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Learning and memory are impaired in the object recognition task during metestrus/diestrus and after sleep deprivation

Joshua Cordeira^{a,*}, Sai Saroja Kolluru^a, Heather Rosenblatt^a, Jenny Kry^a, Robert E. Strecker^b, and Robert W. McCarley^{c,1}

^aDepartment of Biological & Environmental Sciences, Western Connecticut State University, Danbury, Connecticut, USA

^bDepartment of Psychiatry, Harvard Medical School and Veterans Affairs Boston Healthcare System, West Roxbury, MA 02132, USA

^cDepartment of Psychiatry, Harvard Medical School and Veterans Affairs Boston Healthcare System, Brockton, MA 02301, USA

Abstract

Females are an under-represented research model and the mechanisms through which sleep loss impairs cognition are not clear. Since levels of reproductive hormones and the estrous cycle are sensitive to sleep loss and necessary for learning and memory, we hypothesized that sleep deprivation impacts learning and memory in female mice by interfering with the estrous cycle. We used the object recognition task to assess learning and memory in female mice during separate phases of the estrous cycle and after sleep loss. Mice in metestrus/diestrus attended to sample objects less than mice in proestrus/estrus during object acquisition, the first phase of the object recognition task. Subsequently, during the recognition phase of the task, only mice in proestrus/estrus displayed a preference for the novel object. Sleep deprivation for 12h immediately before the object recognition task reduced time attending to sample objects and novel object preference for mice in proestrus/estrus, without changing length of the estrous cycle. These results show that sleep deprived mice in proestrus/estrus had learning deficits and memory impairments, like mice in metestrus/diestrus. Since sleep deprivation did not disrupt the estrous cycle, however, results did not support the hypothesis. Cognitive impairments due to acute sleep loss were not due to alterations to the estrous cycle.

Keywords

Learning; Memory; Object Recognition Task; Sleep Deprivation; Estrous Cycle; Mice

*Corresponding author: Joshua Cordeira, Department of Biological & Environmental Sciences, Western Connecticut State University, 181 White Street, Danbury, CT 06810, United States, Tel. +1 203 837 8940, CordeiraJ@wcsu.edu.

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Conflicts of interest

None

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1. Introduction

The prevalence and consequences of insufficient sleep are not equal for men and women [1,2]. Women report insufficient sleep and sleep problems, most notably insomnia, more often than men [3,4]. And, while it has traditionally been argued that women cope with prolonged wakefulness better than men, evidence indicates that the health outcomes for sleep deprived women may be worse [5,6]. Women are more likely to report difficulties with concentration and memory after sleep loss [7]. The reasons for this disparity are unknown and warrant further investigation.

A well-established task used to assess learning and memory in rodents is the object recognition task [8–12]. Animals attend to and investigate a pair of identical sample objects during an initial acquisition period. Successful recall of this learned information then allows for the ability to discriminate between a familiar and novel object during the recognition phase of the task. A natural preference to investigate the novel object is used as a measure of recognition memory, which serves as a model for episodic-like memory in humans [13].

Sleep loss has been shown to impair recognition memory in the object recognition task but its impact on attention and learning in this task is less clear. For example, in the object recognition test, when sleep deprivation is placed immediately after acquisition, mice display deficits in recognition memory [14,15]. This is presumably due to interference with memory consolidation or retrieval. However, it has not been reported how sleep loss before the acquisition phase impacts attention, learning, and subsequent recall in the object recognition task. This is despite the fact that pre-training sleep deprivation impacts attention and acquisition in other learning and memory tasks in rodents and humans [5,16–18]. Moreover, experiments examining the cognitive consequences of sleep loss are mostly limited to males. These results support a need for careful study of both learning and memory behavior in the object recognition task, especially for sleep deprived females.

Female reproductive behavior and cognitive health are regulated by sex hormones, which are sensitive to sleep deprivation and may account for impairments in learning and memory. Naturally cycling levels of estradiol and progesterone signal transition through the estrous (reproductive) cycle [19]. These hormones are also necessary for object recognition memory [20]. For example, removing their principle source by ovariectomy impairs recognition memory but this can be rescued by administration of estradiol before or after acquisition [20–22]. Sleep deprivation reduces estradiol levels in gonad-intact mice and prolongs the estrous cycle in mice and rats [23,24]. Together, these data suggest that the negative effects of sleep loss on learning and memory in females may be due to interaction with estradiol and the estrous cycle.

Here, we hypothesized that sleep deprivation before acquisition impacts learning and memory in female mice by interfering with the estrous cycle. We first used the object recognition task to investigate the cognitive consequences of sleep loss in gonad-intact, naturally cycling female mice. Mice were tested during proestrus/estrus (P/E), stages of estrous characterized by elevated levels of estradiol, and again during metestrus/diestrus

(M/D) when circulating levels of estradiol are comparatively low. We carefully examined learning behavior during the acquisition phase of the object recognition task by measuring time attending to sample objects, and evaluated recognition memory using novel object preference. Finally, we sleep deprived mice for 12 hours before object acquisition to examine the impact of sleep loss on the learning and memory processes taking place during acquisition and recognition in P/E females.

2. Materials and Methods

All procedures were carried out in accordance with the guidelines established by the National Institutes of Health for the Care and Use of Laboratory Animals and were pre-approved by the Institutional Animal Care and Use Committee of Western Connecticut State University.

2.1. Mice

Adult (8–10 weeks old), gonad-intact, female C57BL/6 mice were purchased from Charles River Labs. Mice were group housed, habituated to a temperature controlled room ($24\pm 2^{\circ}\text{C}$) with a 12h/12h light/dark cycle, and provided *ad libitum* access to food and water for the duration of testing. Mice were handled 5 minutes daily for 2 weeks prior to and during experimentation to minimize stress. Stage of the estrous cycle was assessed daily throughout this time frame. Only those mice with regular 4–6 day cycles were included in experiments. All behavioral testing took place within the room they were housed at the beginning of the dark period between ZT12–ZT2, roughly the peak time of mouse activity.

2.2. Estrous cycle

The mouse reproductive (estrous) cycle is well described [25–27]. It lasts approximately 5 days and is divided into 4 stages: proestrus, estrus, metestrus, and diestrus. Defined fluctuations in circulating levels of reproductive hormones signal transition through these stages which are characterized by changes in the ratio of nucleated epithelial cells, cornified squamous epithelial cells, and leukocytes present in vaginal smears. Estradiol levels in the bloodstream and brain are generally highest during proestrus and estrus and lowest during metestrus and diestrus.

Stage of estrus was evaluated by daily assessment of cell types present in a vaginal smear, as described [28–30]. Cells were collected daily via vaginal gavage with sterile water and immediately applied to a glass slide. Samples were air-dried, stained with methylene blue, and cell types were observed using a light microscope. Stage of the estrous cycle was determined using the following criteria: proestrus – mostly nucleated epithelial cells, estrus – mostly cornified epithelial cells, metestrus – cornified epithelial cells and leukocytes, diestrus – mostly leukocytes.

2.3. Experimental Design

Experiment 1: A group of cycling female mice ($n = 15$) was tested in the object recognition task to examine learning and memory behavior in different phases of the estrous cycle. Mice were tested twice: once during proestrus or estrus (P/E) and once during metestrus or

diestrus (M/D), in a counterbalanced, repeated measure design. Tests were separated by 1–2 weeks and used different objects. Object pairs and their locations were also counterbalanced. Task performance using repeated measures is not subject to decay using different objects [21,27,31].

Experiment 2a: A group of cycling female mice (n=4) underwent surgery for an EEG/EMG headmount for the sole purpose of measuring sleep and wake time during at rest (control) and when subjected to 12h sleep deprivation (SD).

Experiment 2b: A group of cycling female mice (n = 15) was subjected to 12h sleep deprivation immediately before the acquisition phase of the object recognition test, to measure its impact on attention, acquisition (learning) and recall (memory) during proestrus/estrus. No mice in M/D were tested after sleep deprivation because mice in M/D showed poor object recognition without sleep deprivation.

2.4. Object Recognition Task

2.4.1 Apparatus—Object acquisition and recognition were assessed in a commercially available, plexiglass, open field arena (27.3 cm × 27.3 cm × 20.3 cm; Med Associates, Inc.) designed for such purpose. The arena was located inside a sound attenuation chamber (Med Associates, Inc.) to eliminate visual and auditory distractions from testing in the same room they were housed. Small, washable plastic toys different in color and shape but similar in size (approximately 5 cm × 3 cm × 2 cm) were selected as sample objects. Sample objects were pilot-tested in advance to confirm that mouse interaction with the objects was comparable between objects and pairings (data not shown). Novel and familiar object pairs were randomly assigned, and their positions were counterbalanced to further reduce possible effects of object or place preference/aversion. Objects were secured to opposite corners of the arena (~4cm from the wall) to ensure it could not be displaced by a mouse. Test sessions were recorded using an overhead, infrared video monitoring system (Med-Associates, Inc.) Between sessions and animals, the arena and objects were cleaned with 70% ethanol to eliminate olfactory cues.

2.4.2 Task Procedure—The object recognition task was performed by dividing testing into 2 phases: acquisition and recognition, as described by [9,10,31]. The test was performed without a habituation phase, which has been reported reduce experimental time without interfering with task performance [10]. Beginning at ZT12, for the acquisition phase, a single mouse was placed into the arena with two identical sample objects for a period of up to 10 minutes to allow for a criterion level of investigation and familiarization of the objects. After a 1-hour delay, the mouse was returned to the testing arena for recognition, when the mouse was presented with one of the familiarized sample objects and a novel object, for up to 10 minutes of possible investigation. Mice were tested with a 1h inter-trial interval, as opposed to a 24h retention, to ensure that both phases of the task were conducted within the same phase of the estrous cycle. Total time spent investigating the objects during each phase was limited to a criterion of 20s with a maximal time of 10 minutes to reach criterion to standardize exploratory behavior, as previously described [10,32].

2.4.3 Analysis of Behavior—Time spent investigating objects was assessed manually from recorded test sessions by an investigator blinded to test condition. Object investigation was defined as a mouse directing the nose toward, touching or sniffing within 1 cm of the object but not standing, sitting, or chewing on the object. Average time spent attending to objects per investigation during acquisition was used as a measure of object-based attention. Percentage of time spent with the novel object during novel object recognition was used as an index of object recognition memory.

2.5. Sleep Deprivation

One week before testing, mice in the sleep deprivation groups were acclimated to a cylindrical polypropylene cage with bedding and freely available food and water, similar to their home-cage environment. Twelve hours of sleep deprivation (ZT0-ZT12) preceding object recognition testing was achieved using the Automated Sleep Deprivation System for Mice (Pinnacle Technologies, Inc.). Mice stayed awake by stepping over a bar which continuously rotated at ~6 revolutions per minute and switched directions every 10 seconds. Mice in diestrus or proestrus were subjected to sleep deprivation to be tested during proestrus/estrus, when the estrous cycle advanced.

2.5.1. Polysomnography—A cohort of mice were implanted with electrodes under isoflurane anesthesia to measure EEG and EMG activity in order to assess vigilance state at rest and during sleep deprivation only. These mice were not tested for object recognition. A prefabricated EEG/EMG headmount (Pinnacle Technologies) was positioned on the skull by four stainless steel, epidural screw electrodes: A/P +1.5mm, M/L $\pm$ 1.5mm and A/P -3.5mm, M/L $\pm$ 1.5 mm to monitor EEG activity. Stainless-steel, Teflon coated wires were bilaterally inserted into the nuchal muscles to monitor EMG activity. The headmount was secured to the skull with dental cement.

After 2 weeks of post-operative recovery, mice were acclimated to the sleep chamber with lightweight recording cables connected to the headmount for at least 3 days. Digitized EEG and EMG signals were collected, using Sirenia Acquisition software (Pinnacle Technologies, Inc.) for a 24h baseline, a 12h sleep deprivation (Z0-ZT12), and a 3h recovery recording.

2.5.2. Vigilance state scoring and analysis—Wake, NREM sleep, and REM sleep vigilance states were visually scored offline from recorded wave forms in 10s epochs [33]. The following standard criteria were used: Wake - low-voltage, high-frequency EEG; high-amplitude EMG, NREM sleep - high-voltage, low-frequency EEG; low-amplitude EMG, and REM sleep - low-voltage, high frequency EEG; very low-amplitude EMG. Data with artifact (due to movement or scratching) were excluded from analysis. We observed that mice were able to sleep for brief periods during the 12h sleep deprivation. So, to be conservative in the estimates of total sleep time, the entire epoch was classified as NREM sleep if a 10s epoch had 3s or more of NREM sleep. The duration of time spent in each vigilance state was measured and sleep deprivation was compared to baseline using paired, within-subject measures.

2.6. Statistical Analysis

Data for Experiment 1 were grouped based on whether the mouse was tested in proestrus or estrus (P/E) and metestrus or diestrus (M/D), as has been performed previously in rats [11], according to relative levels of estradiol. Mice failing to reach the criterion 20s of object exploration during either of acquisition or recognition phases of the test (~10% of animals) were excluded on the basis that a minimum amount of exploration is required for stable performance [34]. To determine whether time spent with each sample object differed significantly from the chance value of 10s, a One Sample T-Test was used. This type of t-test was used because time spent investigating one sample object is not independent from the time spent investigating the other sample object (e.g. since total investigation time must equal 20s, then any time spent investigating one sample object reduces time spent investigating the other [10,32].) Paired T-Tests were conducted to compare time per investigation and novel object preference values for mice in P/E and M/D. Paired T-Tests were conducted on data in Experiment 2a, comparing minutes in each vigilance state from baseline to sleep deprivation. Independent T-Tests were conducted on data comparing sleep deprived mice tested in P/E from Experiment 2b to the rested mice in P/E (historical controls) from Experiment 1. The alpha level for statistical significance was $p < 0.05$. Bonferroni corrections were applied to correct for multiple comparisons, as appropriate.

3. Results

3.1. Experiment 1: Effects of estrous cycle stage on cognitive performance in the object recognition task

3.1.1. Object acquisition—Naturally cycling female mice were tested in the object recognition task during proestrus or estrus (P/E), when circulating levels of estradiol are high, and again during metestrus or diestrus (M/D), when circulating levels of estradiol are comparatively lower. During object acquisition, the first phase of the object recognition task, mice investigated two identical sample objects.

Neither sample object was investigated more or less than chance (50%), regardless of whether mice were in P/E or M/D (P/E: $t(14) = 0.91$, $p = \text{n.s.}$; M/D: $t(14) = 0.46$, $p = \text{n.s.}$, data not shown). This indicated that there was no side preference during testing. The average duration for each investigation of the sample objects was influenced by stage of the estrous cycle. Mice in P/E attended to sample objects longer than during M/D. Whereas mice in P/E attended to sample objects for an average of $0.77 \pm 0.04\text{s}$ per investigation, mice in M/D attended to sample objects for an average of $0.63 \pm 0.03\text{s}$ per investigation ($t(14) = 2.624$, $p < 0.05$, Fig. 1A).

3.1.2. Object recognition—After a 1-hour delay, mice were tested in the object recognition phase of the object recognition task. Mice investigated one familiarized sample object and one novel sample object. Preference for the novel object was used as an index for recognition memory.

Stage of the estrous cycle impacted novel object preference. Mice in P/E showed greater preference for the novel object than during M/D. Specifically, mice in P/E investigated the

novel object for $65.7 \pm 3.3\%$ of the total time spent investigating the sample objects. This was significantly greater than the percent expected by chance (50% ; $t(14) = 4.67$, $p < 0.001$, Fig. 1B). Mice in M/D displayed only a $54.4 \pm 2.6\%$ preference to investigate the novel object. This was significantly less than when mice were in P/E ($t(14) = 2.494$, $p < 0.05$, Fig. 1B) and not different from chance (50% ; $t(14) = 1.62$, $p = \text{n.s.}$, Fig. 1B). Regression analysis was performed on pooled data from P/E and M/D. Preference to investigate the novel object positively and significantly correlated with the average time spent per object investigation during object acquisition ($r^2 = 0.30$, $n = 30$, $p < 0.001$). A scatter plot summarizes these results (Fig. 1C).

3.2. Experiment 2: Effects of pre-training sleep deprivation on estrous cycle and cognitive performance in the object recognition task

3.2.1. Sleep deprivation—Sleep deprivation produced prolonged wakefulness. Mice were awake for $89.1 \pm 1.6\%$ of the 12h sleep deprivation period (for a total of $86.6 \pm 1.3\%$ of the 24h preceding testing). The average time awake during the light period was significantly increased with sleep deprivation ($t(3) = 20.86$, $p < 0.001$; Fig. 2A). Whereas mice at rest were awake for an average of 223.5 ± 11.7 minutes, during sleep deprivation, mice were instead awake for 641.6 ± 11.3 minutes. Sleep deprivation also significantly reduced NREM and REM sleep times (Fig. 2A). At baseline, mice spent 422.4 ± 15.0 minutes and 74.0 ± 6.1 minutes in NREM and REM sleep, respectively. Time in NREM sleep was reduced to 75.3 ± 10.7 minutes ($t(3) = 17.23$, $p < 0.001$) by sleep deprivation. Time in REM sleep was reduced to 0.3 ± 0.3 minutes ($t(3) = 11.34$, $p < 0.001$).

3.2.2. Estrous cycle—Sleep deprivation for 12h did not disrupt length of the estrous cycle. The time to complete one full cycle before sleep deprivation (5.33 ± 0.19 days) was identical to the time to complete one full cycle after sleep deprivation (5.37 ± 0.20 days; $t(14) = 0.14$, $p = \text{n.s.}$; 2B).

3.2.3. Object acquisition after sleep deprivation—Cycling mice were sleep deprived for 12 hours immediately before object acquisition to assess the impact of prior sleep loss on cognition. Since only mice in P/E successfully distinguished between novel and familiar objects (Fig. 1B), we only tested mice in P/E after sleep deprivation. Because the stage of estrous can proceed quickly, mice in diestrus or proestrus were subjected to 12h sleep deprivation and subsequently tested during proestrus/estrus when the estrous cycle advanced.

Sleep deprived mice tested in P/E did not show preference for either of the identical sample objects ($t(14) = 1.70$, $p = \text{n.s.}$, data not shown), again indicating lack of side preference. Each object investigation lasted an average 0.57 ± 0.03 s in duration. This was significantly less than mice in P/E without sleep deprivation, used as historical controls ($t(14) = 3.95$, $p < 0.001$; Fig. 2C). This indicates that sleep deprivation impaired object acquisition.

3.2.4. Object recognition after sleep deprivation—Unlike control mice in P/E, preference for the novel object ($49.2 \pm 3.4\%$) among sleep deprived mice in P/E did not differ statistically from chance (50% ; $t(14) = 0.22$, $p = \text{n.s.}$; Fig. 2D). Importantly, sleep

deprived mice preferred the novel object significantly less than control mice ($t(28) = 3.615$, $p < 0.001$; Fig. 2D). This indicates that sleep deprivation impaired object recognition memory.

4. Discussion

The objective of this study was to use the object recognition task to investigate how the stage of the estrous cycle and sleep deprivation influence cognition in female mice. Based on evidence that sleep deprivation impairs the estrous cycle and that stage of estrous is known to regulate object recognition performance, we hypothesized that sleep deprivation impacted learning and memory by interfering with the estrous cycle. We found that both learning and memory were impaired when mice were in M/D. Sleep deprivation compromised the cognitive performance of mice in P/D but did not alter the estrous cycle, indicating that sleep deprivation impaired cognition independent of an effect on estrous cycle. Thus, the hypothesis was not supported by the results.

The results of this study are important for several reasons. First, the results show that the object recognition task is a useful tool to assess acquisition performance (and attention by inference), not just recognition memory. The results also identify stage of the estrous cycle and sleep deprivation as key factors regulating learning and confirm established roles for these factors in recognition memory. Further, the results add to our understanding of the physiology serving cognition in a historically male-biased literature and introduce new discussion regarding the complexity and practicality but also importance of testing gonad-intact females in the object recognition task.

Careful examination of investigatory behavior during acquisition revealed a measurable learning deficit for mice in M/D. We found that the average time spent per object investigation was shorter for mice in M/D than P/E (Fig. 1A). This significantly correlated with novel object preference during object recognition (Fig 1C), demonstrating that not only was time per object investigation sensitive to stage of estrous, it was moderately predictive of recognition memory. These results confirm previous reports that pre-training administration of estradiol enhances learning and memory [20,22,35,36] in the object recognition task. Typically, though, only recognition memory is assessed and used to simultaneously evaluate learning and memory in the object recognition task. Our results allow for the unique distinction between effects on acquisition (learning) and recall (memory) by identifying a measurable impairment in time spent per investigation during learning.

We view time spent per investigation during acquisition as a possible measure of attention and a key part of the learning and memory process. In fact, several studies contend that attention is a required cognitive skill for object recognition [9,37–39]. And, the impaired time per object investigation we observed for mice in M/D and in P/E mice after sleep deprivation is consistent with reported deficits in sustained attention for rats in diestrus [40] or after sleep deprivation [16,17]. Accordingly, reduced time per object investigation during acquisition may reflect an attention deficit. Since anxiety and responsiveness to stress peak during diestrus [41,42], it is possible that these attention deficits were stress-induced. Never-

the-less, without first attending to and learning about the sample objects, mice were unable to subsequently discriminate between the familiar and novel object. We suggest that impaired attention during acquisition compromised learning and contributed to recognition memory deficits.

The deficits in recognition memory support established roles for sex hormones in object recognition. During the second phase of the object recognition task, mice in P/E, but not M/D, successfully discriminated between a novel and familiar object. In other words, only during P/E, when estradiol levels are high, were mice able to remember the familiar object and recognize the novel object. This is consistent with a rich literature demonstrating that exogenously applied and endogenous levels estradiol regulate object recognition performance in mice and rats [11,22,27,43,44] (but see [20] for review). Our results are especially in line with those from Walf et al. [27] who showed that (coincident with relative estradiol levels in the brain) gonad-intact mice in proestrus, but not diestrus, had intact recognition memory. Noteworthy is the fact that progesterone also benefits object recognition and the combined signaling with estradiol during the estrous cycle may help mediate cognition [20,45]. In sum, our results lend further support to the conclusion that the levels of naturally cycling and physiological levels of sex hormones during P/E (signaling in the body and brain) are required for optimal recognition memory in female mice.

Prior sleep was also necessary for intact learning and memory, but not because it influenced the estrous cycle. We observed that 12h sleep deprivation before testing impaired cognition in the object recognition task, like reports using alternative learning and memory tasks [46,47]. Sleep deprived mice in P/E exhibited difficulties similar to rested mice in M/D. However, the number of days to complete a full cycle before and after sleep deprivation were nearly identical, with no indication of prolonged diestrus (Fig 2B). This is unlike previous reports that 96h or 15d of REM sleep deprivation establishes long periods of diestrus in rats [24] or mice [23], respectively. The difference may be attributed to our use of a shorter and less stressful 12h (NREM and REM) sleep deprivation protocol which impaired cognition without altering the estrous cycle. Interestingly, these results are consistent with clinical research showing that while young women perform better on attention and memory tasks compared to post-menopausal women, both groups are equally impaired by 25h acute sleep deprivation, and hormone replacement is ineffective at rescuing cognitive impairments [48,49]. Thus, our combined work indicate that acute sleep deprivation negatively impacts learning and memory independent from the reproductive cycle.

This study contributes to a limited body of literature investigating the cognitive consequences of sleep loss in females, who are so far under-represented in biomedical research [50,51]. Ironically, a main reason for this bias may be due to the fluctuation of sex hormone levels occurring during the reproductive cycle. Its influence on behavior may complicate study design and data interpretation. Our work and that of others, however, demonstrate that the effects of sex hormones on the brain are not limited to reproductive behaviors and are worth more detailed investigation. To that end, the object recognition task is a useful tool to help investigate learning and memory behaviors. While the task may be more practically suited for efficient screening of male or ovariectomized female mice, our

results reaffirm the importance of using it to help reveal the complex physiology underlying cognitive health in gonad-intact females.

5. Conclusions

Overall, our results demonstrate that learning and memory performance in the object recognition task were negatively impacted: 1) during stages of the estrous cycle characterized by low levels of circulating estradiol and 2) by 12 hours of pre-training sleep deprivation. Data support known effects on recognition memory performance, identify distinct roles for the estrous cycle and sleep during learning, and highlight their importance in the maintenance of overall cognitive health in a gonad-intact female model.

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Highlights

- Object acquisition (learning) is impaired during metestrus/diestrus.
- Object recognition (memory) is impaired during metestrus/diestrus.
- 12h of pre-training sleep deprivation impairs object acquisition and recognition.
- 12h of sleep deprivation does not alter estrous cycle length.

Figure 1.

Stage of the estrous cycle impacted learning and recognition memory performance during the object recognition task.

A) Mice in M/D attended to sample objects less during the acquisition phase of the object recognition task. The average time spent per object investigation was reduced.

B) Recognition memory was impaired during M/D. Novel object preference during M/D was less than during P/E and reduced to chance during the object recognition phase of the object recognition task.

C) A scatterplot shows the relationship between object acquisition and recognition performance, revealing a positive correlation between time per object investigation and novel object preference.

[Values are means $\pm$ SE, P/E – Proestrus/Estrus, M/D – Metestrus/Diestrus, $n = 15$ each, * $p < 0.05$ One-Way TTest, ** $p < 0.05$ Paired TTest]

Figure 2.

Sleep deprivation for 12h impaired acquisition and recognition memory performance in proestrus/estrus mice, without altering the estrous cycle.

A) Sleep deprivation increased time awake and reduced the duration of NREM and REM sleep.

B) Length of the estrous cycle was unchanged before and after sleep deprivation.

C) Sleep deprivation reduced the average time spent per object investigation during the acquisition phase of the object recognition task, indicative of impaired learning.

D) Recognition memory was impaired by pre-training sleep deprivation, as shown by a reduced preference to investigate novel objects during the object recognition phase of the object recognition task.

[Values are means $\pm$ SE, C – Control, SD – Sleep Deprivation, n = 15 each, * p < 0.05 One-Way TTest, ** p < 0.05 TTest]