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

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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.

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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; Diekelmann & 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 (Diekelmann, 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

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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, Schramek, & 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 & Neuhaus, 1977; Rosenberg, 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
Twenty-two male Long Evans rats (Janvier, Le Genest-Saint-Isle, France), 9–10 weeks old at the beginning of the experiments, were used. Animals were housed individually 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.). 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.
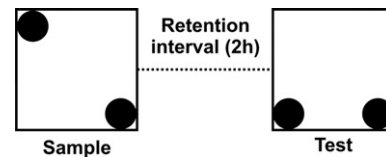

Fig. 1. Schematic drawing of the object-place recognition task. Two objects are presented in an open field during a Sample trial, and after a 2-h retention interval the same objects are presented again in a Test trial. Now, one of the objects is displaced to another corner. If an animal remembers the position of the objects from the Sample trial, due to rodents' innate preference for novelty, it explores the displaced object longer than the stationary one.

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 sojourn time the Sample trial was terminated and 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.