Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters

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Abstract

Sleep has been shown to play a facilitating role in memory consolidation, whereas sleep deprivation leads to performance impairment both in humans and rodents. The effects of 4-h sleep deprivation on recognition memory were investigated in the Djungarian hamster (Phodopus sungorus). Because sleep during the first hours after daily torpor has many similarities to recovery from sleep deprivation, the effects of spontaneous torpor on object recognition were also assessed.

A 4-h sleep deprivation, starting immediately after an object learning task, diminished the ability of the hamsters to: (1) discriminate between an already encountered object (target) and a novel object presented in a novel context, (2) retrieve a target within a complex spatial scene, and (3) detect a spatial rearrangement of familiar objects in a familiar context. Plasma stress hormone levels were similar in sleep-deprived and control hamsters. The occurrence of a daily torpor episode during retention was associated with impaired old–new object discrimination performance in the more effortful complex spatial scene task only, and in a two-object choice situation in a novel context no torpor-induced deficit was found.

Our results show that post learning sleep deprivation and daily torpor induce a deficit in familiar object retrieval performance in a complex spatial scene, while sparing familiarity-based recognition and novelty processing. Sleep deprivation during the first 4 h of memory consolidation hampered also recency memory for discrete objects. Stress was not a factor contributing to the sleep deprivation-induced impairment.

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Keywords: Djungarian hamster; Daily torpor; Sleep deprivation; Recognition memory; Stress; Hormones; ACTH; Corticosterone; Cortisol; Behavior

1. Introduction

There is a major renewal of interest in the relationship between sleep and memory. Significant advances have been made in the understanding of the molecular and physiological mechanisms underlying these brain functions. Various approaches have been used to study the changes in sleep after learning, and the consequences of sleep deprivation on the formation, expression and retrieval of memories [1–3]. In humans sleep seems to play a facilitatory role on the consolidation of procedural and declarative memories [4–8], whereas sleep deprivation leads to their impairment [9,10]. In rodents, REM sleep is increased after task learning [11–15], and sleep deprivation leads to impairment of various forms of memory (e.g., [16–20]). However, because of methodological differences, potential confounding effects of stress, and controversial evidence, the fundamental relevance of sleep in memory processing has been questioned [21].

We investigated the relationship between sleep and memory processes underlying recognition of objects in the Djungarian hamster. Four-hour sleep deprivation in this photoperiodic species leads to an increase in slow-wave activity (SWA) in non-rapid eye-movement (NREM) sleep [22,23]. This effect is observed independently of whether sleep deprivation is performed during summer or winter [23–26]. SWA in NREM sleep is a reliable predictor of sleep intensity [27,28] that increases proportionally to the duration of previous wakefulness and gradually declines during sleep. Under winter photoperiod (LD 8 h light:16 h dark) the Djungarian hamster spontaneously exhibits daily torpor, a hypometabolic state lasting several hours [29]. The remarkable post-torpor increase of SWA in NREM sleep closely resembled recovery after sleep deprivation leading to the hypothesis that also during daily
torpor a sleep deficit is accumulated [23–25]. In hamsters approximately 6 h of torpor and 4 h of sleep deprivation induced a comparable increase in SWA [25] attaining baseline levels within 3 or 6 h of recovery [22,26]. Hibernation, another hypothermic condition resembling sleep deprivation [30,31], diminished performance of ground squirrels in conditioning tasks [32].

Recognition is a universal ability to remember, i.e. to judge that something has occurred previously [33]. It is based on the capacity to detect familiarity or recency, and to discriminate and recollect specific features of objects, persons or events previously encountered in the same or in a different situational context [34]. Familiarity-based recognition of objects is based on the perception that an item has been encountered previously (“familiarity vs. novelty”). Rats and mice explore the familiar object less and show more interest when they encounter something new [35,36]. Recency-based recognition refers to memory of the temporal order of item presentation (“old vs. new”). Three one-trial discrimination learning tasks with different degree of difficulty of stimulus discrimination, were designed to examine the effects of sleep deprivation performed during the early memory consolidation phase and of spontaneous daily torpor on memory retrieval for discrete objects. Because emotional arousal can influence performance, and sleep deprivation may be stressful, plasma levels of pituitary and adrenal stress hormones were measured in control hamsters subjected to the learning task only and compared with hamsters subjected to the learning task and to sleep deprivation.

2. Methods

2.1. Animals

The Cantonal Veterinary Office of Zurich approved all experimental procedures. Adult Djungarian hamsters (Phodopus sungorus; n = 114, 65 males and 49 females), weighing 36 ± 7 g (SD), were kept individually in Macrolon cages (36 × 20 × 35 cm) with food and water available ad libitum. The Djungarian hamster is a photoperiodic rodent [37], therefore all experiments were performed during the winter months. The animals were raised in a natural photoperiod during summer, and then acclimated to winter conditions with an 8:16 h light/dark cycle (light onset at 9 a.m.; approximately 30 lx) and 14 °C ambient temperature.

As soon as the hamsters commenced to adapt to the winter photoperiod, as indicated by body weight reduction (33 ± 7 g) and a change in fur color towards white (index at surgery 3.1 ± 0.1; scale ranging from 1 to 6) [38], they were implanted intraperitoneally (i.p.) with temperature-sensitive transmitters (model X-M, Mini-mitter) under deep anaesthesia (Ketamine, KETALAR® 75 mg/kg, Parke-Davis; Xylasine, ROMPUN® 4 mg/kg, Bayer, i.p.). At least 4 weeks were allowed for recovery. Behavioral testing was performed in a temperature-controlled chamber under dim red lighting conditions (< 5 lx) during the dark period at the age of 6–10 months. Individuals were exposed to one of three behavioural tasks.

2.2. Behavioral tasks

Three learning tasks were adapted from the rat object recognition memory test, which is based on the spontaneous tendency of rodents to explore novel objects more than familiar ones [35]. The tasks comprised a study phase, delay phase and test phase. Before the study phase, all hamsters were familiarized with the experimental context on one or two consecutive days. They were placed individually in the empty enclosure for 10 min. The floor of the enclosures was covered with soiled wood chippings collected from cages of several hamsters, in order to saturate it with odors of conspecifics. Familiarization, study and test phases were performed at the same time of day and kept constant for individual hamsters. In the study phase, the animals were exposed for 5 min to a sample stimulus, consisting of objects. After a delay phase of 24 h, in the test phase, the hamsters were exposed for 5 min simultaneously to an object previously encountered in the study phase (target item) and new choice items. Nine sets of 3 or 4 identical objects (16–18 cm³) differing in shape, color and texture and with no biological relevance were used. The objects and their spatial location were randomised among the animals. Video recordings were obtained throughout the experimental phases.

The first behavioral task tested memory for a discrete object. The study phase was performed in a T-shaped enclosure (grey plastic, 25 cm high), comprising three distinct compartments (10 cm × 10 cm) individualized by three removable partitions and a central zone (20 cm × 10 cm). In the course of the experiment, the location and orientation of the enclosure within the test room were kept constant, thus defining a spatial attribute to three compartments (West, East and South). After two familiarization days, the hamsters were exposed to a single novel object (“novel” because it was never seen before; served as target) located in the middle of the West compartment during the study phase. To minimise the use of spatial and contextual cues to retrieve object information, the test phase was run in a novel rectangular enclosure (30 × 20 × 20 cm) subdivided into 6 inter-connected compartments (10 × 10 cm). A duplicate of the target object (familiar or old) was placed in the middle of the Northwest compartment and presented together with another “novel” object placed in the Northeast compartment. The animals were introduced into the enclosure by the Southwest compartment.

The second task tested memory for a complex scene by exposing a new group of hamsters to a triplet of objects in the T-enclosure, which served as the spatial context for both the study and the test phase. In the study phase, a different novel object was placed in the middle of each of the three compartments. In the test phase, the hamsters were exposed to a new triplet of objects consisting of an identical copy of the original object (target) located in the West compartment and two other different novel objects.

The third task tested memory for the spatial location of an object using a square enclosure (75 × 75 × 37 cm) throughout the experiment. After a single 10-min familiarization session, during the study phase hamsters were allowed to investigate...
two identical copies of a novel object placed in two adjacent corners. In the test phase, two new identical copies of the object were used: one was placed in its original location (target) and the other into another corner (choice copy). The position of the target was counterbalanced among the animals.

2.3. Sleep deprivation

Hamsters were subjected to sleep deprivation by “gentle handling” [22,39] for 4 h immediately following the study phase, thereby interfering with the early phase of memory consolidation. The effect of this duration of sleep deprivation on recovery sleep is comparable to a 6-h sleep deprivation in consolidation. The effect of this duration of sleep deprivation handling” [22,39] for 4 h immediately following the study phase and lasted between 4 and 17 h (6.4 T n = 22).

2.4. Daily torpor

To identify and verify the regular occurrence of torpor episodes, body temperature was continuously recorded for several days at 5-min intervals. Torpor usually occurred after light onset, the rest phase of this species, and approximately at the same time of day within individuals. The beginning or the end of a torpor episode was defined as body temperature below or above 32 °C, respectively [24,42]. Behavioral testing started 1–7.5 h after dark onset, at least 3 h after the end of torpor, once the hamsters exhibited at least two torpor episodes per week.

Despite limiting the timing of the interventions to the beginning of the dark period and their short duration (< 10 min), only 22 of 72 hamsters exhibited torpor spontaneously the day after the study phase. They were assigned to the “torpor groups”. The torpor episodes occurred 8–17 h after the study phase and lasted between 4 and 17 h (6.4 ± 0.4 h, n = 22).

The remaining animals, whose body temperature did not decrease below 32 °C between the study and test phase, served as controls.

2.5. Plasma ACTH, cortisol and corticosterone assays

Hamsters were placed individually in a T-enclosure 10 weeks after the test in a square enclosure (n = 32). They were exposed to three different novel objects for 5 min and killed by decapitation for blood collection 30 min (n = 8) or 4 h later (n = 7). Two groups were subjected to 30 min or 4 h sleep deprivation, starting immediately after exposure to novelty, and were killed thereafter (n = 8 per group). An undisturbed control group (n = 8) was kept in individual home-cages and killed at the same time of day as the animals used for the 30-min time point. Trunk blood was collected in K2E EDTA K2 tubes (Vacuette® 2 ml, Greiner Bio-One Vacuette Schweiz GmbH) at 0 °C, centrifuged at 2600×g at 4 °C for 15 min. The supernatant was stored at −80 °C in cryotubes for later analysis. Plasma ACTH, cortisol and corticosterone concentrations were determined by radioimmunoassay (ACTH kit, Nichols Institute Diagnostics, Bad Vilbel, Germany; cortisol kit, Diagnostic Systems Laboratories, Webster, USA; corticosterone kit, MP Biomedicals, Costa Mesa, USA).

2.6. Variables and statistics

Locomotor activity and interaction with objects were quantified by visual off-line scoring of the tapes. Locomotion was defined as the number of crossings of 4 virtual lines in the T-enclosure or of 6 compartments of the rectangular enclosure. The exploratory behavior towards objects was quantified by counting the number of investigations (approach of the nose to a distance < 2 cm and/or contact with the objects) and their duration. An object exploration ratio, i.e. the difference in exploration time of the target and novel object(s) divided by the total time spent exploring all objects, was calculated. A ratio > 0 for 2 objects (or 0.33 for 3 objects) indicated a greater exploration of the novel object(s) and a ratio < 0 (or 0.33), a greater exploration of the target.

Behavioral data were analysed by ANOVA (unweighted means or hierarchical solution depending on group size) with group (control vs. sleep deprivation or control vs. torpor) as between subject factor and object or phase (study vs. test) as within subject factor. Post-hoc Tukey’s pair-wise comparisons were performed whenever appropriate. For the analysis of plasma hormone levels a two-way ANOVA with group and time as between subject factors was used. Whenever the Levene’s test for homogeneity of variance was significant, data were subjected to decimal logarithm transformation. The normality of distribution was assessed with the procedure Univariate (SAS) that performs the Kolmogorov-Smirnov, Shapiro-Wilk, Anderson-Darling or Cramer-von Mises tests depending on the sample size. Unpaired or paired t-tests were used for between and within group comparisons, respectively. Non-parametric Kruskal-Wallis and Wilcoxon signed-rank tests were used when the sample sizes were equaled six or if data were not normally distributed. Results were expressed as mean ± SE.

3. Results

The object recognition memory test was developed for rats, mice and humans. Its adaptation for hamsters showed that they approach the objects to the same extent as 129/SvJ mice tested in a similar paradigm, and stay twice longer investigating them (Table 1).

3.1. Effects of sleep deprivation on recognition memory

3.1.1. Effects of sleep deprivation on memory for discrete objects

The impact of sleep deprivation on familiarity and recency-based memories for a discrete object (target) encountered previously in a familiar T-enclosure was examined in a simple
two-object choice situation in a novel context (rectangular enclosure).

Exploratory behavior towards the target revealed a significant phase effect on the number of investigations [$P<0.001$] and their duration [$P<0.001$, after logarithmic transformation] (Fig. 1a and b). The overall group effect was not significant for either variable [number, $F_{(1, 25)}=1.05$; duration, $F_{(1, 25)}=0.12$]. Interaction was significant for duration [$F_{(2, 20)}=6.60$, $P<0.02$] but not for number [$F_{(1, 25)}=2.19$]. As expected, the duration of target exploration decreased from study to test ($P<0.01$, Tukey), indicating familiarity detection, and was similar in both groups (Fig. 1b). Moreover, at study and test the control and sleep-deprived animals did not differ (Fig. 1b).

At test the exploration ratio of sleep-deprived hamsters differed from controls [$I_{(11,73)}=2.21$, $P<0.05$] (Fig. 1c). Although both groups explored preferentially the novel object (mean exploration ratio elevated above chance), the sleep-deprived animals displayed a lower level of discrimination, suggesting an impaired recency-based recognition of the target.

A further analysis examined whether the animals could detect object novelty, i.e. a novel object presented in a novel context. The novel object exploration decreased from study to test [number, $F_{(1, 25)}=24.79$ and duration, $F_{(1, 25)}=17.90$, $P<0.001$] and was similar in the two groups [number, $F_{(1, 25)}=0.65$ and duration, $F_{(1, 25)}=0.07$] (data not shown). Furthermore, irrespective of the group, the number of investigations of the two objects at test ($22\pm1$, $n=27$) was significantly higher than that of one object at study ($18\pm1$, $n=27$) [$F_{(1, 25)}=13.48$, $P<0.001$], but their duration was unchanged from study to test ($47\pm3$ s and $49\pm3$ s, respectively, $n=27$) [$F_{(1, 25)}=0.99$].

In summary, sleep-deprived hamsters processed the target as familiar, and detected object novelty in the novel context to the same extent as controls, but their old–new object discrimination performance was significantly less accurate, suggesting an altered recency memory.

### 3.1.2. Effects of sleep deprivation on memory for a complex spatial scene

The consequences of the 4 h sleep deprivation on retrieval and discrimination performance for a specific object, presented within a complex scene, consisting of a triplet of objects spatially arranged in a familiar T-enclosure were evaluated. Already at study, the objects located in the West or East compartment were explored to the same extent, whereas those in the South compartment were approached and investigated less ($P<0.05$, Tukey after ANOVA object: number, $F_{(2, 20)}=8.01$, $P<0.018$ and duration, $F_{(2, 20)}=5.95$, $P<0.035$). However, there was no effect of group [number, $F_{(1, 10)}=2.87$; duration, $F_{(1, 10)}=0.76$] or group×object interaction [number, $F_{(2, 20)}=2.14$; duration, $F_{(2, 20)}=0.30$]. Despite the differences at study, the exploration of the two novel objects did not differ between study and test, and was comparable in the two groups (data not shown).

Familiarity-based recognition in this complex scene was evaluated by comparing the exploratory behavior towards the

### Table 1

<table>
<thead>
<tr>
<th>Objects</th>
<th>Djungarian hamsters ($n=55$)</th>
<th>129/SvJ mice ($n=26$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Duration (s)</td>
<td>Number</td>
</tr>
<tr>
<td>O1</td>
<td>12.8 ±0.5</td>
<td>40.1 ±1.9</td>
</tr>
<tr>
<td>O2</td>
<td>12.9 ±0.7</td>
<td>37.0 ±1.7</td>
</tr>
<tr>
<td>O3</td>
<td>11.3 ±0.7</td>
<td>35.8 ±2.1</td>
</tr>
</tbody>
</table>

Spontaneous exploratory behavior towards novel objects presented in a familiar T-enclosure. Mean values (± SE) of the number of investigations and their duration (in seconds) for three novel objects (O1, O2 and O3) during a 5-min study phase. The exploratory behavior of adult 129/SvJ mice tested in very similar conditions is shown for comparison (L. Prut and F. Crestani, unpublished).

![Fig. 1](image-url)

Fig. 1. Effects of 4-h sleep deprivation on the exploratory behavior towards a target object encountered 24 h earlier at study. The test consisted of a simple two-object choice task in a novel context (a, b and c) or a new complex scene in a familiar context (d, e and f). (a) The number of investigations of the target decreased from study to test [phase, $F_{(1, 25)}=36.28$, $P<0.001$] similarly in controls ($n=18$) and hamsters, which were sleep deprived by “gentle handling” immediately after study (Sleep depr.; $n=9$). (b) Likewise, the duration of target investigations was decreased at test in both groups [phase, $F_{(1, 25)}=87.57$, $P<0.001$]. (c) At test, the exploration ratio was reduced in sleep-deprived hamsters compared to controls. (d) Sleep-deprived hamsters ($n=6$) failed to decrease the number of target investigations at test in contrast to controls ($n=6$). (e) The duration of target investigations decreased from study to test in controls but not in sleep-deprived animals. (f) At test, sleep-deprived hamsters did not differentiate between the target and the two new novel objects, in contrast to control animals, which showed a clear preference for novel objects, as indicated by the increase in the exploration ratio from study to test. Mean values±SE: *$P<0.05$ and **$P<0.01$ versus control, unpaired t-test and Kruskal-Wallis, respectively. The dashed line represents the chance level (0.33).
target object at study and test. The two groups differed significantly in the duration of target investigations \(F_{(1, 19)}=19.39, P<0.001\). In contrast to controls \((P<0.05,\) Wilcoxon signed-ranks test), in sleep-deprived hamsters the mean number and duration of target investigations failed to decrease from study to test (Fig. 1d and e).

In addition, sleep-deprived hamsters differed from controls in their old–new object discrimination performance. The mean exploration ratios of the two groups differed significantly at test \([H_{(1)}=8.31, P<0.004]\), but not at study \([H_{(1)}=0.23]\). At test control hamsters explored preferentially the two novel objects \((P<0.05)\), whereas sleep-deprived animals showed no preference (Fig. 1f). The exploration ratio increased significantly from study to test in control animals only \((P<0.05, Wilcoxon)\).

Thus, sleep-deprived hamsters were unable to discriminate the object encountered previously when it was presented again, but within a new complex scene after a 24-h delay.

### 3.1.3. Memory for object spatial location in sleep-deprived hamsters

The effects of sleep deprivation on the formation of object location memory were assessed by subjecting control and sleep-deprived hamsters at test to a spatial rearrangement of two objects that had been encountered previously in a familiar square enclosure. At study, both groups explored the two copies of the novel object to the same extent \([number, group: F_{(1, 19)}=1.10, object: F_{(1, 19)}=0.15, group \times object: F_{(1, 19)}=0.01; duration, group: F_{(1, 19)}=1.28, object: F_{(1, 19)}=0.07, group \times object: F_{(1, 19)}=1.69]\) (data not shown).

Familiarity processing was evaluated by comparing the exploratory behavior towards the object placed at the target location at study and at test. The mean number and duration of investigations decreased significantly from study to test \([P=0.05\) and \(P<0.001, respectively] in the two groups \([group: number, F_{(1, 19)}=0.69, duration, F_{(1, 19)}=1.63; group \times phase interaction: number, F_{(1, 19)}=0.58, duration, F_{(1, 19)}=3.81]\), indicating intact familiarity detection of the location (Fig. 2a and b).

At test sleep-deprived hamsters approached and investigated the object positioned at a new location significantly less than the controls \((P<0.05, Tukey after group \times phase interaction: number, F_{(1, 19)}=4.57, P<0.046\) and duration, \(F_{(1, 19)}=5.92, P<0.025\) (Fig. 2c and d) due to a phase-dependent decrease in the mean duration of investigations of this object in sleep-deprived hamsters only \((P<0.05)\) (Fig. 2d). Moreover, the old–new object location discrimination differed between the two groups \([group \times phase interaction: F_{(1, 19)}=9.19, P<0.007\); group: \(F_{(1, 19)}=0.78\) and phase: \(F_{(1, 19)}=0.24]\). At test, in contrast to sleep-deprived animals, control hamsters preferentially explored the object associated with the new spatial location \((P<0.05)\); at study there was no difference between the groups (Fig. 2e). The total exploration of the objects decreased from study to test \((32.3 \pm 2.8\) s and \(20.2 \pm 2.1\) s, respectively, \(n=21) [F_{(1, 19)}=33.68, P<0.001]\) irrespective of the group \(F_{(1, 19)}=0.45\), indicating intact familiarity-based memory of the objects.

In summary, sleep-deprived hamsters processed the two objects as familiar, but in contrast to controls, they failed to distinguish them on the basis of their spatial location.

### 3.1.4. Plasma stress hormones, novelty and sleep deprivation

Plasma levels of ACTH, cortisol and corticosterone were measured in hamsters subjected to object and contextual novelty followed or not by a 30-min or 4-h sleep deprivation. Hamsters exposed to novelty showed comparable plasma levels of ACTH, cortisol and corticosterone as undisturbed animals which remained in their home-cage \([H_{(1)}=0.40, H_{(1)}=0.00 and H_{(1)}=1.13, respectively]\) (Table 2). An additional 30 min or 4 h sleep deprivation did not increase the hormone levels significantly (Table 2) \([ACTH: group, F_{(1, 27)}=0.02, group \times time, F_{(1, 27)}=0.25; cortisol: group, F_{(1, 27)}=2.34, group \times time, F_{(1, 27)}=1.09 and corticosterone: group, F_{(1, 27)}=1.11, group \times time, F_{(1, 27)}=1.39; after logarithmic transformation]\).

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Absence of effect of novelty and sleep deprivation on plasma ACTH, cortisol and corticosterone levels. Hamsters were exposed to a novel enclosure containing three different novel objects for 5 min. Blood samples were collected 30 min (familiarity detection, and did not differ between the torpor and values

Corticosterone (was similar in the two groups [group: number, (control: 48.3 0.62) and the novel context (control: 54.2 1.3 s; F(1, 41) = 7.70, P < 0.01). This group bias was independent of the quality of objects or their spatial location [F(2, 82) = 0.07]. However, despite these initial group differences, both control and torpor hamsters explored the two novel objects similarly at study and test (data not shown). Moreover, the mean number and duration of target investigations decreased from study to test [Fig. 3d and e; P < 0.001], indicating familiarity detection and were similar in control and torpor hamsters [number, F(1, 41) = 0.72 and duration, F(1, 41) = 0.03].

In contrast to familiarity detection, old–new object discrimination within the test phase differed between controls and torpor hamsters. In both groups the exploration ratio changed from study to test (phase: P < 0.001; group: F(1, 41) = 2.05; group × phase interaction: F(1, 41) = 1.89). The mean exploration ratios close to chance level (= 0.33) at study, increased significantly at test [Fig. 3f; controls: I(31) = 7.52, P < 0.001; torpor group: I(10) = 2.66, P < 0.05, paired t-test]. However, the increase was significantly smaller in torpor hamsters compared to controls [I(19.96) = 2.02, P = 0.05; confirmed by a Kruskal-Wallis test, H(1) = 4.13, P < 0.05]. In contrast to controls [I(31) = 5.69, P < 0.001], in torpor hamsters the ratio difference between study and test failed to reach significance [Fig. 3f] [I(10) = 1.99].

Locomotion of the two groups was similar at study (control, 51.3 ± 3.5, n = 14; torpor, 42.7 ± 2.3, n = 7) and at test (46.6 ± 3.2 and 50.6 ± 3.3, respectively) [group, F(1, 19) = 0.35; phase, F(1, 19) = 0.20; group × phase, F(1, 19) = 3.21].

In summary, the hamsters, exposed to two complex scenes sharing in common a specific object, manifested a selective reduction of exploration towards this object at test, irrespective

Table 2

<table>
<thead>
<tr>
<th>Novelty + Sleep deprivation</th>
<th>Baseline</th>
</tr>
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<tbody>
<tr>
<td>ACTH (pg/ml)</td>
<td>372.1 ± 143.0</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Corticosterone (µg/dl)</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

Absence of effect of novelty and sleep deprivation on plasma ACTH, cortisol and corticosterone levels. Hamsters were exposed to a novel enclosure containing three different novel objects for 5 min. Blood samples were collected 30 min (n = 8) or 4 h (n = 7) later. Additional two groups (n = 8) were subjected to 30 min or 4 h sleep deprivation after novelty exposure. Hamsters, which provided the baseline hormonal levels, were left undisturbed in their home-cages (n = 8). Mean values ± SE.

3.2. Effects of daily torpor on recognition memory

In a separate series of experiments run in parallel with the sleep deprivation experiments, we tested whether spontaneous bouts of daily torpor would affect object recognition memory.

3.2.1. Effects of daily torpor on memory for discrete objects

The consequences of a post-learning torpor episode on familiarity- and recency-based object recognition were assessed in a simple two-object choice task. The mean number and duration of target investigations decreased significantly from study to test [P < 0.001] (Fig. 3a and b), indicating a clear familiarity detection, and did not differ between the torpor and control group [number, F(1, 27) = 0.68; duration, F(1, 27) = 0.20].

At test, control and torpor hamsters displayed similar mean exploration ratios [I(15.9) = 0.38; Fig. 3c]. Both groups displayed a marked bias for exploring the novel object, as expected for intact recency-based memory.

The novel object presented at study elicited more exploration than the novel object presented at test [number, F(1, 27) = 32.67, P < 0.001 and duration, F(1, 27) = 8.78, P < 0.006], but this effect was similar in the two groups [group: number, F(1, 27) = 0.30, duration, F(1, 27) = 1.01; group × phase interaction: number, F(1, 27) = 0.00, duration, F(1, 27) = 0.07] (data not shown). The difference in the number of items between study (one) and test (two) had no effect on the overall amount of object exploration. Thus, the number of object investigations increased significantly from study to test (18 ± 1 versus 22 ± 1, n = 29) [F(1, 27) = 9.63, P < 0.005], but their duration was stable (49.1 ± 2.7 s versus 49.4 ± 3.3 s, n = 29) [F(1, 27) = 0.00; group and group × phase interaction on both variables: n.s.]. Therefore, control and torpor hamsters explored the objects in the novel context to the same extent as the original target in the familiar context, suggesting that motivation was unaltered from study to test. In addition, the novelty of the context did not affect object processing in either group.

The levels of locomotion were similar both in the familiar (control: 48.3 ± 3.0, n = 18; torpor: 45.5 ± 3.5, n = 11; t(23.08) = 0.62) and the novel context (control: 54.2 ± 2.2; torpor: 53.7 ± 4.1; t(15.78) = 0.11).

Therefore, familiarity- and recency-based memory for a discrete object, as well as novelty processing, were unaltered by a post learning daily torpor experience in a simple two-object choice situation in a novel context.

3.2.2. Effects of daily torpor on memory for a complex spatial scene

The capacity of torpor hamsters to retrieve a discrete object already encountered at study, when it was presented within a new complex scene in the familiar T-enclosure was investigated. Already at study, torpor hamsters differed from the controls approaching the objects in the East compartment significantly more often (16.5 ± 2.0, n = 11) than the controls (11.2 ± 0.8, n = 32; P < 0.05, Tukey after group × object interaction: F(2, 82) = 4.26, P < 0.05), reflecting a spatial bias rather than an object preference due to the object randomisation. No difference occurred between the two other objects (data not shown). Torpor hamsters investigated the objects significantly longer than controls (mean value per object in torpor group: 46.0 ± 2.4 s; controls: 39.1 ± 1.3 s; F(1, 41) = 7.70, P < 0.01). This group bias was independent of the quality of objects or their spatial location [F(2, 82) = 0.07]. However, despite these initial group differences, both control and torpor hamsters explored the two novel objects similarly at study and test (data not shown). Moreover, the mean number and duration of target investigations decreased from study to test [Fig. 3d and e; P < 0.001], indicating familiarity detection and were similar in control and torpor hamsters [number, F(1, 41) = 0.72 and duration, F(1, 41) = 0.03].

In contrast to familiarity detection, old–new object discrimination within the test phase differed between controls and torpor hamsters. In both groups the exploration ratio changed from study to test [phase: P < 0.001; group: F(1, 41) = 2.05; group × phase interaction: F(1, 41) = 1.89]. The mean exploration ratios close to chance level (= 0.33) at study, increased significantly at test [Fig. 3f; controls: I(31) = 7.52, P < 0.001; torpor group: I(10) = 2.66, P < 0.05, paired t-test]. However, the increase was significantly smaller in torpor hamsters compared to controls [I(19.96) = 2.02, P = 0.05; confirmed by a Kruskal-Wallis test, H(1) = 4.13, P < 0.05]. In contrast to controls [I(31) = 5.69, P < 0.001], in torpor hamsters the ratio difference between study and test failed to reach significance [Fig. 3f] [I(10) = 1.99].

Locomotion of the two groups was similar at study (control, 51.3 ± 3.5, n = 14; torpor, 42.7 ± 2.3, n = 7) and at test (46.6 ± 3.2 and 50.6 ± 3.3, respectively) [group, F(1, 19) = 0.35; phase, F(1, 19) = 0.20; group × phase, F(1, 19) = 3.21].

In summary, the hamsters, exposed to two complex scenes sharing in common a specific object, manifested a selective reduction of exploration towards this object at test, irrespective
of the experience of torpor. However, torpor hamsters showed diminished old–new object discrimination performance at retrieval.

### 3.2.3. Duration of daily torpor and body temperature

The duration of torpor between the study and test phase was $6.4 \pm 0.4$ h, and body temperature dropped to $20.1 \pm 0.6$ °C (range $18.3–24.5$ °C). Neither minimal torpor body temperature nor torpor duration correlated with the exploration ratio in the two learning tasks (temperature: $R = 0.16$ and $R = 0.25$, duration: $R = 0.21$ and $R = -0.16$, respectively, $n = 11$ per group; Pearson product-moment correlation; pooled data of the two torpor groups: temperature: $R = 0.32$, duration: $R = -0.17$, $n = 22$). There was a positive correlation between the duration of the interval between the study phase and the onset of the torpor episode and the ratio of exploration at test ($R = 0.46$, $F_{(2,0)} = 5.25$, $P = 0.032$, $n = 22$). Hence, better performance correlated with a longer consolidation interval, but not with torpor duration or body temperature.

### 4. Discussion

Exploratory activity towards objects is often used in rodents to investigate the memory processes involved in recognition [36,43]. Recognition can be inferred from the changes in exploratory behavior towards an object encountered previously, when it is presented simultaneously with novel ones [34,44].

A post-learning sleep deprivation affects familiarity and recency processes underlying object recognition in rodents differentially. The sleep deficit incurred immediately after object encoding did not interfere with the formation of a familiarity-based memory for this object. Sleep-deprived and control animals displayed similar reduced exploration of the target object at second occurrence in a simple two-object choice situation 24 h after encoding (Fig. 1a,b). Detection and processing of object novelty were not altered by prior sleep deprivation because the animals exhibited relatively high curiosity for the novel object at test. On the other hand, the natural attraction of hamsters for novelty may have confined the decreased responsiveness to the target object, rendering the familiarity processing questionable. However, the significant phase-dependent decrease in novel object exploration seen in both control and sleep-deprived groups suggests that Djungarian hamsters are capable to learn in one trial an absolute class concept ‘biologically meaningless objects’ and to form a familiarity-based memory for other items belonging to the same class [45]. In contrast, the sleep deprivation experience led to a recency memory detriment, as seen by the diminished old–new object discrimination ability (Fig. 1c). Because the test was performed in a novel context, the use of contextual or self-motion cues to retrieve specific features of the target object was limited [46]. Therefore, the target object was the most recent to be recognized item.

The negative effects of sleep deprivation on object recognition increased with the level of difficulty of the task. When subjected to a complex scene task in a familiar context, sleep-deprived hamsters displayed a deficit in familiarity-based memory at retrieval. They explored the target object as much as the two other novel objects (Fig. 1). In contrast, controls were able to fully recognize the familiar target (Fig. 1d,e), indicating their ability to process and identify three different objects of a spatial scene as separate entities. Therefore, sleep deprivation could have interfered with the formation of a stable memory for the whole scene, which requires the knowledge of the specific features of the objects and their spatio-temporal relationships [47]. Thus, despite the presence of the target, at test sleep-deprived animals might have encoded the new scene as a novel one, due to an impaired ability to retrieve the whole scene. To
test this possibility, the ability of hamsters to detect a spatial rearrangement of familiar objects in a familiar context was evaluated. Sleep deprived animals failed to discriminate between two familiar identical objects on the basis of the familiarity or novelty of their spatial location (Fig. 2e), but processed them as already seen, indicating intact familiarity encoding (Fig. 2a,b).

Thus, depending on the difficulty of the discrimination task, a sleep deprivation hampers recency memory for discrete objects and retrieval of scene memory, while sparing object familiarity-based recognition and novelty processing.

Processes underlying new memories initially persist in a fragile state and consolidation occurs over time [48]. The time window for consolidation of specific learning tasks in rats after REM sleep deprivation of different duration and timing was lasted up to 20 h (for review [5]). It is unknown how long the consolidation phase needs to be for the object recognition paradigm we applied. Therefore, it cannot be excluded that familiarity and novelty detection, which were intact after the early, 4-h sleep deprivation, could be sensitive to a longer sleep deprivation or a sleep deprivation performed during a later phase of memory consolidation.

The selective cognitive deficit could be either attributed to an overall lack of sleep or of slow waves during sleep, postulated to be associated with optimal conditions for synaptic downscaling [49]. Alternatively, it could result from a proactive interference effect due to the ongoing sensory stimulation during retention (e.g., [50, 51]). In humans, waking activities during retention can interfere with the process of consolidation and lead to a memory detriment (e.g., [52]), whereas restful waking facilitates learning and provides similar benefits as sleep [53].

An acute stress experience at learning can disturb memory formation and processing at retrieval [54]. In mice stressful experience after encoding impaired object recognition [55]. On the other hand, in rats 48 h sleep deprivation activated the hypothalamo-pituitary adrenal (HPA) axis, elevating plasma ACTH and corticosterone levels [56]. However, the 4 h of sleep deprivation following an exposure of the hamsters to novelty did not influence the plasma levels of cortisol, corticosterone and ACTH. Therefore, it is unlikely that stress contributed to the object memory deficits seen in the hamsters after sleep deprivation.

Interestingly, the occurrence of daily torpor during retention is associated with a selective impairment in scene memory retrieval due to the deficit in target recognition within a complex spatial scene (Fig. 3). However, intact familiarity- and recency-based recognition and object novelty processing argue against potential detrimental effects of sleepiness [57, 58], reduced attention or vigilance associated with torpor on object information processing. The changes in body temperature during torpor did not play a major role in the memory impairment. Thus, the exploration ratios at test were independent of both body temperature reached during torpor and torpor duration. Exposure to a novelty induces long-term potentiation (LTP), a basic physiological mechanism implicated in memory formation [59, 60]. Once established, LTP becomes insensitive to variations in temperature [61, 62]. It is possible that torpor has important consequences on the brain, comparable to the transient depression of transcriptional initiation in liver during torpor [63].

The scene memory retrieval deficit seen in torpor hamsters is analogous to that induced by sleep deprivation, suggesting the existence of detrimental factors common to both conditions. The occurrence of daily torpor later in the retention period certainly contributed to the less pronounced cognitive impairment after torpor compared to sleep deprivation. Due to the limited amount of hamsters and the influence of the adaptation procedures on the occurrence of torpor, the timing and duration of sleep deprivation could not be matched to the torpor episode. Moreover, spontaneous torpor epochs can last up to 17 h that is above the duration of sleep deprivation that Djungarian hamsters tolerate without stress. These difficulties preclude the comparison between the effects of sleep deprivation and torpor.

Sleep enhances explicit recollection of words in humans, but has no effect on implicit judgement of familiarity [64]. Our data are consistent with this study, and provide additional evidence that a transient sleep deficit during an early (first 4 h) or late time window (8–17 h) of the retention period has no effect on item familiarity and novelty processing but impairs recency and spatial scene memory retrieval of objects, depending on the difficulty of the task. Thus, the sleep deficit would not interfere with the formation of object memories but rather with cognitive abilities, which are essential for their retrieval.

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