep at all.

4. Discussion

Results of Experiment 1 indicate that rats in the morning after a retention interval presumably spent in sleep accomplished the object-place recognition task whereas they failed to do so when tested after an Evening retention interval which was presumably predominated by active wakefulness. These results are in accordance with human data showing improved retention for hippocampus-dependent declarative memory after an interval of sleep in comparison to a period of wakefulness (e.g., Plihal & Born,
Table 1
Sleep architecture in minutes for the retention interval between Sample and Test trials and for corresponding Morning Baseline period.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Morning S</th>
<th>Evening S</th>
<th>Morning SD</th>
<th>Evening SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>40.31 ± 5.56</td>
<td>57.51 ± 4.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.36 ± 2.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116.96 ± 0.28</td>
<td>108.19 ± 7.75</td>
</tr>
<tr>
<td>NREM</td>
<td>57.26 ± 4.47</td>
<td>47.97 ± 3.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.06 ± 2.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24 ± 0.23</td>
<td>0.64 ± 0.32</td>
</tr>
<tr>
<td>REM</td>
<td>15.63 ± 1.44</td>
<td>8.59 ± 1.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.37 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>PreREM</td>
<td>4.24 ± 0.41</td>
<td>3.35 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.82 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>TST</td>
<td>77.13 ± 5.83</td>
<td>59.91 ± 4.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.25 ± 2.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24 ± 0.23</td>
<td>0.64 ± 0.32</td>
</tr>
</tbody>
</table>

Notes: Values are given in mean ± SEM. TST = total sleep time, REM = rapid eye movement sleep, NREM = NonREM sleep, PreREM = pre-rapid eye movement sleep. Duration of all sleep stages and TST differed significantly between Baseline, Morning and Evening conditions (p < 0.001). Bonferroni corrected pairwise comparisons revealed significant differences between Morning and Evening condition for NREM, REM, TST (p < 0.001) and between Baseline and Evening condition for all stages and TST (p < 0.001). Morning and Baseline condition differed only in the duration of REM (p < 0.001), while TST and duration of Awake failed to reach significance (p = 0.067 and 0.066, respectively). Sleep architecture of SD sessions were not included in analysis. N = 15.

Table 2
Sleep architecture in percentage of TST for the retention interval between Sample and Test trials and for corresponding Morning Baseline period.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Morning S</th>
<th>Evening S</th>
</tr>
</thead>
<tbody>
<tr>
<td>NREM</td>
<td>74.45 ± 1.22</td>
<td>79.87 ± 1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.71 ± 2.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>REM</td>
<td>19.92 ± 1.25</td>
<td>14.24 ± 1.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.74 ± 1.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PreREM</td>
<td>5.62 ± 0.40</td>
<td>5.89 ± 0.60</td>
<td>7.55 ± 1.50</td>
</tr>
</tbody>
</table>

Notes: Values are given in mean ± SEM. REM = rapid eye movement sleep, NREM = NonREM sleep, PreREM = pre-rapid eye movement sleep. Proportion of NREM and REM differed significantly between Baseline, Morning and Evening conditions (p < 0.001). Bonferroni corrected pairwise comparisons revealed significant differences between Morning and Evening condition for NREM and REM (p < 0.05 and p < 0.001, respectively). The same prevailed for comparison between Morning and Baseline condition (p < 0.01) and Evening and Baseline condition (NREM: p < 0.01, REM: p < 0.001). Sleep architecture of SD sessions were not included in analysis. N = 15.

1997). However, Experiment 1 taking advantage of the regular 24-h rest-activity cycle did not dissociate effects of sleep from those of the circadian rhythm. It is known that activity and expression of some enzymes and genes involved in memory consolidation, underlie circadian oscillations (Dolci et al., 2003; Eckel-Mahan et al., 2008; Wang et al., 2009). However, studies of circadian effects on learning and memory yielded mixed results, with some revealing better recall when subjects were trained and tested during the active phase whereas others found no effect of time-of-day of training on recall (Chaudhry & Colwell, 2002; Valentiniuzzi et al., 2001). Indeed, many studies on circadian influences on memory functions did not aim at disentangling effects of sleep from the observed circadian changes (e.g., Devan et al., 2001; Van der Zee et al., 2008; Wisor et al., 2002).

To better distinguish between circadian effects and those of sleep, in Experiment 2 we sleep deprived animals and compared the effects on memory retention with those following sleep during the same times of the day. The main finding is that a significant memory for the object place in the sleep as compared to the SD group was only found for animals with undisturbed sleep in the Morning retention interval. If circadian factors alone sufficed to enhance memory consolidation performance after Morning SD should likewise have been observed.

Morning sleep as the essential factor mediating consolidation of object-place memory, is indicated by the finding that the Morning retention interval with undisturbed sleep generated significant object-place memory, whereas in the undisturbed Evening condition sleep did not prove sufficient to consolidate memory on object positions. In addition to the almost three fold longer time spent in sleep during the undisturbed Morning as compared to the Evening retention interval, the EEG in particular during NonREM sleep differed markedly between the Morning and evening retention intervals: Strikingly more slow wave activity occurred during Morning intervals. Post-learning slow wave activity including the slow oscillations and delta activity have been revealed to be critical for the consolidation of hippocampus-dependent memories as well as for providing the temporal framework for neuronal replay (Crunelli & Hughes, 2010; Marshall & Born, 2007; Marshall, Helgadottir, Mölle, & Born, 2006).

Interestingly, spindle power was higher in the undisturbed Evening than Morning sleep interval which may reflect a circadian rhythm which is similarly observed in humans showing increased stage 2 spindle activity towards the end of the nocturnal sleep period (Aeschbach, Dijk, & Borbely, 1997). Considering evidence that increased spindle activity has shown to be a reliable indicator of “good learning” (Fogel, Smith, & Beninger, 2009; Schabus et al., 2004), at first glance, the higher spindle activity in the evening retention interval appears to be contradictory to the chance level object recognition performance after this interval. However, the morning-to-evening increase in spindles may primarily reflect a circadian process. Most importantly, session spindle activity after the Sample trial in the Morning was significantly higher than during a respective baseline morning interval. This pattern confirms that learning prior to sleep can stimulate spindle activity during subsequent sleep, as shown before in humans and rodents (Eschenko, Mölle, Born, & Sara, 2006; Fogel et al., 2009; Gais, Mölle, Helms, & Born, 2002). Results furthermore show that this increase in spindle activity after learning on a hippocampus-dependent object recognition task is confined to the slow wave sleep-rich period in the beginning of the rest as compared to the active period. Together these findings suggest that spindles contribute to hippocampus-dependent memory consolidation, but only when occurring conjointly with intense slow wave activity. In addition, to this
constellation of oscillatory EEG activity differential hormonal levels between Morning and Evening sessions underlying e.g. circadian regulation may have contributed to the consolidation effect (Atkinson & Waddell, 1997; Bertani et al., 2010; Born & Wagner, 2009; Kalus, Kneib, Steiger, Holboher, & Yassouridis, 2009). The differential sleep drive between morning and evening and associated modifications in brain neurochemistry are also to be considered (Vyazovskiy, Achermann, & Tobler, 2007).

We also found that in the morning sleep session rats spent more time in REM sleep than in the Evening sessions. Many studies have shown the importance of REM sleep for successful memory consolidation in rats (Hennewin et al., 2007; Smith, 1996). Furthermore, there is evidence that the sequential occurrence of both NonREM and REM sleep are necessary for plasticity processes during sleep to take place (Ambrosini & Giuditta, 2001; Giuditta, 1985; Ribeiro & Nicolelis, 2004; Ribeiro et al., 2007). It could thus be argued that the increased REM sleep during the Morning sleep interval further added to the improved memory performance in this condition. However, an immediate contribution of REM sleep to the enhanced retention of object place memories appears to be unlikely: Firstly, in previous studies (Smith, 1996; Smith & Rose, 1996) the effects of REM sleep on memory processing appeared to be often restricted to “REM windows” which usually occurred with longer delays after learning, compared to the 2-h post-learning retention interval of the present study. Secondly, most previous studies demonstrating an immediate contribution of REM sleep to memory consolidation used tasks with a strong emotional, i.e., aversive, component like fear conditioning (Datta, Mavanji, Ulloor, & Patterson, 2004) and it might be this emotional component that makes these tasks particularly sensitive to the effects of REM sleep (Born & Wagner, 2009; Nishida, Pearsall, Buckner, & Walker, 2009). Thirdly, compared with baseline sleep, morning sleep after the Sample trial was characterized by a significantly lower amount of REM sleep, which does not support an essential role of REM sleep for consolidation of the object place memories.

We show here that sleep critically supports consolidation of a hippocampus-dependent task which is consistent with previous studies investigating the influence of sleep on memory consolidation in rats (Graves, Heller, Pack, & Abel, 2003; Hagewoud et al., 2010; Smith & Rose, 1996; Smith et al., 1998). On the other hand, some of these studies have also shown that other versions of the same task not relying on hippocampal processing did not benefit from sleep (Graves et al., 2003; Smith et al., 1996; Smith et al., 1998). A few studies in mice (Palchikova, Winsky-Sommerer, Meerlo, Durr, & Tobler, 2006; Palchikova, Winsky-Sommerer, & Tobler, 2009; Rolls et al., 2011) have revealed an effect of sleep deprivation on novel-object recognition performance, a task considered not to essentially involve hippocampal function (Bussey et al., 2000; Mumby et al., 2002). Whether differences in the species or in the methods (e.g., duration of the retention interval) are responsible for these divergent findings, cannot be answered here. To our knowledge, of the many studies on object recognition by rats (reviewed in Dere, Huston, & De Souza Silva, 2007), up until now only two studies have investigated the influence of sleep or sleep loss on object presentation. In both these studies, however, the effect of novel object presentation on subsequent brain electric activity was the focus, without any testing for recognition performance (Schifferholz & Aldenhoff, 2002; Ribeiro & Nicolelis, 2004). As we did not perform comparison on the object-place recognition task with performance on a non-hippocampus-depending task, the specificity of the observed effects for hippocampus-dependent memory remains to be further explored.

Our findings are consistent with concepts of hippocampus-dependent memory consolidation during SWS that build on findings both in rodents and humans (Diekelmann & Born, 2010; Marshall & Born, 2007). These concepts assume an active system consolidation process in which memories that temporally encode into hippocampal networks, are reactivated during SWS to be redistributed towards long term storage sites preferentially residing in neocortical areas (Gais et al., 2007; Rasch, Buchel, Gais, & Born, 2007; Wilson & McNaughton, 1994). For this process, both slow wave activity and sleep spindles seem to play a crucial role (Sirotta, Cicsvari, Buhl, & Buzsaki, 2003). Slow oscillations group spindle activity (Mölle, Bergmann, Marshall, & Born, 2011; Mölle, Eschenko, Gais, Sara, & Born, 2009; Steriade, 2006) and their enhancement through transcranial direct current stimulation resulted not only in an improved memory consolidation for declarative material but also increased spindle activity (Marshall et al., 2006). Aside from electrical field constellations, i.e., slow oscillation-driven spindle activity, transfer of hippocampal memory information into long term storage sites is linked to specific constellations of neuromodulatory activity, notably of low acetylcholine during SWS (Gais & Born, 2004; Hasselmo, 1999; Hobson & Pace-Schott, 2002).

It is to note, that the 2 h retention interval used here is well suited for investigating effects of sleep on so called “intermediate memory” (Kesner & Hunsaker, 2010), but one cannot infer our findings to longer retention intervals. Also, studies on brain electric activity such as replay suggest that early post-acquisition sleep is a most sensitive period (Ramadan, Eschenko, & Sara, 2009; Wilson & McNaughton, 1994). Future experiments should focus on possibilities to tune the experimental procedure in a way, which extends the retention interval so as to use it as long-term memory test, i.e. for retention delays >24 h.

5. Financial disclosure

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