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Research report

Sleep-dependency of episodic-like memory consolidation in rats

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HIGHLIGHTS

- We tested the influence of sleep on the episodic-like memory consolidation in rats.
- Sleep is critical for maintaining an episodic-like memory over an 80-min retention period.
- Sleep is critical to episodic-like memory by maintaining the binding of an event into its spatio-temporal context.
- Sleep supports memory for the spatio-temporal context per se, but not for the event.
- Sleep is only effective when it occurs shortly after encoding.

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ABSTRACT

Episodic memory refers to the recollection of a representation that binds together into a unique past experience "what" happened, "where" and "when". Sleep has been identified as a state that optimizes the consolidation of newly acquired memory. To determine if sleep is important for the consolidation of episodic-like memory, we tested rats on an episodic-like memory task requiring the binding of an object memory into a spatio-temporal context, as well as retention of its individual components, using separate tests of novel-object recognition ("what"), object-place recognition ("where") and temporal memory ("when"), respectively. The 80-min retention interval between encoding of the task and retrieval testing covered either a period of regular morning sleep or sleep deprivation or a period of evening wakefulness. Sleep during the retention interval, compared with the other two retention conditions, significantly enhanced retrieval in the episodic-like memory task as well as in the object-place recognition and temporal memory tasks. In fact, when the rats stayed awake during the retention interval, there was no significant memory left at retrieval testing for the learnt object place and temporal memory. Sleep did not benefit novel-object recognition memory which unlike the other components of episodic-like memory is considered not to critically rely on the hippocampus. In an additional delayed sleep condition, episodic-like memory in rats which had stayed awake during the first 80-min interval after encoding, was not recovered when they were allowed to sleep during a subsequent 80-min interval. Our results suggest that sleep specifically supports the aspects in episodic memory most closely linked to hippocampal function, i.e., the binding of an event into spatio-temporal context as well as the spatio-temporal context itself. Sleep is particularly effective when it occurs shortly after encoding.

1. Introduction

In humans episodic memory is formed when individual events upon their one-time occurrence become bound to the specific temporal context in which they take place [1,2]. Accordingly, the investigation of episodic-like memory in nonhuman animals refers to their ability to remember the "what", "where" and "when" components of a unique episode, which can be assessed at the behavioral level [3–5]. Essential to the formation of a genuinely episodic memory is the "binding" of the three components into an episode. Encoding and storage of episodic memory information critically relies on the hippocampus [6–8]. Episodic memories can be the basis of semantic memories (for facts) arising from the repeated encoding or activation of overlapping episodic memories [9].

Numerous findings have demonstrated that sleep contributes to memory consolidation, presumably by promoting the integration of newly encoded memory representations into preexisting long-term memories [10]. Major evidence in favor of an active

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consolidation of memory established during sleep involves observations of neuronal replay of hippocampal activity patterns during slow wave sleep that occurred during a preceding learning experience, indicating that consolidation involves the reprocessing of fresh memories within the same hippocampal networks that were used for encoding them [11–13]. Based on the predominance of replay activity in the hippocampus leading replay also in other brain regions (e.g., [14]), it was proposed, in conjunction with related findings, that sleep specifically supports consolidation in the declarative memory system that as a whole, comprising episodic and semantic memory, was linked to hippocampal function [10,15,16]. However, it remains unclear whether the consolidating effect of sleep is restricted to specific aspects of these memories, presumably those most closely linked to hippocampal function [17–20].

Encoding representations of an experienced event at its first occurrence, episodic memory is at the core of memory formation. Nevertheless, the offline processing of episodic memory during sleep has so far not been thoroughly investigated. Two previous human studies suggested that sleep strengthens episodic memory [21,22], although these studies used repeated presentations of the materials to be learnt or explicitly instructed the participants to learn the material which increases the probability of overlapping semantic memory processing. A most recent study in humans, pointed towards a beneficial effect of a midday nap selectively on context memory, whereas item memory remained unchanged [23]. The lack of studies of episodic-like memory in animals is owed to the fact that the episodic memory concept arose from human research [24] and only recently, experimental tasks have been developed that model episodic memory in animals [4,25–29]. Here, we employed an object-recognition based approach to episodic-like memory developed by Kart-Teke et al. [4,5] to dissociate the effects of post-encoding sleep (versus wakefulness) on the consolidation of the different components of such memories in rats. The approach comprises a non-stressful, one-trial based test of an episodic-like memory requiring the binding of an object memory into a spatio-temporal context, as well as separate tests of episodic memory components, i.e., of novel-object recognition ("what" memory), object-place recognition ("where" memory) and temporal memory ("when" memory). The approach also allowed for comparing retention of genuinely hippocampus-dependent memory, i.e., episodic-like memory, with hippocampus-independent memory such as object recognition relying on parahippocampal structures, although the latter is still a matter of debate (for review see [30,31]).

2. Methods

2.1. Animals

Eighteen adult male Long Evans rats (Janvier, Le Genest-Saint-Isle, France, 260–310 g) were used for the experiments. A subset of these animals ($n = 12$) participated in supplementary experiments to explore effects of delayed sleep on the recovery of episodic-like memory. Rats were housed individually, kept on a 12 h light/12 h dark cycle (lights on at 6 a.m., lights off at 6 p.m.), and had unrestricted access to water and food throughout the experiment. 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.

2.2. Design and procedures

To test retention of episodic-like memory and its components, the rats were tested on four tasks in the following order: (i) novel-object recognition, (ii) object-place recognition, (iii) temporal memory and (iv) episodic-like memory, with an interval of at least 2 days between the tasks. Testing on each task consisted of a Sample phase (encoding), followed by a Retention period of ~80 min, and a subsequent Test phase (retrieval). Each rat participated in three retention conditions, in which the 80-min retention interval between the Sample and Test phase was filled either with (i) normal morning sleep (Sleep), (ii) morning sleep deprivation (S-Deprivation), or (iii) normal evening wakefulness (Wake). The order of retention conditions was balanced across rats, with the conditions separated by six to seven days. Different task versions were used for each retention condition.

The Sleep retention condition was performed during the light phase (between 8 a.m. and 13 p.m.). During the retention interval the rats were left undisturbed in their home cages, and sleep was assessed based on video recorded behavior and a tracking software (ANY-Maze, Stoelting Europe, Dublin, Ireland) by standard visual procedures validated in previous studies [32]. Basically, sleep was scored when the animal displayed a typical sleep posture and kept immobile for at least 5 s. Scores indicated an average of 20.9 ± 1.6 min of sleep during the 80-min retention interval, with the first bout of sleep occurring 42.3 ± 2.8 min after the Sample phase. The S-Deprivation condition was performed during the same time of the rest phase as the Sleep condition (8 a.m.–13 p.m.). Deprivation of sleep was accomplished by "gentle handling" to minimize stress during the enforced waking and to exclude locomotion as confounding variable. The procedures involved tapping on the cage, gently shaking the cage or if necessary disturbing the sleeping nest [19]. No intense stimulation was used. Inspection of video records did not indicate any signs of startle or freezing behavior (like a clear arrest of ongoing activity) beyond normal orienting behavior induced by these procedures. The Wake condition was performed during the active phase (8 p.m.–1 a.m.) and did not involve systematic stimulation as the rats spontaneously increase wakefulness during this phase of the circadian cycle [33].

2.2.1. Habituation and memory tasks

After handling during 4 consecutive days for 4 min, the rats were brought into the testing room 3 times once a day, placed into the center part of the open field, and allowed to explore for 10 min. The rats were always brought into the testing room in their home cage which was placed near to the testing apparatus.

Beginning on the day after habituation, rats were tested on four different tasks using the object recognition paradigm to examine the individual components of episodic-like memory (novel-object recognition, object-place recognition and temporal memory) and the episodic-like memory task per se. The Sample phase(s) for each task allowed the rat to explore two (or four, respectively) objects in the testing box until it had accumulated at least 15 s of exploration time for each object within an interval of 2–5 min. Exploration was 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. The temporal memory task and the episodic-like memory task included two Sample phases with the second conducted in exactly the same way as the first Sample phase but with a different arrangement of objects. The interval between the first and second Sample phase was 20 min in order to avoid pro or retroactive interference and that the animal fell asleep. For the Test phase, the rats were placed in the testing arena to allow exploration for another 3 min. The location of objects during the Sample and Test phases was randomized across rats and across the different tests within an individual rat. Objects were positioned at least 10 cm equidistant from the walls to avoid that the animal's preference to stay in corners biased exploration times.

2.2.2. Novel-object recognition ("what" memory)

To test whether rats were able to recognize a familiar object a standard novel-object recognition task was used [34]. In the Sample phase, two identical objects were presented. In the Test phase, one object was the same as one of the two objects seen in the Sample phase while the other object was novel (see Fig. 1 for an illustration of the tasks). Relatively enhanced exploration time for the novel object indicates memory for the familiar object.

2.2.3. Object-place recognition ("where" memory)

In the Sample phase the rat is presented with two identical objects. During the Test phase one of these objects is displaced to a new position while the other object remains at the same location as during the Sample phase. Relatively enhanced exploration time for the displaced object indicates memory for the location of the non-displaced object.

2.2.4. Temporal memory ("when" memory)

This task consists of two 3-min Sample phases separated by a 20-min interval [35] during which the rat was placed into the home cage, and the testing box was cleaned and reconfigured. (A 3-min phase duration was chosen based on pilot studies indicating that rats on average needed this time to accumulate 15 s exploration for each object.) In the first Sample phase, two identical objects are presented and in the second Sample phase, two different identical objects are presented at the same locations. During the Test phase, one of the objects from each Sample phase is presented (in its original position). That is, during the Test phase, the rat was presented with two familiar objects that differed in the temporal order in which they had been encountered (in the Sample phases). Relatively enhanced exploration of the earlier presented object indicates temporal order memory.

2.2.5. Episodic-like memory task

This task also contains two Sample phases and a final Test phase [4]. The length of the phases and of the interval between the Sample phases was the same as in the temporal memory task. In the two Sample phases the animals were presented with different sets of four identical objects, designated as "old-familiar" (first Sample phase) and "recent-familiar" objects (second Sample phase), respectively. In

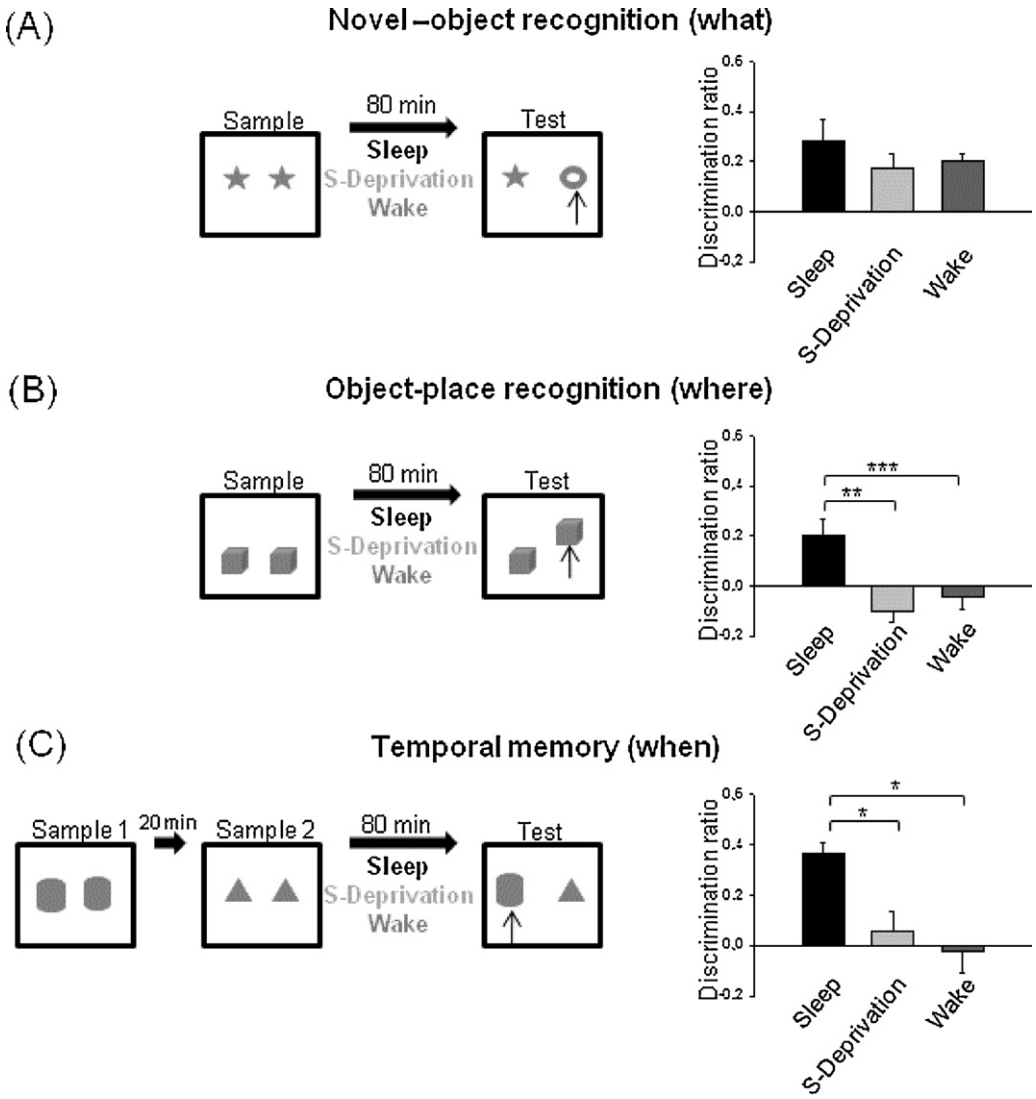

Fig. 1. Evaluation of components of episodic-like memory in a novel-object recognition task (A), an object-place recognition task (B), and a temporal memory task (C). The tasks comprised one or two Sample phases where the rat explored the objects, followed by an 80-min retention interval during which the rats slept (Sleep condition), were deprived of sleep (S-Deprivation condition), or were spontaneously awake (Wake condition). Then memory for the Sample phase was tested by examining the exploration pattern in the Test phase. In the novel-object recognition task memory retention in the Test phase is indicated by the rat's preferential exploration of a novel object (arrow) over a familiar object, with reference to the objects of the Sample phase. In the object-place recognition task, memory retention is indicated by relatively enhanced exploration time for the displaced (arrow) compared to the non-displaced object. In the temporal memory task memory for the temporal order is indicated by the relatively enhanced exploration time of the object presented earlier, i.e., in the first of two Sample phases (arrow). Bar diagrams on the right indicate memory performance in the Test phase expressed as mean (±SEM) discrimination ratios for the three tasks and retention conditions. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, for pair-wise comparisons between retention conditions. *Note*: only with sleep during the retention interval rats in the Test phase showed significant memory for the object-place and temporal order, whereas sleep was not critical for retention of objects in the novel-object recognition task.

the Test phase a mixed set was presented consisting of two old-familiar and two recent-familiar objects. One of the two old and recent-familiar objects, respectively, was placed at the same location as in the Sample phase ("old-familiar stationary" and "recent-familiar stationary"). The other two objects were placed in new locations ("old-familiar displaced" and "recent-familiar displaced"). Relatively enhanced exploration of the "recent-familiar displaced" object in comparison with the "recent-familiar stationary" object indicates memory for the spatial context, and relatively enhanced exploration of the "old-familiar stationary" object in comparison with the "recent-familiar stationary" object indicates memory for the temporal context binding the episode.

2.3. Apparatus and object stimuli

Tasks were performed in a room with a noise-generator providing masking noise. The open field (80 cm × 80 cm W × 40 cm H) used was made of grey PVC. Behavior of the rat was monitored by a video camera, and exploration was analyzed offline by an experienced researcher using the ANY-maze tracking system. The upper side of the open field arena was open, so that the rat could perceive visual cues outside which were in the same position during the whole experiment (two rectangles at the north wall, two other rectangles at the east wall, and a square at the west wall). After each phase, the apparatus and objects were cleaned with water containing 50% ethanol. Objects for exploration were made by glass and were heavy enough not to be moved by the rat. They differed in height (10–15 cm), base diameter (8–10 cm), color and shape. Pilot studies ensured that the rats could discriminate the different objects and did not show any preference for one of the objects.

2.4. Data analysis

For each retention condition and memory task, the time a rat spent exploring each object during the Test phase was converted into a discrimination ratio which indicated the respective episodic-like memory component based on the general formula [(time at novel − time at familiar)/(time at novel + time at familiar)], where "novel" refers to the novel object on the novel-object recognition task, the displaced object on the object-place recognition task, and the old- familiar object on the temporal memory task, and "familiar" to the respective other object. For the episodic-like memory task we calculated two discrimination ratios: i.e., [(recent-familiar displaced − recent-familiar stationary)/(recent-familiar displaced + recent-familiar stationary)] reflecting the "where" memory as indicated by longer exploration time for the recent-familiar object in the novel place compared with the one not displaced, and [(old-familiar stationary − recent-familiar

stationary)/(old-familiar stationary + recent-familiar stationary)] which reflects the "when" memory as indicated by longer exploration time for the object that was experienced earlier (first Sample phase) in the same location. 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 on each task was determined.

Results are reported as the mean ± SEM. Statistical analyses were performed using SPSS 18.0 for Windows. To evaluate discrimination ratios determined for each task, we used analyses of variance (ANOVA) with retention conditions (Sleep, S-Deprivation, Wake) as repeated measures factor. For the episodic-like memory task two additional ANOVA were run on the exploration time for each object, one including an additional repeated measures factor "object" (old-familiar stationary, recent-familiar stationary, old-familiar displaced, recent-familiar displaced) and the other including separate "temporal" (old-familiar stationary, recent-familiar stationary) and "spatial" factors (old-familiar displaced, recent-familiar displaced). In the latter ANOVA significance of the interaction between the temporal and spatial factors can be considered a straightforward statistical indicator of the binding of the two components into an episodic memory. Where appropriate, Greenhouse-Geisser corrections of degrees of freedom were applied in the ANOVAs. Only if an ANOVA indicated significance for main or interaction effects of interest, these were followed by post hoc *t*-tests (uncorrected for multiple comparisons). Discrimination ratios for each group were also compared with chance level performance (zero) using one-sample *t*-tests. A $p < 0.05$ was considered significant.

3. Results

3.1. Individual components of episodic-like memory

Sleep during the retention interval, in comparison with sleep deprivation (S-Deprivation) or wakefulness (Wake), did not affect performance in the Test phase on the novel-object recognition task ($p = 0.39$, for condition main effect; Fig. 1A). One-sampled *t*-test revealed for all three retention conditions discriminations ratios significantly above chance level, confirming that memory for the familiar object was present (Sleep: $t(17) = 3.43$, $p < 0.001$, S-Deprivation: $t(17) = 2.88$, $p = 0.021$, Wake: $t(17) = 5.88$, $p < 0.001$).

In contrast, discrimination ratios for the object-place recognition and temporal memory tasks indicated that spatial and temporal order memory was preserved only after the Sleep retention interval, but not after the other two retention conditions (object-place recognition: $F(2, 34) = 16.10$, $p = 0.001$; temporal memory: $F(2,24) = 6.48$; $p = 0.01$). Post hoc *t*-tests revealed the Sleep condition differed significantly from both the S-Deprivation and Wake condition in the object-place recognition task ($t(17) = 3.44$, $p = 0.009$ and $t(17) = 6.90$, $p < 0.001$, respectively; Fig. 1B) and in the temporal memory task ($t(17) = 3.08$, $p = 0.015$ and $t(17) = 2.38$, $p = 0.044$, Fig. 1C). In fact, for both tasks only after the Sleep retention interval discrimination ratios significantly differed from chance level (object-place recognition: $t(17) = 3.12$, $p = 0.014$, temporal memory: $t(17) = 8.88$, $p < 0.001$), whereas discrimination ratios for the S-Deprivation and Wake conditions indicated that rats had completely forgotten respective spatial and temporal memories ($p = 0.50$ and $p = 0.79$).

3.2. Episodic-like memory task

The rats' performance during the Test phase of the episodic-like memory task overall revealed the typical object exploration pattern with longer exploration time for the old-familiar stationary than recent-familiar stationary object (indicating temporal memory) and longer exploration time for the recent-familiar displaced than recent-familiar stationary object (indicating spatial memory; $F(3, 42) = 9.98$, $p < 0.001$, for main effect of object, Fig. 2). The episodic nature of the expressed memory integrating temporal and spatial components was confirmed also by significance of the interaction between spatial and temporal components of the task ($F(1, 17) = 30.09$, $p < 0.001$). The pattern, however, was differentially expressed depending on the retention condition ($F(6, 57) = 3.71$, $p = 0.013$, for object × retention condition, and $F(2, 32) = 16.19$, $p = 0.001$, for spatial × temporal component × retention condition). Indeed, paired *t*-tests between objects revealed that only after retention Sleep exploration time was significantly longer for the old than recent-familiar stationary object ($t(17) = 3.81$, $p < 0.001$) and exploration time was also significantly longer for the displaced than stationary recent-familiar object ($t(17) = 4.24$, $p < 0.001$), confirming the presence of temporal-spatial context memory after the 80-min retention interval. In contrast, in the S-Deprivation condition rats showed only significant "where" memory, i.e. longer exploration time for the displaced than stationary recent-familiar object ($t(17) = 2.19$, $p = 0.044$), but no temporal memory ($p = 0.16$), and in the Wake condition rats showed neither significant spatial context memory ($p = 0.20$) nor significant temporal context memory ($p = 0.55$).

Analyses of discrimination ratios for the "where" and "when" components of episodic-like memory confirmed the superior retention performance in the Sleep condition ($F(2,32) = 21.09$, $p < 0.001$, for main effect of retention condition

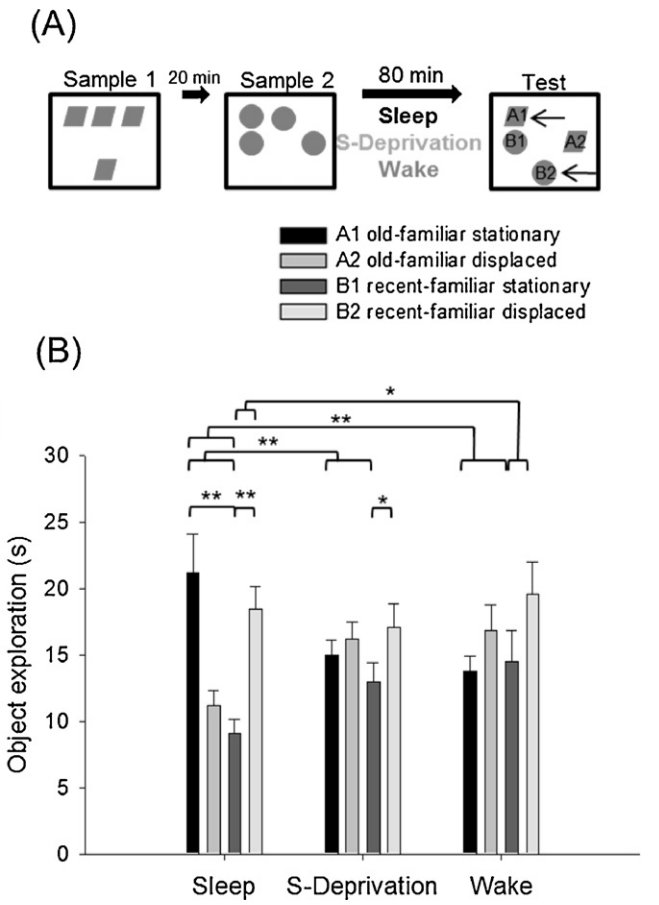

Fig. 2. Episodic-like memory task (A). An example arrangement of objects is illustrated for the two Sample phases and the Test phase which were separated by an 80-min retention interval of Sleep, sleep deprivation (S-Deprivation), or spontaneous wakefulness (Wake). During the Test phase, two "old-familiar" objects and two "recent–familiar" objects of the first and second Sample phase, respectively, are presented at the same ("stationary") or different ("displaced") locations, as compared to the Sample phases. A1: "old-familiar stationary"; A2: "old-familiar displaced"; B1: "recent-familiar stationary"; B2: "recent-familiar displaced". Sample and Test phases took 3 min each. (B) Memory performance during the Test phase indicated by the mean (±SEM) exploration time for each object and retention condition. $^{*}p < 0.05$; $^{**}p < 0.01$, for pairwise comparisons between objects and retention conditions. *Note*: distinct preference pattern of object exploration for the Sleep condition (B2 > B1 indicating "where" memory; A1 > B1 indicating "when" memory), which is absent in the S-Deprivation and Wake conditions, except the B2 > B1 for the S-Deprivation condition.

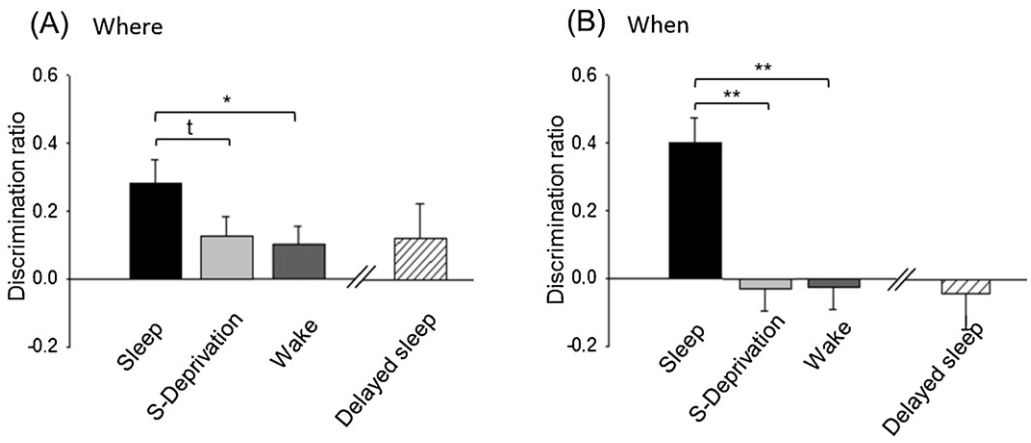

Fig. 3. Mean (±SEM) discrimination ratios indicating spatial ("where") (A) and temporal ("when") (B) context memory in the episodic-like memory task for the Sleep, S-Deprivation and Wake retention conditions. Rightmost bars ("Delayed sleep") indicate discrimination ratios in supplementary experiments where rats were allowed to sleep during a second 80-min retention interval after they had been sleep deprived during the first 80-min retention interval. (t) $p < 0.1$, $^{*}p < 0.05$; $^{**}p < 0.01$, for pair-wise comparisons between retention conditions of the main experiment. *Note*: enhanced spatial and temporal context memory for the Sleep condition in comparison with both S-Deprivation and Wake conditions. Delayed sleep that follows a first 80-min retention period of sleep deprivation does not recover memory.

in an 2 "Where"/"When" discrimination ratios × 3 Sleep/S-Deprivation/Wake conditions ANOVA). The discrimination by condition interaction effect was not significant ($p = 0.37$). Pairwise comparisons between retention conditions confirmed that following the Sleep retention interval "when" discrimination ratios were higher than following both the sleep deprivation ($t(17) = 3.93$, $p < 0.001$) and wake retention intervals ($t(17) = 3.55$, $p = 0.002$). "Where" discrimination ratios following sleep were significantly higher than following the Wake condition ($t(17) = 2.14$, $p = 0.04$), and tended to be also superior to those in the S-Deprivation condition ($p = 0.09$, Fig. 3). Moreover, in the Sleep condition, both "where" and "when" discrimination ratios were clearly above chance level ($t(17) = 4.22$, $p = 0.001$; $t(17) = 4.74$, $p < 0.001$; respectively). In contrast, these ratios did not reach the 5%-level of significance in both the S-Deprivation condition ($p = 0.07$ and $p = 0.66$, respectively) and in the Wake condition ($p = 0.09$ and $p = 0.69$), indicating that only when the rats slept in the 80-min retention interval they were able to consolidate a spatio-temporally integrated episodic-like memory.

Analyses of the total time spent exploring objects in the Test phase or Sample phase and the duration of the Sample phase did not differ between conditions for any of the tasks ($p > 0.25$, for all relevant comparisons; see Table 1).

3.3. Time-window of sleep consolidation

The main experiments revealed that rats could not retain an integrated episodic-like memory when they did not sleep during an 80-min interval after the Sample phases. In supplementary experiments (performed in a subgroup of 12 rats started one week after the main experiment) we explored whether sleep would recover these memories if it occurred not until after a 80-min period of wakefulness. Thus, rats after a first 80-min interval of sleep deprivation and a first Test phase underwent a second 80-min retention interval where they slept (Delayed sleep), and then were tested in a second Test phase. As in the main experiment, rats after the first retention interval of sleep deprivation did not exhibit any significant episodic-like memory, i.e., both "where" and "when" discrimination ratios were not significantly above chance level ($t(11) = 1.22$, $p = 0.25$; $t(11) = -0.39$, $p = 0.70$, respectively). Importantly, when the rats slept during the subsequent (i.e., delayed) 80-min retention interval exploration performance at the second Test phase remained unchanged, indicating that they did not recover the episodic-like memory of the Sample phases. Again both "where" and "when" discrimination ratios did not differ from chance level ($t(11) = -0.33$, $p = 0.75$; $t(11) = -0.60$, $p = 0.56$, respectively; $p > 0.62$ for differences between first and second Test phase, Fig. 3). To exclude that in these supplementary trials the first Test phase interfered with memory recovery during the subsequent 80-min interval of sleep, two further controls were run where the rats slept both in the first and second 80-min retention interval but, in one of these conditions the Test phase after the first 80-min interval was omitted. Analyses of the final Test phase confirmed the presence of episodic memory in both the condition with and without a Test phase after the first 80-min sleep retention interval ("where" discrimination ratio: $t(9) = 4.70$, $p = 0.001$; $t(9) = 2.97$, $p = 0.016$; "when" discrimination ratio: $t(9) = 2.99$, $p = 0.015$, $t(9) = 2.74$, $p = 0.023$, respectively) and, importantly, there was no difference in object exploration between the conditions ($p > 0.77$, for condition main effect and condition × discrimination ratios interaction in an ANOVA across both conditions). In combination, these results suggest that once the rats

Table 1
Time spent exploring objects during the Sample and Test phases.

Tasks	Sleep		S-Deprivation		Wake	
	Sample phase	Test phase	Sample phase	Test phase	Sample phase	Test phase
Episodic-like memory	59.83 ± 3.39	60.56 ± 4.12	68.14 ± 3.00	66.56 ± 3.98	59.47 ± 2.64	62.12 ± 4.78
Novel-object recognition	42.64 ± 4.48	43.47 ± 4.41	48.88 ± 7.73	50.47 ± 4.02	46.31 ± 5.55	49.25 ± 5.01
Object-place recognition	41.23 ± 3.68	40.18 ± 3.01	42.23 ± 3.59	44.46 ± 3.63	43.46 ± 4.02	46.48 ± 4.63
Temporal memory	42.53 ± 6.45	43.78 ± 6.15	39.90 ± 2.68	41.36 ± 3.29	37.84 ± 3.98	40.71 ± 5.12

Mean (±SEM) total time (in s) spent exploring objects in the Sample and Test phase for the four different memory tasks and the 3 retention conditions (Sleep, S-Deprivation, Wake). There were no significant differences between the retention conditions. Also, retention conditions did not differ with regard to the duration of the Sample phases (not shown); $p > 0.25$, for all relevant comparisons.

had forgotten the episodes of the Sample phases during wakefulness, delayed sleep does not recover this memory.

4. Discussion

While a strengthening effect of sleep on memory is well-established, recent research in humans has suggested that memory consolidation during sleep in particular supports associative integration and binding in memory [36–38], i.e., key processes underlying the formation of episodic memory. However, systematic investigations of the effect of sleep on episodic memory consolidation per se are scarce, and whether consolidation during sleep supports specifically the binding of contextual ("where", "when") and item ("what") memory aspects of an episode has been examined so far neither in humans nor in rodents. Whereas previous studies (e.g., [20]) were restricted to the isolated examination of just one aspect contributing to episodic memory (mostly the spatial component) the present experiments go beyond those studies by examining the effects of sleep on the consolidation of an integrated episodic-like memory, together with a separate evaluation of influences on its components. The two main finding are: (i) only when the rats slept during the 80-min retention interval between the Sample and the Test phase did they preserve an integrated memory for the episodes experienced during the Sample phases, i.e., recall testing (in the Test phase) showed that they were able to bind the experienced event (item) into a spatio (where) – temporal (when) context. This finding demonstrates that sleep is of critical importance for the consolidation of episodic-like memory. (ii) Separate testing of episodic-like memory components showed that sleep was critical only for the consolidation of associative learning components, i.e., sleep preserved the association between item and spatial context (object-place recognition) and the association between item and temporal context (temporal memory task). When the rats did not sleep in the retention interval these associations were forgotten. In contrast, memory for the item per se (novel-object recognition) did not benefit from sleep. These findings agree with evidence in rodents and humans indicating a robust effect of sleep in strengthening spatial and temporal associative memories (e.g., [17,18,20,39,40]). A previous study using the same episodic-like memory task as here in Wistar rats, revealed behavioral signs of preserved episodic across a retention interval, which like in the Wake condition of our study was positioned in the first half of the dark period [41]. However, sleep was not monitored and also the retention interval in that study was shorter (60 min) than that of the present study, hampering the straight forward integration of those results. Assuming that place-object and temporal order memories are hippocampus-dependent whereas object-recognition memory is not, the data of the present study also tie in with previous studies from rats and humans suggesting that sleep selectively supports the consolidation of hippocampus-dependent aspects in memory [16,42–45]. Our supplementary studies suggest that episodic-like memory benefits from sleep mainly if sleep occurs shortly after encoding, (i.e., here within 80 min after encoding) which is consistent with previous studies likewise suggesting a limited time window after encoding in which hippocampal memories profit from sleep [42]. In demonstrating a fundamental impact of sleep on the maintenance of episodic-like memory, our data underscore the importance to account for this brain state in any experimental assessment of episodic-like memory.

Although many human studies have quite compellingly demonstrated an enhancing effect of sleep on hippocampus-dependent declarative memory, evidence that this benefit for hippocampal memory may be specifically related to its episodic features is scarce [23,46]. In rodents, studies of the effects of sleep on episodic-like memory in the strict sense are completely lacking although some findings pointed towards preferential benefits from sleep for memories involving hippocampal function [19,42,44,47]. Against this background, the present study in rats aimed to elaborate on the unique features of episodic-like memory, by assessing how sleep affects the retention of a singularly experienced event contextually integrated in time and space. Our task approach was based on spontaneous object exploration that has been widely used to evaluate multiple aspects of memory [4,35,48]. It bears several methodological advantages, mainly because it relies on an unconditioned preference thereby preventing the induction of any rule learning or semantic memory [34]. Additionally, it does not require water or food deprivation, is not stressful and less arousing than tasks like fear conditioning or the Morris water maze task that is based on negative reinforcement [4,48–50]. Thus confounds by emotional and stress-related learning processes are avoided (e.g., [51–53]), and conditions are achieved most closely comparable with those of episodic memory assessment in humans [54]. Indeed our results that sleep selectively preserves spatio-temporal binding of episodic-like memory while leaving unaffected object recognition per se, well fits with recent findings in humans where sleep enhanced spatial context memory whereas item memory remained unchanged [23].

Interestingly, in this study sleep overall appeared to profit memory for the temporal context ("when" discrimination ratio) somewhat more consistently than memory for spatial context ("where" discrimination ratio), although respective ("where"/"when") interaction effects failed to reach significance. Whereas following sleep deprivation "when" discrimination ratios for the episodic-like memory task were clearly inferior to those after sleep, the difference in the same direction for "where" discrimination ratios between these conditions did not reach significance. This pattern is reminiscent of findings by Pierard et al. [55] who observed in mice after 10 h of total sleep deprivation a distinct suppression of serial context memories whereas effects on spatial context memory were not significant. These less consistent outcomes for spatial context memory might partly reflect task specific characteristics, as far as it is known that when animals cannot use hippocampus-dependent strategies based on distal allocentric cues to solve a spatial task, they tend to shift to a non-hippocampus-dependent strategy based on the striatal processing of proximal egocentric cues [17,47,56]. Such change in the strategy can be caused by hippocampal damage and might account for the less consistent effects after sleep deprivation. Such changes in strategy have been also observed after stress [57]. Yet, stress as a confounding factor can be excluded here, as a non-stressful procedure of sleep deprivation (slightly tapping on the cage and gently shaking the cage) was employed. Moreover, such confound would not explain that rats in the S-Deprivation condition did in fact exhibit complete forgetting of any object-place memory when measured separately.

The idea has been discussed for some time that the same consolidation process applies to all declarative memories whether episodic/contextual or semantic/schematic [6,9]. The present data may be taken to argue against this view, if it is assumed that the binding into spatio-temporal context is a key feature of episodic memory in both rats and humans, genuinely linked to hippocampal function, whereas memory for an item per se shares the non-contextual features of semantic memory, represented in non-hippocampal (e.g., perirhinal and also neocortical) structures [30,58–64]. In this perspective, our data support the notion that only the consolidation of hippocampus-dependent contextual memories critically relies on sleep whereas consolidation of non-contextual object recognition memories does not. Corroborating this conclusion two previous studies in rats found the consolidation of context fear conditioning which involves hippocampal function, to critically depend on sleep [42,44]. In contrast, cued

fear conditioning which does not require hippocampal function, did not profit from sleep (see also [19]). There have been also apparently divergent findings (e.g., in mice) of a sleep-induced enhancement in object recognition memory [65]. Yet, such sleep-induced increments in object recognition rather than reflecting a direct beneficial action of sleep on these memories might represent secondary effects owing to the use of tasks with high contextual memory demands. In fact, in the adult brain any enhancements of preexisting semantic memory, like new semantic memories, might basically originate as an extract from episodic memories repeatedly replayed during sleep [9,66].

Episodic memory consolidation can be divided into different critical time periods [67]. Here we evaluated intermediate-term episodic-like memory, with a duration from minutes to hours. In our supplementary experiments, sleep that occurred after the first 80-min retention interval, failed to recover any episodic memory. Together with previous work [23,42], these findings point towards a particular importance of sleep for intermediate-term consolidation. The memory benefit linked to sleep occurring soon after encoding, might reflect effects of reduced non-specific interference, i.e., sleep protecting the fresh episodic-like memory to become overwritten in hippocampal circuitry by new information [68]. However, protection from interference is not a sufficient explanation given that the rats during the retention interval were kept in a highly familiar box with no new information to be encoded. There is strong evidence that neuronal reactivations of hippocampal memories occur at a highest rate during the early hours of slow wave sleep only shortly after the encoding experience [11,69,70]. These reactivations are causative for the strengthening of hippocampal memory, presumably by initiating a system consolidation process that transforms intermediate memories into long-term memories [10,13,71]. Hence, the critical sleep-dependency of intermediate-term episodic-like memory observed here probably reflects an additional active role of sleep in upholding these memories, serving beyond a merely strengthening influence, the extraction of invariant and semantic aspects in these memories.

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