Behavioral/Cognitive

The Emergence of New Schema Memory Requires Sleep

Maximilian Harkotte,[1,2] Fabian Heimel,[1] Stoyan Dimitrov,[1] Marion Inostroza,[1,3] and Jan Born[1,3,4,5]

[1]Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen 72076, Germany, [2]International Max Planck Research School for the Mechanisms of Mental Function and Dysfunction, Graduate Training Centre of Neuroscience, University of Tübingen, Tübingen 72076, Germany, [3]German Center for Mental Health (DZPG), Partner Site Tübingen, Tübingen 72076, Germany, [4]German Center for Diabetes Research (DZD), Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen (IDM), Tübingen 72076, Germany, and [5]Center for Integrative Neuroscience, University of Tübingen, Tübingen 72076, Germany

Schema memory refers to generalized knowledge that is formed across multiple episodes containing regularities. Schema memory is thought to be formed in an active systems consolidation process that transforms individual episodic representations into neocortically anchored schema representations and that is facilitated by sleep. Here we show in rats that sleep is indeed critical for the emergence of a new schema memory. Male rats ($n = 20$) were trained on an elaborated version of the object–place recognition task, which allowed for abstraction of a spatial rule across eight encoding episodes spaced 20 min apart without intermittent sleep. During each episode, animals freely explored two objects in an open-field arena. Following the encoding phase, animals either slept or were kept awake for 2 h, after which they remained undisturbed for 22 h before schema memory for the spatial rule was assessed. Only animals that slept during the 2 h postencoding window exhibited schema memory. Prior knowledge conflicting with the spatial rule prevented schema memory formation. c-Fos expression assessed at retrieval indicated that successful schema recall was supported by a more sparsely activated yet highly interconnected network comprising, among others, the medial prefrontal cortex and hippocampus. Our findings highlight the critical role of immediate postencoding sleep in forming new spatial schema memory.

Key words: **memory consolidation; object–place recognition; schema memory; sleep**

Significance Statement

Schema representations integrate regularities abstracted from multiple episodes. Whether and how the formation of schema representations benefit from sleep is still unclear, partly because evoking abstraction processes across episodes within a single wake period in animal models is challenging. Using an object–place recognition-based schema memory task, we show for the first time in rats that sleep critically supports schema memory formation. Rats that slept, but not those kept awake for 2 h after encoding the eight task episodes, expressed schema memory 24 h later. Schema recall after sleep was associated with a sparse but distinctly more coordinated network activation, mainly comprising the medial prefrontal cortex and hippocampus. Our findings highlight sleep's role in newly forming distributed spatial schema representations.

Introduction

Consider a party at a colleague's apartment you have never visited before. Even without knowing the exact floorplan, you would likely have little difficulty locating the bathroom, drawing on general knowledge about how apartments are typically arranged. This intuition arises from having experienced many different apartments in the past, allowing the brain to abstract and store regularities across the experienced episodes. Such generalized knowledge structures, known as mental schemas (Bartlett, 1932; Gilboa and Marlatte, 2017; Farzanfar et al., 2023; Sekeres et al., 2024; Bein and Niv, 2025), can be defined as patterns of knowledge in long-term memory that capture regularities and rules derived from multiple experiences, shaping the perception and learning of new information (Ghosh and Gilboa, 2014).

Schema memory formation is mainly studied in humans, where evidence points to an important role of sleep (Wagner et al., 2004; Lewis and Durrant, 2011; Lerner and Gluck, 2019; Lacaux et al., 2021). However, human subjects typically possess well-established prior schema knowledge that can unpredictably influence experimental outcomes. Rodent models offer the advantage of having full control over prior experience, beyond

Received Sept. 24, 2025; revised Nov. 28, 2025; accepted Dec. 30, 2025.

Author contributions: M.H., M.I., and J.B. designed research; M.H. and F.H. performed research; M.H. and S.D. analyzed data; M.H. and J.B. wrote the paper.

This study was supported by grants from the Deutsche Forschungsgemeinschaft to J.B. (FOR 5434) and the European Research Council to J.B. (ERC AdG 883098 SleepBalance). M.I. is supported by the Hertie Foundation (Hertie Network of Excellence in Clinical Neuroscience). We thank Anuck Sawangjit, Julia Fechner, Enea Tosadori, and Niels Niethard for the valuable discussions. We thank Lisa Bastian and Enea Tosadori for sharing code to perform correlational network analyses.

The authors declare no competing financial interests.

Correspondence should be addressed to Jan Born at jan.born@uni-tuebingen.de.

This paper contains supplemental material available at: https://doi.org/10.1523/JNEUROSCI.1810-25.2025

https://doi.org/10.1523/JNEUROSCI.1810-25.2025

Copyright © 2026 the authors

enabling the invasive study of underlying neural mechanisms. Indeed, adult rodents are capable of forming schema-like representations from multiple episodes (Tse et al., 2007, 2011; Benchenane et al., 2010; Harkotte et al., 2022; Abdou et al., 2024). For example, Abdou et al. trained mice in an arena with five interconnected compartments, where in each trial only two compartments were accessible and entering one, but not the other, was rewarded with a sugar treat. Over multiple days, animals learned ordered compartment–reward associations (A > B, B > C, C > D, D > E). When later presented with a novel choice between Compartments B and D, mice preferred B, indicating that the animals could infer the underlying hierarchy. Strikingly, this knowledge emerged only after a 24–72 h delay and only when animals had a 4 h sleep opportunity following the final training session. However, this and similar tasks (Tse et al., 2007; Benchenane et al., 2010) are limited as they require training across several days. Manipulating sleep only after the final training session may simply promote consolidation of that last episode, essentially adapting it to a preexisting schema rather than integrating information across episodes.

Hence, a more definitive test of sleep's role in schema formation requires tasks that induce schemas from multiple episodes within a single wake period. Indeed, building on classical object–place recognition (OPR), tasks have been developed that minimize prior training requirements and allow to study how schema memories emerge across just a few episodes (Genzel et al., 2019; Harkotte et al., 2022). In our own work (Harkotte et al., 2022), we exposed adult rats to either four or eight encoding episodes in which they freely explored an open-field arena containing objects arranged according to a spatial rule. When tested 24 h later, animals showed schema memory for the rule only after encoding eight—but not after four—episodes, underlining the importance of integrating across multiple episodes for schema memory formation. Testing memory specifically for the most recent of the eight episodes indicated that memory for the individual episode had decayed.

Here, we used this OPR-based schema paradigm to test the effects of sleep on the formation of a novel spatial schema. Sleep strengthens episodic memory in an active systems consolidation process that transforms hippocampally represented episodic memory into long-term memory preferentially residing in neocortical networks (Lutz et al., 2025). This transformation is also thought to foster schema abstraction. To test this, rats were exposed to eight episodes of the schema memory task without intermittent sleep. Following this encoding phase, animals either slept or remained awake for 2 h, and schema memory was tested 24 h later. A control study assessed potential confounds of preexisting knowledge on formation of the schema memories, and we also assessed c-Fos activity to identify brain networks implicated in the retrieval of schema memories.

Materials and Methods

Animals. Forty-seven adult male Long–Evans rats (Janvier Labs), aged 9–12 weeks in the beginning of the experiment, were used in this study. The rats were housed in groups of 2–4 per cage, with *ad libitum* access to food and water. They were maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.). Prior to behavioral testing, the animals were handled daily for 10–15 min over 5 consecutive days. All experimental procedures were conducted in accordance with European animal protection laws and policies and were approved by the Baden-Württemberg state authorities.

Apparatus and objects. An elaborated version of the OPR task was conducted in a quadratic open-field arena (80 × 80 × 40 cm) made of gray PVC. The arena was dimly lit with an illumination level of 20–30 lux and supplemented with masking white noise at 60 dB. A camera (Logitech C920) was mounted above the arena to record the trials. Distal spatial cues included the camera, posters affixed to the walls of the testing room, various objects (spheres, egg boxes, etc.) hanging from the ceiling, and surrounding curtains. Next to the arena but separated by the curtains, a resting box (35 × 35 × 45 cm) made of gray PVC and filled with bedding material was used to keep the animals during the postencoding phase and sleep was monitored using a second camera.

One set of four and nine pairs of glass objects of various shapes and sizes (height, 15–30 cm; bottom diameter, 7–12 cm), filled with sand of different colors, were used in the experiments (Fig. S1*A*). The objects were sufficiently weighted to prevent the rats from moving them during testing. To avoid olfactory interference, we thoroughly cleaned the arena and objects with a 70% ethanol solution after each episode.

Experimental design. The study comprised a main experiment and an additional control experiment. The main experiment comprised two groups of animals, a sleep group ($n = 10$) in which the animals slept during the 2 h postencoding retention interval and a wake group ($n = 10$) in which the animals were kept awake during this interval. To enable comparisons of c-Fos expression with a home-cage (HC) control baseline condition, an additional group of six animals underwent the experimental protocol in the same way as the sleep group, except that these animals did not undergo the retrieval phase of the experiment but, instead, were transported from the animal facility to another room, where they were kept awake in their HC for 10–15 min (i.e., for the duration of the retrieval phase in the main experiment).

To investigate how prior knowledge influenced schema memory formation in the arena context, a control study was performed in two additional groups of animals ($n = 10$ for the sleep and $n = 11$ for the wake condition). To manipulate prior knowledge, both groups of this experiment underwent a slightly modified habituation protocol (Fig. 2*A*), before being subjected to the same encoding and retrieval phases as in the main experiment. Specifically, animals were not habituated to an empty arena, as in the main experiment, but were repeatedly exposed to four identical objects placed in the corners of the arena during all three habituation sessions. This procedure established a spatial regularity (objects occupying corner locations), thus generating a spatial knowledge conflicting with the spatial rule to be acquired in the encoding phase of the experiment 2 d later.

Experimental procedures and task. Prior to the experiments, animals were habituated to the testing room and open-field arena. For this purpose, the animals were brought to the testing room inside their HC on 3 consecutive days. After spending at least 20 min in the testing room, each animal was placed in the open-field arena, facing a different wall of the arena during each habituation session. The animals were then allowed to freely explore for 10 min, after which they were placed into a resting box, in which the animals were left undisturbed for 4 h with ad libitum access to water. Afterward, animals were brought back to their HC and to the animal facility where they were kept.

The experiment proper included an encoding phase, a postencoding retention interval, and a retrieval phase (Fig. 1*A*). During the encoding phase, animals were brought back to the lab and subjected to eight consecutive encoding episodes, each separated by a 20 min interval. In each episode, a different pair of identical objects was placed in two of the eight possible locations within the arena (equidistant from the arena walls). Critically, one specific location in the arena was consistently occupied by an object across all encoding episodes, while two other locations were occupied by objects in an alternating way, i.e., every other episode. This design introduced a spatial rule across the encoding episodes: one location was always occupied, whereas two locations were only partially occupied (Fig. 1*A*). In each episode, the animal was placed in the open-fi eld arena facing a different wall to encourage the formation of an allocentric spatial representation. Each encoding episode lasted 5 min. During the 20 min intervals between episodes, the animals were returned to their HC (with their cage mates). Note that because the animals were tested in parallel, following an alternating encoding schedule, they were

not in completely undisturbed conditions during these interepisode intervals. Specifically, while one animal underwent an encoding episode, its cagemate remained in the HC and vice versa. During the 20 min interepisode interval, the second animal was then taken from the HC, subjected to its own encoding episode, and returned afterward. Consequently, the HC was opened and closed approximately every 5–7 min, creating experimental conditions under which sleep was highly unlikely to occur. Animals were also video recorded during the entire encoding phase, and these videos were later used to confirm that animals did indeed not sleep during these intervals. After completion of the eight episodes of the encoding phase, the animals were placed into the resting box where they spent the 2 h postencoding retention interval either sleeping in undisturbed conditions or being kept awake.

To enforce wakefulness during the 2 h postencoding period in the wake condition, we employed a standard gentle handling procedure which is known to minimize stress even when applied over longer periods (Palchykova et al., 2006; Hagewoud et al., 2010). The procedure involved softly tapping on the resting box or, if necessary, gently shaking it. Intense stimulation was strictly avoided, and neither the rat nor its bedding materials were touched throughout the procedure. Interventions were applied whenever a rat closed its eyes (with or without adopting a sleep posture) and remained immobile for >5 s. On average, 39 ± 14 interventions per animal were required to prevent sleep during the 2 h postencoding interval. Video recordings confirmed that no signs of startle or freezing behavior occurred during the procedure.

After the 2 h postencoding interval, the animals were returned to their HC and left undisturbed for 22 h, before they were brought back to the test room and tested for their memory of the spatial rule. During this retrieval phase, a pair of novel objects was placed in the arena. One object was positioned in a location that had been partially occupied during encoding, while the other was placed in a location that had never been occupied during any of the encoding episodes. Thus, although both objects were completely new to the animals, the location of one object conformed with the spatial rule, while the other violated it, providing a test of the animals' memory of the spatial rule from the previous day (Fig. 1*A*).

All experiments were conducted between 8:00 A.M. and 3:00 P.M. The locations of the objects and the types of objects used were randomized across the encoding episodes to minimize potential biases.

Behavioral assessment. To assess memory performance, exploratory behavior directed toward the objects during both the encoding and test trials was visually scored using tracking software (ANY-maze, Stoelting) after all experiments were completed. Object exploration was defined as the rat being within 1 cm of an object, with its nose directed toward the object and engaging in active exploratory behaviors such as sniffing. Leaning on the object without sniffing or being >1 cm away from the object was not considered object exploration. All scoring was performed by the same experienced experimenter, who was blinded to the experimental condition. To assess memory retrieval for the spatial rule (schema memory), a discrimination ratio (DR) was calculated using the following general formula:

$$(\text{exploration time for rule violating object} - \text{exploration time for rule conforming object}) / (\text{exploration time for rule violating object} + \text{exploration time for rule conforming object}).$$

A positive DR indicates memory for the spatial rule, whereas a DR of zero reflects no exploration preference. Additionally, total object exploration times (across both objects), the distance the animals travelled, and rearing time during the retrieval phase were assessed as indicators of locomotion and nonspecific arousal. Object exploration times and distance traveled were also calculated to analyze the animal's behavior during the encoding episodes.

Sleep during the retention interval was assessed using standard visual scoring procedures of video recordings (Pack et al., 2007; Sawangjit et al., 2018). 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. Validity of this visual scoring procedure was demonstrated in previous studies in rats and mice, consistently providing an agreement with conventional EEG-/EMG-based sleep scoring (van Twyver et al., 1973; Pack et al., 2007). Additionally, the behavioral sleep scoring approach was validated against standard EEG-/EMG-based sleep scoring, in a separate group of five animals tested in comparable conditions (Sawangjit et al., 2018), which revealed a similarly high agreement of 89% across all scored periods as in those previous studies (Fig. S2). Importantly, the percentage of cases where behavioral scoring indicated wake but EEG-/EMG-based scoring indicated sleep was negligible (<2.5%) indicating that behavior-based scoring of sleep can be used to safely exclude the presence of sleep.

To obtain estimates for the time the rats spent in substates of active and quiet wakefulness during the 2 h postencoding interval, additionally, the total duration of immobility periods was analyzed. Immobility was scored behaviorally whenever the animal was almost completely motionless for >2 s (using ANY-maze's automated immobility detection with 95% sensitivity).

c-Fos immunocytochemistry. c-Fos immunocytochemistry was done as previously described (Dimitrov et al., 2026). Briefly, animals of the main experiment including the HC control group were transcardially perfused 90 min after the end of the retrieval phase (or corresponding interval of the HC control). The 90 min interval coincides with the peak of c-Fos protein expression following neuronal activation (Bisler et al., 2002; Zangenehpour and Chaudhuri, 2002). Perfusions were done using phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were postfixed for 16–24 h at 4°C and then stored in PBS at 4°C until further processing. All further analyses were done by a blinded experimenter who was unaware of the individual rat's experimental condition. Coronal slices (70 µm) at the respective bregma levels (see below) were cut with a vibratome (HM 650V, MICROM). During initial optimization of the protocol, it was confirmed that c-Fos labeling intensity was comparable between the surface (section borders) and the center across the 70 µm thickness, indicating adequate antibody diffusion throughout the tissue (see also Dimitrov et al., 2026). For immunostaining, free-floating sections were blocked with PBS containing 10% horse serum and 0.3% Triton X-100 for 90 min. Afterward, the sections were incubated with 0.2% Triton X-100 in PBS containing mouse monoclonal anti-GAD67 (1:1,000 dilutions; Sigma-Aldrich, #MAB5406) and guinea pig monoclonal anti-c-Fos (1:1,000 dilutions; Synaptic Systems, #226 308) primary antibodies for 48 h at 4°C. Incubation with anti-GAD67 was included to allow future analyses of inhibitory network activity, which was not part of the present study. Sections were washed with PBS (four times) and then incubated with 0.2% Triton X-100 in PBS containing donkey anti-mouse Alexa Fluor 488 (1:1,000 dilution; Thermo Fisher Scientific) and donkey anti-guinea pig Alexa Fluor 647 (1:500 dilution; Jackson ImmunoResearch Laboratories) secondary antibodies for 24 h at 4°C. Samples were incubated with NeuroTrace (1:200 dilutions; Thermo Fisher Scientific), washed with PBS, mounted on gel-coated slides, and coverslipped with Vectashield antifade mounting medium (Vector Laboratories).

Regions of interest (ROIs) were defined based on the literature about hippocampal and cortical regions known to be involved in the formation of spatial memories and anatomically determined according to the rat brain atlas (Paxinos et al., 2015). For each ROI, one coronal slice of each brain from all three experimental conditions was used. ROIs and their distance from the bregma were (see also Fig. S3*A*) +3.24 mm for the prelimbic (PL), infralimbic (IL), and anterior cingulate (aCG1) cortices; +0.72 mm for the medial cingulate cortices (mCG1/mCG2); −1.80 mm for the anterior granular and agranular retrosplenial cortices (aRSG/aRSA); −3.96 mm for the medial granular and agranular retrosplenial (mRSG/mRSA), the parietal (PAR), perirhinal (PRH), and entorhinal (ENT) cortices, as well as for the hippocampal proximal and distal cornu ammonis 1 (CA1prox/dist), cornu ammonis 3 (CA3), and dentate gyrus (DG) subfields; and −5.76 mm for the posterior granular and agranular retrosplenial cortices (pRSG/pRSA).

Images were obtained using a confocal laser scanning microscope (LSM 710; Carl Zeiss) with a low magnification [plan-apochromat oil

immersion objective, 25×/0.8NA; *z*-stack, 11 slices (50 µm thickness); scaling (per pixel), $0.33 \times 0.33 \times 3.5$ µm]. Settings for laser intensity, gain, offset, and pinhole were optimized initially and held constant throughout the experiment. Automated c-Fos expressing cell counts were performed using the CellProfiler software. Counting was done on z-stacks of the entire slice volume presented as maximum intensity projection. The sizes of the counting frames were 809,200 µm^2 for PL; 578,000 µm^2 for aCG1; 462,400 µm^2 for IL and CA3; 404,600 µm^2 for PAR; 346,800 µm^2 for mCG1, aRSA, mRSA, PRH, CA1prox/dist, DG, and pRSA; 289,000 µm^2 for mCG2, aRSG, mRSG, and pRSG; and 231,200 µm^2 for ENT.

Statistical analyses. Statistical analyses were conducted in R (R Core Team, 2024). None of the rats had to be discarded from analyses due to, e.g., insufficient object exploration during encoding (<1 s per object) or, for animals of the sleep group, insufficient sleep time (<15 min).

For statistical comparisons across conditions, linear mixed models were fitted using the lme4 package (Bates et al., 2015) with individual rats as random effect (random intercept only) and the fixed effects for the group factors. To compare memory performance, for instance, sleep/wake and minute (Minutes 1–5 of retrieval phase) were compared using the following formula:

$$\mathrm{DR} \sim (\mathrm{Sleep/Wake} * \mathrm{Minute}) + (1|\mathrm{animal}),$$

where DR indicated the discrimination ratio over the 5 min retrieval phase. Significance of factors was assessed by removing the respective factor or interaction of two factors step by step from the model and comparing the modified models with the original using likelihood-ratio tests. Relative c-Fos cell counts compared with the HC control, as well as behavioral control parameters—including total distance traveled and total object exploration time during encoding and retrieval phases—were analyzed using the same approach. For post hoc comparisons, two-sided Welch *t* tests were computed. Correlational analyses were based on Spearman correlation coefficients to account for the low number of animals.

To examine c-Fos coactivation patterns, we conducted network analysis separately for the sleep and wake groups of the main experiment. For each brain region, c-Fos–positive cell counts were normalized to the mean count for that region in the HC control group. Pearson's correlation coefficients were computed between all region pairs within each condition. Significant correlations were identified using the sample size-adjusted critical correlation value (two-tailed *t* test; $\alpha = 0.05$; df = n–2). Correlations with $|r| \geq r_{\mathrm{critical}}$ were assigned as edges in a binary adjacency matrix. These matrices were then used to construct undirected, unweighted graphs in igraph (Csárdi and Nepusz, 2006).

To test for differences in network connectivity, we compared edge densities (observed edges/maximum possible edges) between the two condition-specific graphs. The observed difference in edge density was tested against a null distribution from a permutation test ($n = 1{,}000$). For each permutation, edges from both networks were pooled and randomly reassigned between two graphs with the same number of nodes. The difference in edge density was computed for each permutation, and the two-tailed *p* value was computed as the proportion of permuted differences with absolute magnitude greater than or equal to the observed difference.

For all analyses, a $p < 0.05$ was considered significant. Results are reported as means ± SEM and visualized using ggplot2 (Wickham, 2011) and ggpubr (Kassambara, 2025) packages.

Code accessibility. All video files as well as tracking data are available at osf.io/u9h7d/. Analysis scripts are available at github.com/MaxHarkotte/The-emergence-of-new-schema-memory-requires-sleep.

Results

Successful retrieval of schema memory depends on postencoding sleep

We assessed schema memory performance in animals that were habituated to the arena following a standard protocol. The encoding phase of the task comprised eight 5 min episodes, spaced 20 min apart. In each episode, the rat was exposed to a (different) pair of identical objects placed on specific locations. Across the eight episodes, the objects were placed according to a spatial rule such that in all episodes, one object location was always occupied, while the other object alternated between two possible locations. For testing schema memory 24 h later, a novel pair of objects was introduced, one of which violated the rule as it was placed at a location never occupied during the eight encoding episodes (Fig. 1*A*). Successful schema memory was indicated by preferential exploration of the rule-violating object relative to the time the animal spent exploring the rule-conforming object.

Behavioral scoring confirmed that all animals of the sleep group slept during the experimental 2 h retention interval following the encoding (77 ± 9 min, mean ± SEM, sleep onset latency after encoding 26 ± 7 min), whereas all animals of the wake group remained awake during this interval. Gentle handling was applied on average 39 ± 14 times in the wake group, with these interventions not being evenly distributed across the 2 h postencoding interval. Thus, gentle handling was not at all applied during the first 15 min, whereas during the second hour, animals required on average 30 ± 9 interventions, indicating that sleep pressure accumulated toward the end of the postencoding interval. Indeed, locomotion (i.e., traveled distance) during the first 15 min was comparable in the sleep and wake groups (14.80 ± 5.25 m vs 12.22 ± 5.11 m; $t_{(18.0)} = 1.11$; $p = 0.28$), indicating that early in the retention interval, both groups sustained active wakefulness. Further analyses also revealed that, although gentle handling aimed to prevent sleep, it did not stop animals to enter quiet wakefulness per se: Firstly, within the wake group, the average duration of immobility was comparable between the first and last 15 min of the postencoding interval (249.84 ± 154.88 s vs 397.98 ± 319.58 s; $t_{(13.0)} = -1.32$; $p = 0.21$), i.e., the periods with no and the highest number of gentle handling interventions. Secondly, overall distance traveled across the total 2 h interval was comparable between the wake and the sleep groups (27.52 ± 10.47 m vs 28.90 ± 11.23 m; $t_{(17.9)} = -0.28$; $p = 0.78$; see Fig. S1*C*).

At the retrieval phase, only the animals of the sleep group displayed significant schema memory as indicated by DRs that were significantly higher than zero in the first 4 min of the retrieval phase (all $p < 0.05$; 5th min; $t_{(9)} = 2.24$; $p = 0.05$; Fig. 1*B*). Also, DRs of the sleep group were significantly higher than those of the wake group ($\chi^2_{(1)} = 5.54$; $p = 0.02$, for the main effect of sleep). Post hoc comparisons revealed significant group differences for Minutes 2 and 3 ($t_{(16.9)} = 2.80$; $p = 0.01$; $t_{(16.4)} = 2.71$; $p = 0.02$, respectively). Noteworthy, animals of the sleep group also spent more time exploring the arena standing on their hind legs (rearing) during the retrieval phase, in comparison with the wake group ($t_{(13.2)} = 3.26$; $p = 0.006$; Fig. 1*C*, right panel), suggesting an increased orientation toward distal contextual cues located outside the open-field arena. Control measures total exploration time (of both objects) and distance traveled during the retrieval phase were comparable between the groups, ruling out nonspecific differences in arousal ($t_{(11.4)} = 0.98$; $p = 0.45$; $t_{(18.0)} = 1.01$; $p = 0.32$; Fig. 1*C*, left and middle panels, respectively).

As to the encoding phase, behavioral analyses confirmed that none of the animals fell asleep during the 20 min interval between episodes. In no case, an animal adopted a typical sleep position for >10 s during this interval (refer to Fig. S1*B*, for a detailed description of the rats' behavior during these intervals). Importantly, analyses of exploration behavior during the episodes did not reveal any hint that the rats abstracted the spatial rule already during the encoding phase. Firstly, total exploration

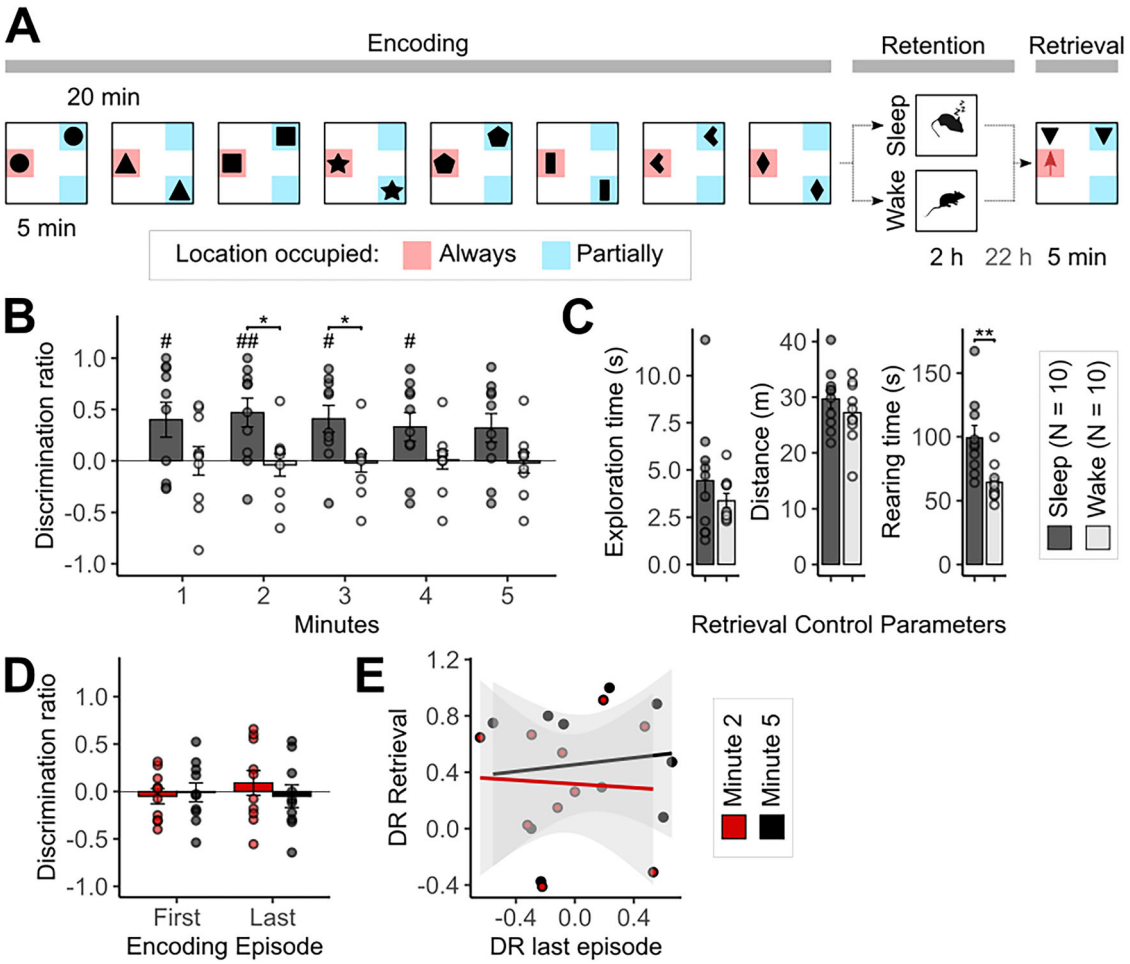


Figure 1. Schema memory formation depends on postencoding sleep. ***A***, Encoding: animals underwent eight 5 min episodes, spaced 20 min apart. Across episodes, one object location was always occupied ("red"), while the other object alternated between two possible locations ("blue"). Retention: afterward, animals either slept or were kept awake for 2 h. Retrieval: memory was tested 22 h later by placing two (novel) objects, one (rule-conforming) at a location partially occupied during encoding and one (rule-violating) at a completely novel location ("red arrow"). ***B***, Mean ± SEM cumulative DRs over the 5 min retrieval phase. Only animals of the sleep group preferentially explored the object in the novel location, indicating that the spatial schema was learned. Asterisks indicate significant post hoc comparisons between sleep and wake groups, hashtags significant DRs above chance (*$p < 0.05$; **$p < 0.01$). ***C***, Mean ± SEM total exploration time (left), traveled distance (middle), and total rearing time (right) during the 5 min retrieval phase. Note, rearing time was increased in the sleep group compared with the wake group (**$p < 0.01$). ***D***, During encoding, DRs of the sleep group showed no preference for either location (always vs partially occupied) in the last episode compared with the first. In addition to the full 5 min interval of the episodes, DRs are shown also for the first 2 min, i.e., the interval with the highest DR at the retrieval test. ***E***, DRs of the sleep group during the last encoding episode did not correlate with DRs at the retrieval phase for the first 2 min or the full 5 min.

time across all eight encoding episodes was comparable between the sleep and wake groups (sleep 5.24 ± 3.30 s vs wake 6.26 ± 4.01 s; mean ± SEM; $\chi^2_{(1)} = 1.57$; $p = 0.21$). Secondly, there was no systematic preference for object placements emerging toward the end of the encoding episodes, as DRs comparing exploration toward always versus partially occupied locations were closely comparable between the first and last episodes ($p > 0.5$) and in both episodes close to zero ($p > 0.5$, for test against chance; Fig. 1*D*). Thirdly, there was also no correlation between these DRs in the last encoding episode and the DR for the retrieval phase ($R = 0.11$; $p = 0.75$; $R = -0.05$; $p = 0.88$, for the second and fifth minute, respectively; Fig. 1*E*).

Preexisting knowledge conflicting with the spatial rule prevents schema memory formation

A control experiment aimed to prove that, in the main experiments, the novel spatial schema formed was indeed uniquely bound to the eight episodes in the arena context. To this end, we tested the effect of prior knowledge conflicting with the spatial schema to be abstracted. Instead to an empty arena (as in the main experiment), the animals were habituated to an arena, where four identical objects were placed in the corners of the arena during each of the three habituation sessions, and the encoding session occurred 2 d after the final habituation day (Fig. 2*A*). In contrast to the main experiment, rats in this condition failed to show schema memory, regardless of whether they slept or were kept awake during the postencoding interval ($\chi^2_{(1)} = 0.02$; $p = 0.89$ for the sleep/wake main effect; Fig. 2*B*). DR did not differ from chance in any group (all $p > 0.1$, two-sided Welch's t tests). Also, control measures of total exploration time during the retrieval ($t_{(18.2)} = 0.18$; $p = 0.86$; Fig. 2*C*, left panel) did not differ between the groups, while distance traveled during the retrieval phase was slightly higher in the Wake condition of the control experiment ($t_{(18.4)} = -2.19$; $p = 0.04$; Fig. 2*C*, right panel). Finally, comparisons between the sleep groups of the control and main experiments confirmed that exploration times during the encoding phase as well as postencoding sleep duration were closely comparable between these groups

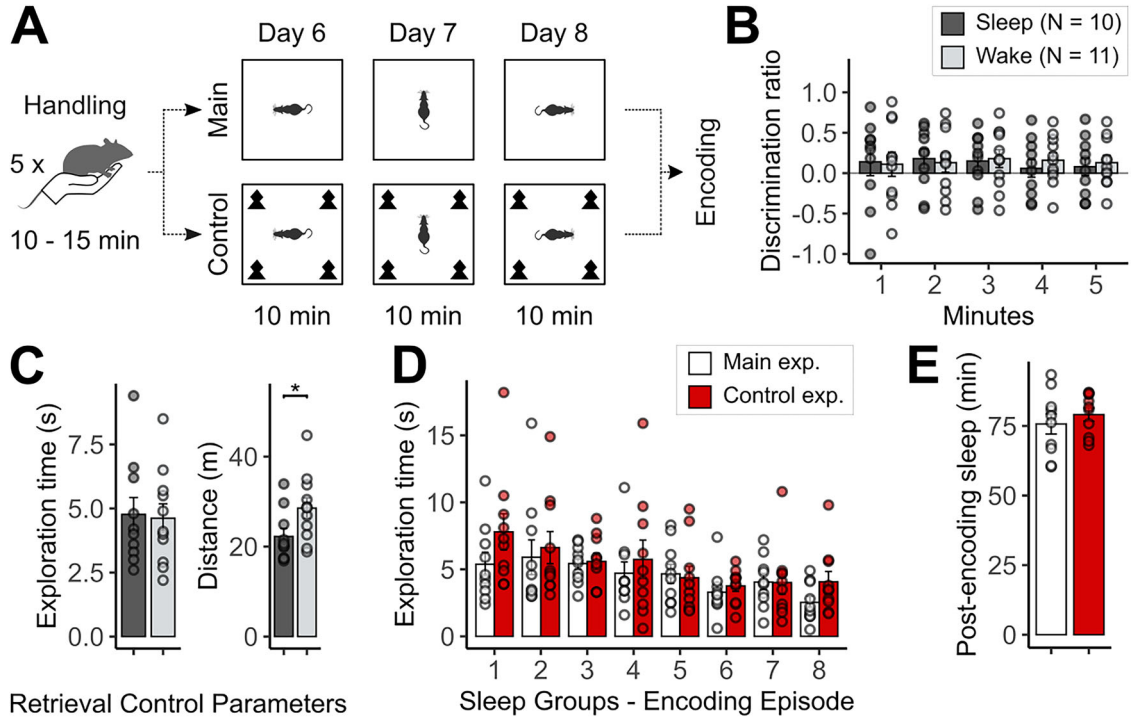


Figure 2. Conflicting prior knowledge prevents schema memory formation. ***A***, Like in the main experiment (Main), animals of the control experiment (Control), after handling, underwent three 10 min open-field habituation sessions (on Days 6–8) before the actual experiments (as described in Fig. 1). Different from the main experiments where the arena was empty, in the control experiments in all habituation sessions, four identical objects were placed in the corners to induce conflicting spatial knowledge. ***B***, Mean ± SEM DRs during the 5 min retrieval phase were not significantly above-chance levels and did not differ between sleep and wake groups of the control experiment, indicating that animals in both groups failed to form schema memory. ***C***, Mean ± SEM total exploration time (left), distance traveled (right) at retrieval for the sleep and wake groups of the control experiment. ***D***, Mean ± SEM total exploration times across the eight encoding episodes and (***E***) postencoding sleep duration was comparable for the sleep groups of the main (white) and control experiment (red).

($\chi^2_{(1)} = 1.00$; $p = 0.32$ for the main effect of experiment type and $t_{(15.5)} = -0.78$; $p = 0.45$ for the sleep duration comparison; Fig. 2*D*,*E*, respectively). Altogether, these results indicate the sensitivity of schema formation to prior knowledge about the arena and, thereby, underline that the schema was indeed newly formed from the eight episodes experienced in the arena context.

Schema memory retrieval is associated with sparse activation of a highly interconnected network of hippocampal and cortical regions

We used c-Fos expression as a marker of neuronal activation during the retrieval phase to investigate whether specific brain regions are differentially engaged during schema memory recall. To this end, we quantified c-Fos–positive cells in the sleep and wake groups of the main experiment and normalized them to a HC control group, which underwent the same learning protocol as the sleep group but was not tested for schema recall. In medial prefrontal cortex (mPFC) areas, c-Fos expression in the sleep group (showing schema memory) was generally comparable to those in the HC group (all $p > 0.05$; Fig. 3*A*). c-Fos levels in the sleep group were even distinctly lower than in the HC group in the hippocampus (all $p < 0.001$; Fig. 3*B*) and retrosplenial cortex (all $p < 0.05$; except for the anterior granular subregion, where $t_{(9)} = -1.75$; $p = 0.114$; Fig. 2*C*). In contrast, in the wake group, c-Fos expression in mPFC was elevated over levels in the HC control (all $p < 0.01$; Fig. 3*A*), the proximal CA1 subregion of the hippocampus ($t_{(9)} = 4.33$; $p = 0.002$; Fig. 3*B*), and the medial and posterior parts of the retrosplenial cortex (all $p < 0.05$; Fig. 2*C*). Significant differences between the sleep and wake groups were observed in the prelimbic ($t_{(14.0)} = -6.50$; $p > 0.0001$), infralimbic ($t_{(15.6)} = -7.86$; $p > 0.0001$), and anterior cingulate subregions ($t_{(16.4)} = -4.12$; $p = 0.004$) of the mPFC ($\chi^2_{(1)} = 17.40$; $p < 0.001$ for the sleep/wake main effect), as well as across all hippocampal subregions ($\chi^2_{(1)} = 30.54$; $p < 0.001$ for the sleep/wake main effect; $p < 0.01$; for all post hoc comparisons). Noteworthy, in the retrosplenial cortex, we observed a gradient of activation along the anterior–posterior axis in the wake group, such that c-Fos counts were progressively higher in posterior regions for both granular and agranular subregions ($\chi^2_{(1)} = 53.35$; $p < 0.001$ for sleep/wake × subregion interaction; Fig. 2*C*). c-Fos levels in parietal, entorhinal, and perirhinal cortices were comparable across all groups ($\chi^2_{(1)} = 3.46$; $p = 0.063$ for the sleep/wake main effect; Fig. 2*D*).

To assess network-level differences, we performed correlational network analysis of c-Fos expression patterns across regions. Based on correlations in c-Fos activity across animals, the analysis assesses to what extent the regions of interest are coactivated during retrieval. We found that interregional functional connectivity during the retrieval phase was markedly higher in the sleep compared with the wake group ($p < 0.001$; permutation testing; Fig. 3*E*; see also Fig. S3*B*). Exploratory analyses for the sleep groups revealed that c-Fos activity in most regions was negatively correlated with behavioral DRs, highest for the parietal cortex ($r = -0.63$). However, none of these coefficients reached statistical significance (corrected for multiple comparisons). Likewise, multiple regression analyses failed to identify key regions predicting behavioral schema memory performance.

Discussion

Studying how novel schema memories emerge is challenging given their dynamic nature, integrating patterns across multiple

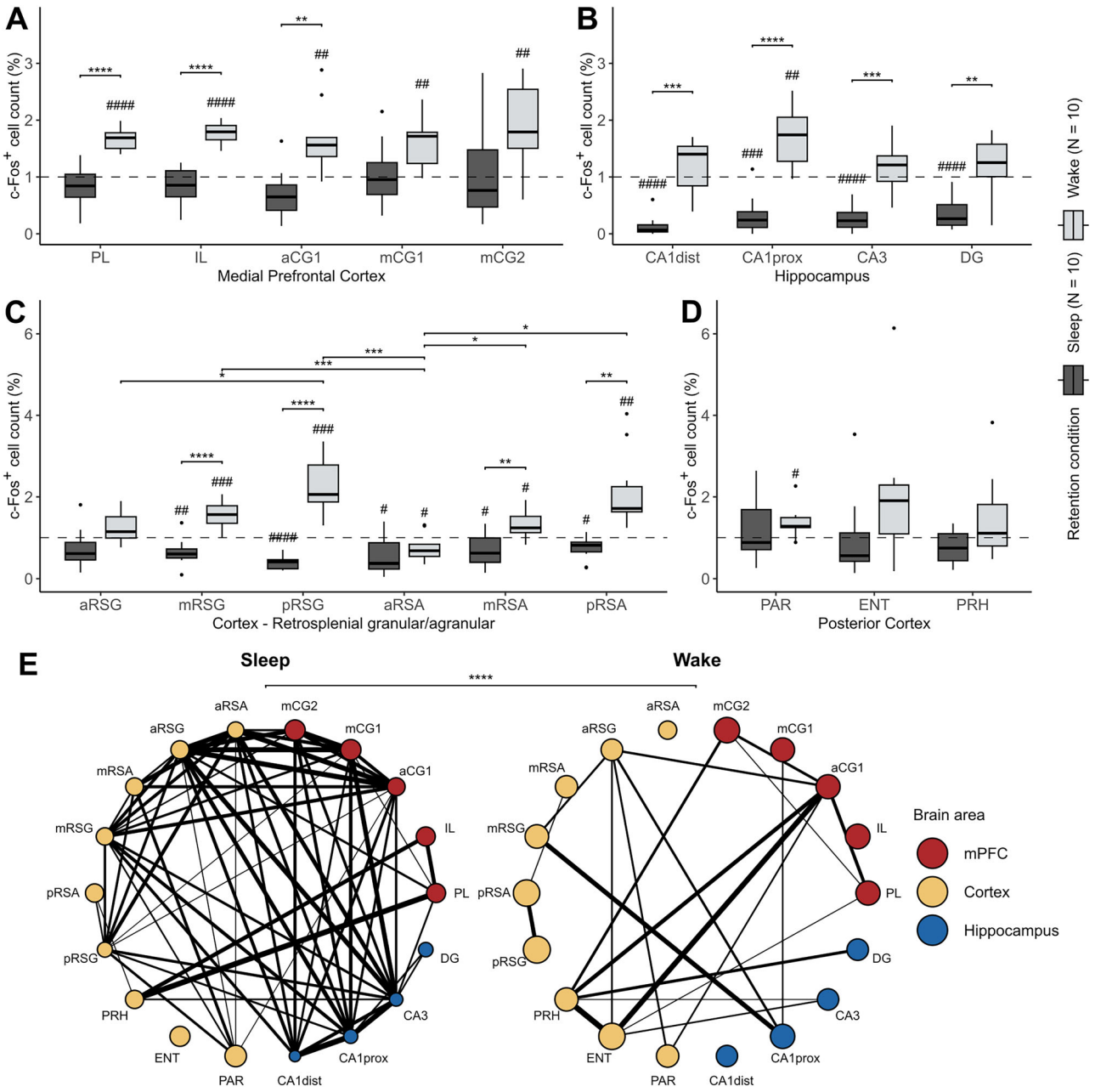


Figure 3. Recall of spatial schema memory is associated with reduced hippocampal c-Fos expression but increased interregional c-Fos correlations. ***A–D***, c-Fos expression in sleep and wake groups of the main experiment, separately shown for subregions of the mPFC (***A***), hippocampus (***B***), retrosplenial cortex (***C***), and posterior cortex ROIs (***D***). c-Fos$^+$ cell counts are shown as percentages of respective c-Fos$^+$ cell count in the HC control group (dashed line). Asterisks indicate significant Bonferroni-corrected post hoc comparisons between groups, hashtags significant cell counts above the HC controls (*$p < 0.05$; **$p < 0.01$). Extreme values (>1.5× the interquartile range) are shown as individual points. ***E***, In the sleep group, a distinctly larger number of ROIs showed correlated c-Fos activity during retrieval than in the wake group ($p < 0.001$, permutation testing). The node size represents c-Fos expression compared with HC controls; edge width indicates correlation strength. All significant correlations were positive. mPFC, medial prefrontal cortex; PL, prelimbic; IL, infralimbic; CG, cingulate gyrus; CA, cornu ammonis; DG, dentate gyrus; PAR, parietal; ENT, entorhinal; PRH, perirhinal; RSG, retrosplenial granular; RSA, retrosplenial agranular; a, anterior; m, medial; p, posterior; dist, distal; prox, proximal.

events (Dudai, 2012; Ghosh and Gilboa, 2014). Schema memories are assumed to arise from episodic representations in hippocampal networks that, over time, are transformed into more abstract representations distributed across neocortical regions, particularly involving the mPFC in conjunction with the medial temporal lobe (Schacter et al., 2012; Sekeres et al., 2018, 2024). This transformation has been linked to active systems memory consolidation processes occurring primarily during postencoding sleep (Brodt et al., 2023; Lutz et al., 2025). However, it remains unclear whether the initial formation of schema memories depends on post-encoding sleep, as previous studies relied on extensive multiday training protocols in which sustained sleep deprivation is not feasible (Tse et al., 2007; Benchenane et al., 2010; Richards et al., 2014; Abdou et al., 2024).

Contrasting with these previous studies, animals of the present study abstracted a novel schema memory from (eight) episodes experienced within a single wake period. Because each encoding episode and the retrieval test involved different object pairs, schema memory formation required not only the abstraction of the spatial rule but also the generalization across objects.

Based on this task paradigm, our results indeed provide first time evidence in adult rodents that initial schema memory formation for a spatial rule critically depends on postencoding sleep: rats allowed to sleep preferentially explored the rule-breaking object and showed increased rearing, an exploratory behavior indicating enhanced spatial orientation toward contextual cues (Lever et al., 2006; Sawangjit et al., 2022; Shan et al., 2023), whereas rats kept awake failed to recall the rule 24 h after encoding. Importantly, no signs of rule learning emerged during encoding itself, as reflected by the lack of preferential exploration in the final encoding episode. These findings support the view that schema memory emerged from sleep-dependent reprocessing of episodic representations (Stickgold and Walker, 2013; Lutz et al., 2025).

Our finding of a sleep-dependent enhancement of schema memory recall 24 h after encoding concurs with findings in humans and animals indicating that schema emergence often occurs with some delay after encoding, allowing offline reprocessing (Wagner et al., 2004; Abdou et al., 2024). However, the timing of abstraction depends on the task and on preexisting knowledge that can facilitate abstracting relevant features. Indeed, studies in humans indicate that rule abstraction can also occur online during encoding (Sweegers and Talamini, 2014; Schlichting et al., 2015; Wagner et al., 2015). Against this backdrop, our results identify sleep as a state that promotes, though may not be strictly required for, schema memory formation.

How prior knowledge affects schema memory formation was indeed assessed in our control experiment. During the habituation sessions of these experiments, the rats learned that objects can be placed in the four corners of the arena and, thus, at locations conflicting with the spatial rule to be abstracted from the eight encoding episodes. This prior knowledge completely abolished the expression of schema memory at the retrieval test in both the sleep and wake groups of this experiment. This failure occurred although object exploration during encoding was equivalent to that in the main experiment, ruling out reduced task engagement. The absence of schema memory in this experiment, hence, most likely reflects the competing prior knowledge formed during habituation that interfered with forming the new spatial schema, which was otherwise readily formed in the main experiments where animals were habituated in an empty arena. Together, our findings highlight that prior knowledge can either facilitate or interfere with the emergence of a new schema. The absence of schema memory after introducing prior knowledge through altered habituation confirms that rats in the main experiment indeed formed a novel schema representation based on the eight encoding episodes, consistent with earlier evidence showing that schema abstraction occurs only after eight, but not four, episodes (Harkotte et al., 2022).

Complementing the behavioral results, c-Fos analysis revealed that retrieval of schema memory after sleep was associated with lower neuronal activation in the infralimbic, prelimbic, and anterior cingulate cortices of the mPFC, as well as the hippocampus, compared with the wake group. The relative increases in c-Fos activity in the wake group in this context likely reflect enhanced processing of novelty in regions implicated in spatial processing (like mPFC, retrosplenial cortex), given that these animals failed to recall the spatial schema (Czajkowski et al., 2014; Kinnavane et al., 2017; Powell et al., 2017; Trask and Helmstetter, 2022). However, the reductions in c-Fos activity in the sleep group, specifically those observed in hippocampal regions, were also significant when compared with the levels in the HC controls. Such reduction in hippocampal activation is indeed consistent with the idea that hippocampal engagement decreases once a schema has been established in neocortical regions, including the mPFC which may inhibit activity in hippocampal networks (Tse et al., 2007; Chudasama et al., 2012; Eichenbaum, 2017; Sekeres et al., 2018). Activity in the prefrontal cortical regions of the sleep group, on the other hand, was comparable to that in the HC controls, which differs from previous findings pointing to an upregulation of immediate early gene expression in the mPFC with activation of schema memory (Tse et al., 2011; Contreras et al., 2024). However, those studies used different activation markers and tasks. Tse et al. (2011), for example, assessed the retrieval-related expression of Zif268 and Arc after the assimilation of new flavor–place-associated memories into an existing schema. Furthermore, in all of these studies (including the present one), memory recall was assessed based on exploration of "novelty" (i.e., here the rule-breaking object) which, in principle, does not allow to discriminate neuronal activity related to memory retrieval from that related to novelty processing. Accordingly, one could speculate that an increase of Zif268 and Arc—immediate early genes related to synaptic plasticity—might reflect the selective recruitment of neuronal ensembles in the mPFC involved in the retrieval and updating of an existing schema, whereas the total number of cells activated, as measured by c-Fos in this study, remains relatively low. Exploratory analyses in which we compared the proportion of inhibitory neurons (double-labeled for c-Fos and GAD67) between the sleep and wake groups relative to that in the HC group did not support the view of an increased inhibitory activity within the mPFC in the sleep group. Whatever the reason is for the discrepancy between these studies, the present lack of a distinct increase in c-Fos activity in mPFC regions during retrieval suggests that successful recall of schema memory is not so much related to the activation of individual regions but rather relies on the coordinate interregional activation at the network level. Supporting this view, our correlational network analysis revealed stronger coactivation across all measured brain regions (including hippocampal and mPFC regions) in the sleep group, suggesting that postencoding sleep promotes a more sparsely activated yet highly interconnected network that supports schema memory recall. However, the causal role of such network in representing and retrieving schema memory remains to be demonstrated.

Indeed, our assessment of c-Fos activity during retrieval remains correlative in nature preventing mechanistic inferences. Our study is likewise limited by the purely behavioral assessment of sleep. This was done to prevent any stress-related confounds on memory behavior that could arise from concurrent recordings of electrophysiological signals. Although we assured the validity of behavioral recordings in differentiating sleep and wake states in a separate sample of rats with EEG and EMG recordings, behavioral scoring alone may not be optimal for a finer differentiation of brain states, like the substates of active and quiet wakefulness, and it does not allow to distinguish between nonrapid eye movement (NonREM) and REM sleep. Using periods of behavioral immobility and locomotion as rough proxy for the substates of quiet and active wakefulness, respectively, we did not find any hints that the gentle handling interventions in the wake animals led to a substantial disruption of quiet wakefulness (Fig. S1C). Although this finding argues against the possibility that the wake group failed to form new schema memory because of a reduced time spent quietly awake, further research seems to be warranted to substantiate this conclusion, given also findings indicating that hippocampal ripples can contribute to memory

consolidation during quite wakefulness (Remondes and Wilson, 2015; Fernández-Ruiz et al., 2019; Wamsley, 2022). As to NonREM and REM sleep, previous work has suggested that NonREM and REM sleep act in complementary ways, with NonREM supporting hierarchical organization of information and mPFC activity, particularly in the anterior cingulate cortex, mediating abstraction processes in both stages (Benchenane et al., 2010; McKenzie et al., 2014; Aly et al., 2022; Abdou et al., 2024). Moreover, it would be interesting to know whether these processes depend on the same mechanisms as episodic memory consolidation during sleep, such as hippocampal replay coordinated with cortical slow oscillations, spindles, and ripples during NonREM sleep (Lutz et al., 2025). Building on the present behavioral findings, future investigations using electrophysiological sleep recordings appear especially promising for addressing these and related issues.

References

Abdou K, Nomoto M, Aly MH, Ibrahim AZ, Choko K, Okubo-Suzuki R, Muramatsu S, Inokuchi K (2024) Prefrontal coding of learned and inferred knowledge during REM and NREM sleep. Nat Commun 15:4566.

Aly MH, Abdou K, Okubo-Suzuki R, Nomoto M, Inokuchi K (2022) Selective engram coreactivation in idling brain inspires implicit learning. Proc Natl Acad Sci U S A 119:e2201578119.

Bartlett FC (1932) *Remembering: a study in experimental and social psychology*. Cambridge, United Kingdom: Cambridge University Press.

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48.

Bein O, Niv Y (2025) Schemas, reinforcement learning and the medial prefrontal cortex. Nat Rev Neurosci 26:141–157.

Benchenane K, Peyrache A, Khamassi M, Tierney PL, Gioanni Y, Battaglia FP, Wiener SI (2010) Coherent theta oscillations and reorganization of spike timing in the hippocampal- prefrontal network upon learning. Neuron 66:921–936.

Bisler S, Schleicher A, Gass P, Stehle JH, Zilles K, Staiger JF (2002) Expression of c-Fos, ICER, krox-24 and JunB in the whisker-to-barrel pathway of rats: time course of induction upon whisker stimulation by tactile exploration of an enriched environment. J Chem Neuroanat 23: 187–198.

Brodt S, Inostroza M, Niethard N, Born J (2023) Sleep-a brain-state serving systems memory consolidation. Neuron 111:1050–1075.

Chudasama Y, Doobay VM, Liu Y (2012) Hippocampal-prefrontal cortical circuit mediates inhibitory response control in the rat. J Neurosci 32: 10915–10924.

Contreras MP, Mendez M, Shan X, Fechner J, Sawangjit A, Born J, Inostroza M (2024) Context memory formed in medial prefrontal cortex during infancy enhances learning in adulthood. Nat Commun 15:2475.

Csárdi G, Nepusz T (2006) The igraph software package for complex network research. Complex Syst 1695:1–9.

Czajkowski R, Jayaprakash B, Wiltgen B, Rogerson T, Guzman-Karlsson MC, Barth AL, Trachtenberg JT, Silva AJ (2014) Encoding and storage of spatial information in the retrosplenial cortex. Proc Natl Acad Sci U S A 111: 8661–8666.

Dimitrov S, Shan X, Born J, Inostroza M (2026) Impact of tissue storage time on immunodetection of c-Fos and GAD67 in the rat brain. J Neurosci Methods 425:110602.

Dudai Y (2012) The restless engram: consolidations never end. Annu Rev Neurosci 35:227–247.

Eichenbaum H (2017) Prefrontal–hippocampal interactions in episodic memory. Nat Rev Neurosci 18:547–558.

Farzanfar D, Spiers HJ, Moscovitch M, Rosenbaum RS (2023) From cognitive maps to spatial schemas. Nat Rev Neurosci 24:63–79.

Fernández-Ruiz A, Oliva A, Fermino de Oliveira E, Rocha-Almeida F, Tingley D, Buzsáki G (2019) Long-duration hippocampal sharp wave ripples improve memory. Science 364:1082–1086.

Genzel L, Schut E, Schröder T, Eichler R, Khamassi M, Gomez A, Lobato IN, Battaglia F (2019) The object space task shows cumulative memory expression in both mice and rats. PLoS Biol 17:e3000322.

Ghosh VE, Gilboa A (2014) What is a memory schema? A historical perspective on current neuroscience literature. Neuropsychologia 53:104–114.

Gilboa A, Marlatte H (2017) Neurobiology of schemas and schema-mediated memory. Trends Cogn Sci 21:618–631.

Hagewoud R, Havekes R, Tiba PA, Novati A, Hogenelst K, Weinreder P, Van der Zee EA, Meerlo P (2010) Coping with sleep deprivation: shifts in regional brain activity and learning strategy. Sleep 33:1465–1473.

Harkotte M, Contreras MP, Inostroza M, Born J (2022) Effects of information load on schema and episodic memory formation. Front Behav Neurosci 16:923713.

Kassambara A (2025) ggpubr: "ggplot2" based publication ready plots. Available at: https://rpkgs.datanovia.com/ggpubr/

Kinnavane L, Amin E, Olarte-Sánchez CM, Aggleton JP (2017) Medial temporal pathways for contextual learning: network c-fos mapping in rats with or without perirhinal cortex lesions. Brain Neurosci Adv 1: 2398212817694167.

Lacaux C, Andrillon T, Bastoul C, Idir Y, Fonteix-Galet A, Arnulf I, Oudiette D (2021) Sleep onset is a creative sweet spot. Sci Adv 7:eabj5866.

Lerner I, Gluck MA (2019) Sleep and the extraction of hidden regularities: a systematic review and the importance of temporal rules. Sleep Med Rev 47:39–50.

Lever C, Burton S, O'Keefe J (2006) Rearing on hind legs, environmental novelty, and the hippocampal formation. Rev Neurosci 17:111–134.

Lewis PA, Durrant SJ (2011) Overlapping memory replay during sleep builds cognitive schemata. Trends Cogn Sci 15:343–351.

Lutz ND, Harkotte M, Born J (2025) Sleep's contribution to memory formation. Physiol Rev 106:363–483.

McKenzie S, Frank AJ, Kinsky NR, Porter B, Rivière PD, Eichenbaum H (2014) Hippocampal representation of related and opposing memories develop within distinct, hierarchically organized neural schemas. Neuron 83:202–215.

Pack AI, et al. (2007) Novel method for high-throughput phenotyping of sleep in mice. Physiol Genomics 28:232–238.

Palchykova S, Winsky-Sommerer R, Meerlo P, Dürr R, Tobler I (2006) Sleep deprivation impairs object recognition in mice. Neurobiol Learn Mem 85: 263–271.

Paxinos G, Watson C, Calabrese E, Badea A, Johnson GA (2015) *MRI/DTI atlas of the rat brain*. London: United Kingdom: Academic Press.

Powell AL, Vann SD, Olarte-Sánchez CM, Kinnavane L, Davies M, Amin E, Aggleton JP, Nelson AJD (2017) The retrosplenial cortex and object recency memory in the rat. Eur J Neurosci 45:1451–1464.

R Core Team (2024) R: a language and environment for statistical computing. Available at: https://www.R-project.org/

Remondes M, Wilson MA (2015) Slow-γ rhythms coordinate cingulate cortical responses to hippocampal sharp-wave ripples during wakefulness. Cell Rep 13:1327–1335.

Richards BA, Xia F, Santoro A, Husse J, Woodin MA, Josselyn SA, Frankland PW (2014) Patterns across multiple memories are identified over time. Nat Neurosci 17:981–986.

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

Sawangjit A, Harkotte M, Oyanedel CN, Niethard N, Born J, Inostroza M (2022) Two distinct ways to form long-term object recognition memory during sleep and wakefulness. Proc Natl Acad Sci U S A 119:e2203165119.

Schacter DL, Addis DR, Hassabis D, Martin VC, Spreng RN, Szpunar KK (2012) The future of memory: remembering, imagining, and the brain. Neuron 76:677–694.

Schlichting ML, Mumford JA, Preston AR (2015) Learning-related representational changes reveal dissociable integration and separation signatures in the hippocampus and prefrontal cortex. Nat Commun 6:8151.

Sekeres MJ, Winocur G, Moscovitch M (2018) The hippocampus and related neocortical structures in memory transformation. Neurosci Lett 680:39–53.

Sekeres MJ, Schomaker J, Nadel L, Tse D (2024) To update or to create? The influence of novelty and prior knowledge on memory networks. Philos Trans R Soc Lond B Biol Sci 379:20230238.

Shan X, Contreras MP, Sawangjit A, Dimitrov S, Born J, Inostroza M (2023) Rearing is critical for forming spatial representations in pre-weanling rats. Behav Brain Res 452:114545.

Stickgold R, Walker MP (2013) Sleep-dependent memory triage: evolving generalization through selective processing. Nat Neurosci 16:139–145.

Sweegers CCG, Talamini LM (2014) Generalization from episodic memories across time: a route for semantic knowledge acquisition. Cortex 59:49–61.

Trask S, Helmstetter FJ (2022) Unique roles for the anterior and posterior retrosplenial cortices in encoding and retrieval of memory for context. Cereb Cortex 32:3602–3610.

Tse D, Langston RF, Kakeyama M, Bethus I, Spooner PA, Wood ER, Witter MP, Morris RGM (2007) Schemas and memory consolidation. Science 316:76–82.

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

van Twyver H, Webb WB, Dube M, Zackheim M (1973) Effects of environmental and strain differences on EEG and behavioral measurement of sleep. Behav Biol 9:105–110.

Wagner IC, van Buuren M, Kroes MC, Gutteling TP, van der Linden M, Morris RG, Fernández G (2015) Schematic memory components converge within angular gyrus during retrieval (Eichenbaum H, ed). Elife 4: e09668.

Wagner U, Gais S, Haider H, Verleger R, Born J (2004) Sleep inspires insight. Nature 427:352–355.

Wamsley EJ (2022) Offline memory consolidation during waking rest. Nat Rev Psychol 1:441–453.

Wickham H (2011) Ggplot2. WIREs Comp Stat 3:180–185.

Zangenehpour S, Chaudhuri A (2002) Differential induction and decay curves of *c-fos* and *zif268* revealed through dual activity maps. Brain Res Mol Brain Res 109:221–225.