RESEARCH PAPER

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Impact of REM sleep deprivation during the acquisition phase on learning and memory performance, neurotrophic and oxidative stress markers in male rats

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During adolescence, the brain is particularly vulnerable to sleep disruption, which may lead to long-term memory deficits and emotional dysregulation. This study investigated the impact of REM sleep deprivation (REM-SD), applied specifically during the acquisition phase of learning, on spatial memory, anxiety-like behavior, and hippocampal neurotrophic and oxidative status in adolescent rats. Male adolescent Wistar Albino rats were subjected to a REM-SD protocol using the modified multiple platform method (MMPM), integrated directly into the training phase of the Morris water maze (MWM). Animals were divided into three groups: Control, Pedestal Control, and REM-SD. Anxiety-like behavior was assessed using the open field (OFT) and elevated plus maze (EPM) tests. Corticosterone levels and hippocampal levels of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), total oxidant status (TOS), total antioxidant status (TAS), superoxide dismutase (SOD), and malondialdehyde (MDA) were analyzed by ELISA. REM-SD during the acquisition phase significantly impaired memory performance in the MWM probe trial. In the OFT test, REM-SD rats exhibited increased anxiety-like behavior, reflected by reduced time spent in the center zone, whereas the EPM demonstrated increased open-arm duration. These behavioral alterations were accompanied by significantly elevated corticosterone levels. REM-SD was associated with reduced hippocampal antioxidant status (decreased TAS and SOD), decreased NGF levels, and increased oxidative status (TOS). Our findings demonstrate that exposure to the MMPM, together with REM sleep disruption and associated stress-related physiological responses, was accompanied by alterations in memory performance, corticosterone levels, oxidative balance, and neurotrophic markers. The observed behavioral changes, including alterations during late acquisition and impaired probe trial performance, may reflect combined effects on learning- and memory-related processes.

Key words: REM sleep deprivation, spatial memory, adolescence, oxidative stress, hippocampus

INTRODUCTION

Sleep is a daily rhythm regulated by the suprachiasmatic nucleus of the hypothalamus and is an essential need for all living (Adamantidis & de Lecea, 2023; Huang et al., 2024). Sleep plays an important role in the maintenance of many physiological functions, including synaptic plasticity, memory consolidation, metabolite removal, energy metabolism, hormone regulation, and the optimal functioning of the immune system (Stickgold & Walker, 2005; Haack et al., 2007; Meerlo et al., 2009; Luyster et al., 2012; Stich et al., 2022). Sleep deprivation can be defined as insufficient sleep and has been associated with cognitive, metabolic, and physiological disturbances in both humans and experimental animals (Dickerman et al., 2016; Xu et al., 2016; Neculicioiu et al., 2023; Amiri, 2023; Zieneldien & Kim, 2024). Studies show that the

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negative effects of sleep disruption on learning and memory differ between young and older individuals. Experimental evidence suggests that the cognitive consequences of sleep disruption may vary across developmental stages. Younger animals appear to be particularly vulnerable to sleep loss, potentially due to ongoing metabolic and neurodevelopmental processes occurring during maturation (Wimmer et al., 2013; Sengupta et al., 2017; Pasula et al., 2018; Konakanchi et al., 2023; Neculicioiu et al., 2023; Zhang et al., 2025). Previous studies indicate that sleep deprivation increases oxidative stress in different brain regions, possibly through the production of reactive oxygen species in both humans and animals (Tufik et al., 2009; Villafuerte et al., 2015; Neculicioiu et al., 2023; Konakanchi et al., 2023). A recent systematic review and meta-analysis of experimental studies in rats and mice further confirmed that sleep deprivation consistently impairs memory performance and alters anxiety and depression-like behaviors, highlighting the broad neurobehavioral consequences of sleep loss in rodent models (Zhang et al., 2025).

REM sleep plays an important role in brain maturation, memory consolidation, and the regulation of neurotransmitter systems. In rodents, sleep architecture consists of alternating non-rapid eye movement (NREM) and rapid eye movement (REM) sleep stages. REM sleep is characterized by prominent hippocampal theta oscillations and neuronal activity associated with synaptic plasticity, emotional regulation, and memory consolidation, making rodent models highly valuable for investigating the neurobiological consequences of REM sleep disruption (Meerlo et al., 2009; Hutchison & Rathore, 2015; Levenstein et al., 2019; Giri et al., 2021). Consequently, adolescent rodents may exhibit increased vulnerability to the cognitive, oxidative, and neuroendocrine consequences of REM sleep disruption compared to mature adults (Sengupta, 2013; Soto-Rodriguez et al., 2016; Murillo-Rodríguez et al., 2021; Kang et al., 2024). Therefore, adolescent rodents provide a particularly relevant experimental model for investigating the neurobiological consequences of REM sleep disruption and its impact on memory-related processes.

REM-SD applied at different durations has been shown to impair contextual memory retrieval and auditory fear memory retention (Nasehi & Zarrindast, 2019). In addition, REM-SD was shown to impair contextual fear memory in a mouse model of fear conditioning (Kim et al., 2021). These findings collectively support the critical role of REM sleep in memory consolidation and hippocampus-dependent cognitive processes in preclinical studies. When sleep deprivation occurs in mammals, a REM rebound effect occurs to compensate for REM sleep loss (Machado & Suchecki, 2016). A significant challenge in REM-SD research is the "REM rebound" effect, a homeostatic mechanism in which the brain increases REM sleep intensity and duration following a period of deprivation. In many rodent learning paradigms, including the MWM and fear conditioning studies, REM sleep deprivation protocols are commonly applied either before behavioral acquisition or immediately after training sessions to investigate the effects of sleep loss on memory consolidation processes (Walsh et al., 2011; Yang et al., 2019; Nasehi & Zarrindast, 2019; Kim et al., 2021). This separation often allows the subject to experience rebound sleep during the testing or consolidation intervals, which may mask the deleterious effects of the initial deprivation rather than truly mitigating them. However, it is important to acknowledge that platform-based REM sleep deprivation paradigms, including the modified multiple platform method (MMPM), may also induce stress-related neuroendocrine and physiological alterations beyond REM sleep loss itself. Furthermore, MMPM predominantly targets REM sleep but may also induce a concomitant reduction in slow-wave sleep, indicating that the method cannot be considered exclusively REM-selective. These methodological considerations have been widely discussed in the sleep deprivation literature and should be considered when interpreting behavioral and molecular outcomes.

In the present study, we aimed to investigate the effects of REM sleep deprivation applied specifically during the learning phase on memory performance in male adolescent rats. By utilizing the MWM task and integrating the REM-SD protocol directly into the training days, we sought to eliminate the rebound sleep confounder. Additionally, we assessed behavioral changes and measured serum corticosterone levels as well as hippocampal levels of NGF, BDNF, MDA, SOD, TAS, and TOS.

METHODS

Animals

A total of 24 male Wistar albino rats (postnatal day 28) were included in the experimental protocol. The experimental procedures were conducted in accordance with the animal welfare regulations adopted by Mersin University. Ethical approval was obtained from the Institutional Animal Care and Use Committee of Mersin University (Approval No. 2023/05, dated April 10, 2023).

The animals were housed in the Physiology Laboratory of Mersin University Faculty of Medicine. They

had unrestricted access to water and standard laboratory chow. The housing conditions were controlled, with temperature and a 12-hour light/dark cycle maintained. Prior to the initiation of the experimental procedures, the subjects were permitted to acclimatize to the laboratory environment for one week. The animals were randomly assigned to three experimental groups (n=8 per group): control (C), pedestal control (PC), and REM-SD. The sample size was determined based on the findings of previous studies that employed REM sleep deprivation paradigms and behavioral outcome measures in rodents. Additionally, ethical principles were considered to minimize animal usage. Investigators were aware of group allocation during animal assignment and experimental procedures due to the nature of the REM sleep deprivation protocol. Behavioral data were collected using an automated tracking system (EthoVision; Noldus Information Technology, Wageningen, The Netherlands). Biochemical analyses were executed using coded samples to minimize observer bias during outcome assessment and data analysis. The experimental procedure is illustrated in Fig. 1.

REM sleep deprivation procedure

The REM sleep deprivation procedure was induced by subjecting rats to the modified multiple platform apparatus throughout the 4-day acquisition phase of the MWM test, with the exception of the daily training sessions. Rats in the PC group were housed in the same apparatus as the REM-SD group but on larger platforms that allowed normal sleep, including REM sleep. This condition was utilized to regulate for environmental exposure and non-specific stress associated with the sleep deprivation apparatus. The control rats remained in their home cages during the experiment (Suer et al., 2011; Sahin et al., 2019). No unexpected health issues were observed during the procedure.

REM sleep deprivation was induced using the modified multiple platform method, which was adapted from Sahin et al. (2021). The animals assigned to the REM-SD condition were placed in a Plexiglass apparatus containing multiple small platforms, each measuring 6 cm in diameter. The apparatus comprised eleven platforms positioned above water maintained at 24 ± 1°C, with the water level adjusted approximately 1 cm below the

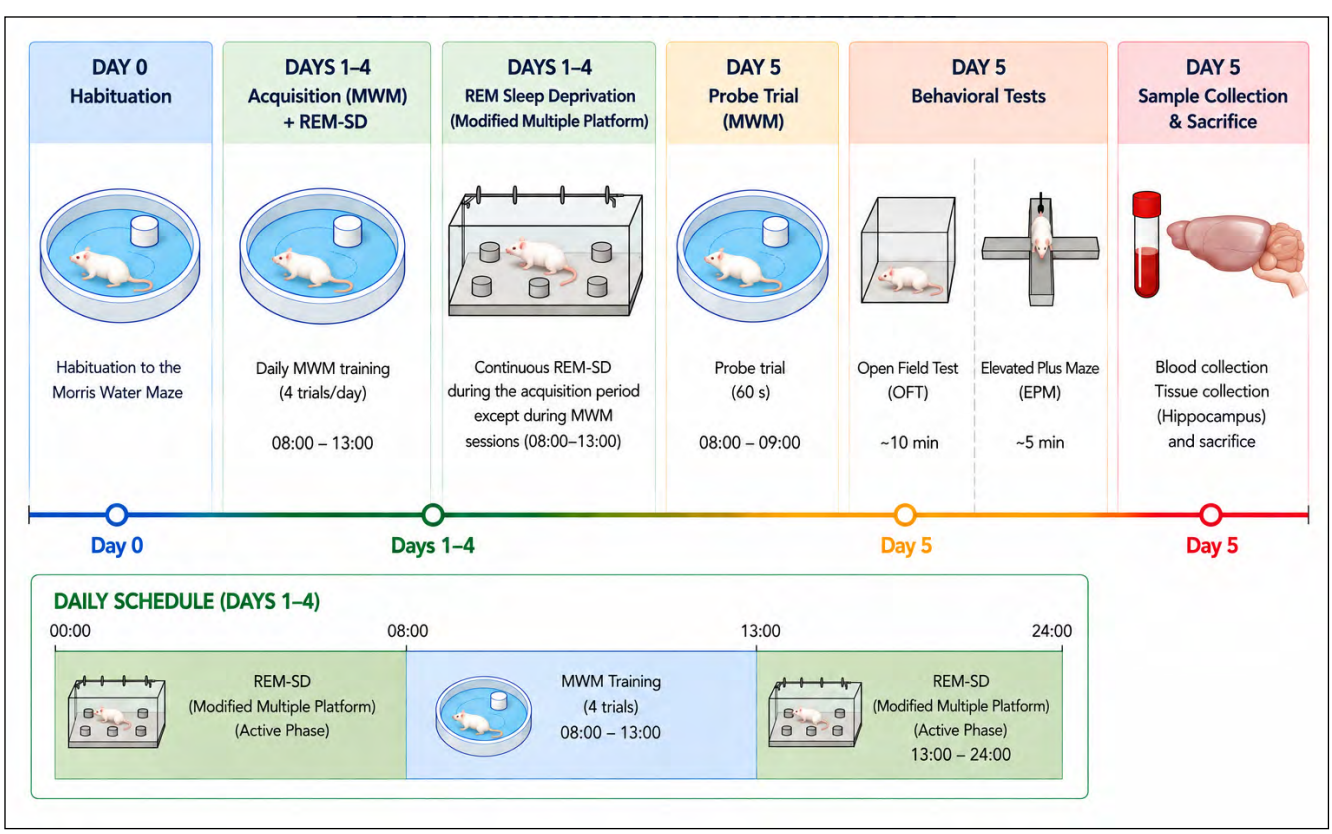

Fig. 1. Experimental timeline of the study. Following habituation on Day 0, rats underwent daily Morris Water Maze (MWM) acquisition trials during Days 1–4 concurrently with REM sleep deprivation using the modified multiple platform method. On Day 5, the probe trial, behavioral tests (OFT and EPM), and sample collection procedures were performed.

platform surface. The animals had unrestricted access to food and water, and were able to move between platforms during the procedure. During REM sleep, a reduction in postural muscle tone may result in contact with the water surface, thereby interrupting REM sleep episodes. Although electroencephalograms (EEGs) and electromyograms (EMGs) were not performed to electrophysiologically confirm the suppression of REM sleep, the modified multiple platform method is widely used as an established experimental paradigm for inducing REM sleep deprivation in rodents (Machado et al., 2004; Suchecki et al., 2000).

Assessment of spatial learning and memory in the Morris water maze test

Spatial learning was assessed using the MWM simultaneously with the REM-SD protocol throughout the four-day acquisition period. The experimental procedure was adapted from the original method described by Morris (1981). Behavioral testing was conducted during the light phase (8:00 a.m.- 1:00 p.m.). The apparatus consisted of a circular stainless-steel tank (150 cm in diameter, 60 cm in height) filled with water maintained at 22 ± 1°C. For analysis, the pool was virtually separated into four equivalent sectors (northeast, northwest, southeast, and southwest) within the tracking software without the use of physical partitions. A submerged escape platform (10 cm in diameter) was placed 1.5 cm below the water surface in the center of the southwest quadrant. At the start of each trial, the subjects were placed into the pool facing the wall. Spatial learning performance was monitored using an automated video tracking system (EthoVision; Noldus Information Technology, Wageningen, The Netherlands) (Sahin et al., 2019).

Learning phase

The MWM protocol was executed over a period of five days, and the experimental paradigm incorporated a visible platform. On the first day of the experiment, the platform was positioned at a depth of 1.5 cm below the water surface in all four quadrants and the central area. When a rat failed to locate the platform within the designated time frame of 120 s, the experimenter intervened by guiding the rat onto the platform using a wooden stick, after which the rat was permitted to rest for a period of 10 s. During the four acquisition days (days 2–5), each rat received five trials per day (60 s per trial, with a 5 min inter-trial interval). Following the conclusion of the training session, the animals were gently dried and returned to their home cages. The platform remained in the southwest quadrant throughout this phase. When the rat found the platform, the trial was terminated. The total distance moved (cm), velocity (cm/s), and escape latency (s) were recorded by the tracking system (Sahin et al., 2019).

Probe trial (Memory assessment)

Spatial memory was assessed during the probe session that was conducted after the acquisition period. The hidden platform was removed from the southwest quadrant, and animals were introduced into the maze from the northern release point for a single 60-second trial. Time spent in the target area was used as an indicator of memory retention for the platform location. Subsequent to the completion of the probe trial, the rats underwent open field and elevated plus maze testing in sequence on the same day. To avoid confounding effects of acute behavioral testing, blood and hippocampal tissue samples were collected within 0–2 min after completion of the behavioral procedures under standardized conditions.

Assessment of locomotor activity and anxiety-related behavior in the open-field test (OFT)

Anxiety-related behavior and locomotor activity were evaluated using the open field test. The apparatus consisted of a black opaque plexiglass arena measuring 100 × 100 × 40 cm. For the purpose of analysis, the tracking software virtually separated the arena into central and peripheral regions. Each animal was individually placed into the arena and allowed to explore freely for 10 min. Following completion of each session, the apparatus was cleaned using 20% ethanol solution. Behavioral parameters including center zone entries, duration spent in the center area, and total distance travelled were automatically recorded using the EthoVision video tracking system (Noldus Information Technology) (Keloglan Musuroglu et al., 2022).

Assessment of anxiety-like and risk-taking behavior in the elevated plus maze test (EPM)

The elevated plus maze was utilized to assess exploratory and anxiety-related responses. The maze comprised two open and two closed arms, constructed from opaque black plexiglass. Open arms measured

50 × 5 cm, whereas enclosed arms had identical dimensions with surrounding 40 cm high walls. Each rat was placed in the maze for 5 min. After each session, the maze was cleaned with 20% ethanol. Behavioral parameters including time spent in the open arms, open arm entries, time spent in closed arms, closed arm entries, total arm entries, distance moved, and velocity were automatically recorded using the EthoVision XT video tracking system (Keloglan Musuroglu et al., 2022).

The collection of hippocampus samples

At the conclusion of behavioral testing, the rats were deeply anesthetized *via* intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) based on body weight. Brain tissues (e.g., hippocampus) were then rapidly removed for subsequent biochemical analyses. The brain tissue was removed and carefully isolated in a petri dish containing ice-cold phosphate buffer solution (PBS buffer) at 4°C and kept on ice. Both right and left hippocampus tissues were collected. The tissues were stored at -80°C until the day of ELISA analysis for NGF, BDNF, MDA, SOD, TAS, and TOS levels.

Enzyme linked immunosorbent assay (ELISA)

Intracardiac blood collection was performed in all experimental groups, including the control group, the pedestal control group, and the REM-SD group. Serum was isolated by means of a centrifugal process (1000 × g, 10 min) and subsequently preserved at –20°C prior to biochemical analysis. The quantification of corticosterone levels was performed using an ELISA assay kit supplied by BT LAB (Catalog No: EA0036Ra). The removal of residual blood from the hippocampal tissues was conducted by rinsing them with ice-cold phosphate-buffered saline (PBS). The subsequent homogenization of the tissues in ice-cold PBS was performed using a tissue homogenizer. The homogenates were subsequently subjected to a centrifugation process at 5000 × g for a duration of 5 min at a temperature of 4°C. Thereafter, the resulting supernatants were meticulously collected for the purpose of ELISA analysis. The levels of NGF, BDNF, MDA, SOD, TAS, and TOS levels were measured using commercial rat ELISA kits (BT LAB, China; Cat. No. E0539Ra, E0476Ra, E0156Ra, E0168Ra, E1710Ra, and E1512Ra) in accordance with the manufacturer's instructions. Absorbance was measured using a microplate reader, and concentrations were calculated from standard curves generated for each assay. Marker concentrations were expressed according to the units provided by the commercial ELISA kits (NGF, BDNF, and SOD: ng/mL; TAS and TOS: U/mL; MDA: nmol/mL). Hippocampal tissues were processed using the same homogenization protocol for all samples. Total protein content was not determined; therefore, analyte concentrations were reported as measured in the tissue homogenates without further normalization.

Statistical analysis

All statistical evaluations were conducted using SPSS. The assumption of normality was examined separately for each group by using the Shapiro–Wilk test. The data are expressed as mean values ± standard deviation. Since all variables met the assumptions of normality, parametric statistical analyses were applied. *Post hoc* multiple comparisons were performed using Tukey's test where appropriate. Within-subject measurements to trial days (first, second, third, and fourth days) in the MWMT were analyzed using repeated-measures ANOVA. The Sphericity assumptions were evaluated during repeated-measures ANOVA analyses, and Greenhouse–Geisser corrections were applied where appropriate. The statistical significance of the results was determined by the p-value, with results deemed to be statistically significant when $p<0.05$. It is imperative to note that no animals or data points were excluded from the analysis. Prior to the initiation of the experiment, inclusion and exclusion criteria were formally established. For all behavioral and biochemical analyses, the exact number of animals included in each experimental group was n=8.

RESULTS

The present study aimed to evaluate the effects of REM sleep deprivation during spatial learning in rats. The assessment of memory performance was conducted using the MWM, locomotor activity and anxiety-related behavior were examined using the OFT and EPM test respectively. Furthermore, serum corticosterone concentrations were measured, and the levels of NGF, BDNF, MDA, SOD, TAS, and TOS in the hippocampus were examined.

REM sleep deprivation damaged the memory function in the MWM test

Learning phase (Acquisition trials: Distance, speed and latency)

A repeated-measures analysis of variance (ANOVA) with Greenhouse-Geisser correction was conducted to

ascertain the effects of group and day on distance traveled during the learning phase. This analysis revealed no significant group × day interaction ($F_{(3.79, 39.82)}$=0.913, η^2_p=0.080, p=0.461). A substantial main effect of day was identified ($F_{(1.90, 39.82)}$=14.766, η^2_p=0.413, p<0.001). Subsequent *post hoc* comparisons across days (collapsed across groups) revealed that distance traveled was significantly higher on day 1 compared to day 3 (p=0.003) and day 4 (p=0.012), and on day 2 compared to day 3 (p=0.007) and day 4 (p=0.032). These findings indicate a progressive reduction in path length over training days, regardless of group (Fig. 2A). No significant group x day interaction was observed ($F_{(4.60, 48.32)}$=1.280, η^2_p=0.109, p=0.298), but a significant main effect of day was present ($F_{(2.30, 48.32)}$=11.094, η^2_p=0.346, p<0.001) for velocity (Fig. 2B).

A repeated-measures analysis of variance (ANOVA) with Greenhouse-Geisser correction was conducted to examine the effects of group and day on escape latency. This analysis revealed a significant group × day interaction ($F_{(4.14, 43.47)}$=4.245, η^2_p=0.288, p=0.005), as well as a significant main effect of day ($F_{(2.07, 43.47)}$=5.374, η^2_p=0.204, p=0.008). Subsequent *post hoc* comparisons across days (collapsed across groups) indicated that escape latency was significantly higher on day 1 compared to the other days (p<0.010), and on day 2 compared to days 3 and 4 (p<0.010). No significant group differences were observed on day 1 ($F_{(2, 21)}$=0.971, p=0.395), day 2 ($F_{(2, 21)}$=1.188, p=0.325), or day 3 ($F_{(2, 21)}$=2.300, p=0.125). On the fourth day, a significant group effect was identified ($F_{(2, 21)}$=3.814, η^2_p=0.266, p=0.038). Subsequent *post hoc* comparisons indicated

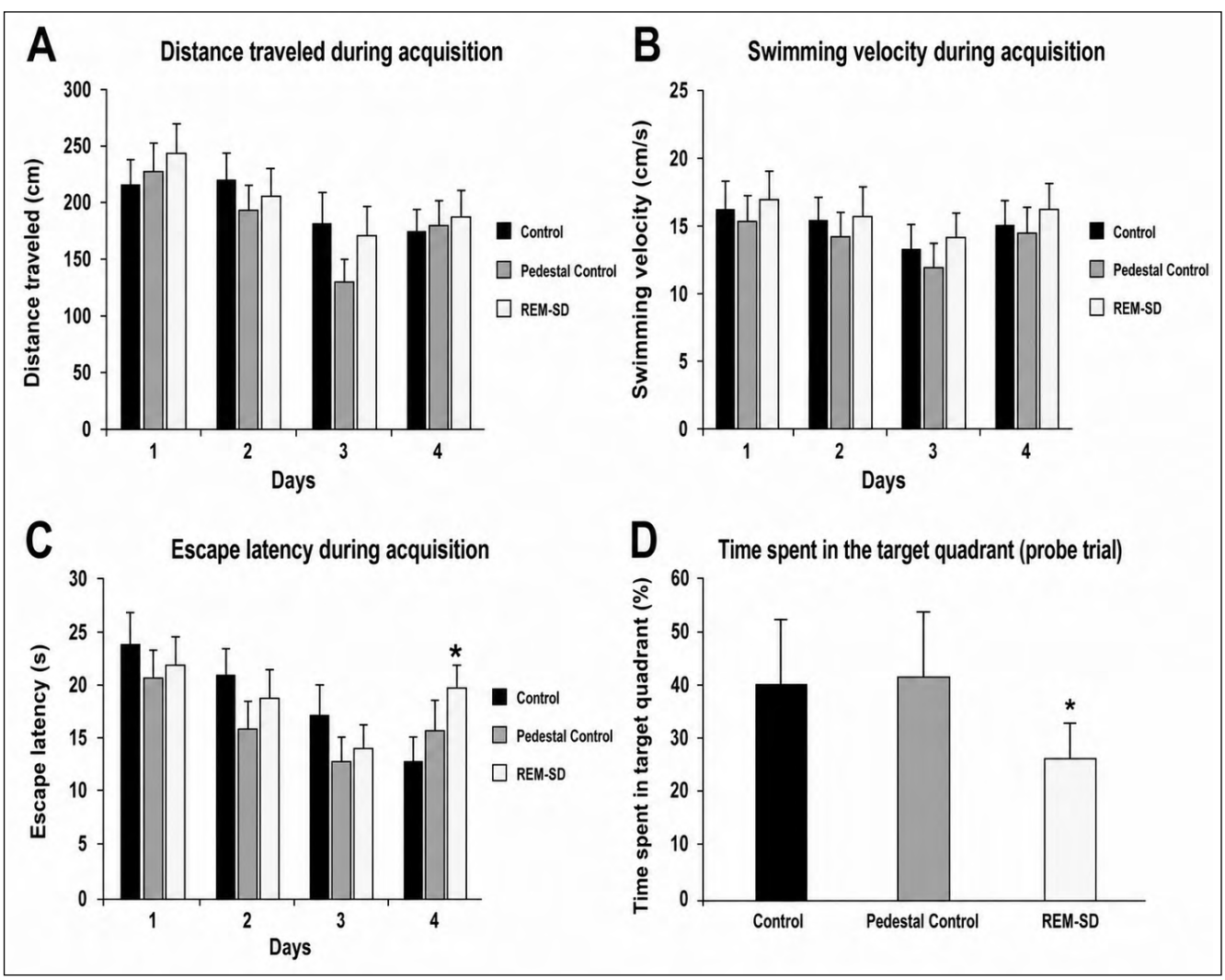

Fig. 2. Morris water maze performance during the acquisition and probe phases. (A) Distance traveled during the 4-day acquisition phase. A significant effect of day was observed (p<0.001). (B) Swimming velocity during the acquisition phase. A significant effect of day was observed (p<0.001). (C) Escape latency during the acquisition phase. A significant effect of day was observed (p<0.001) and *p<0.05 *vs.* Control. (D) Time spent in the target quadrant during the probe test. *p<0.05 *vs.* Control (C).

that the REM-SD group demonstrated a significantly higher escape latency compared to the control group (p=0.033, Cohen's d=1.18) (Fig. 2C).

Probe trials: Percentage of time spent in the target quadrant

On the probe trial (platform removed), a one-way ANOVA revealed significant differences among the three groups in the percentage of time spent in the target quadrant ($F_{(2, 21)}$=4.214, η^2_p=0.286, p=0.030) (Fig. 2D). *Post hoc* comparisons (Tukey's HSD) showed that the REMSD group spent significantly less time in the target quadrant compared to the control group (p=0.026, Cohen's d=1.23) indicating that REM sleep deprivation substantially impaired spatial memory retention. No significant differences were observed between the REM-SD and pedestal control groups or between the control and pedestal control groups (p>0.05).

Behavioral assessment of anxiety-like behavior and locomotor activity in OFT

To determine whether REM-SD affects anxiety-like behavior, all groups were subjected to OFT. In the open field test, the center entries, time spent in the center and the total distance moved were analyzed as behavioral outcomes. A one-way analysis of variance (ANOVA) revealed that there were no statistically significant group differences in distance moved ($F_{(2, 21)}$=1.68, η^2_p=0.138, p=0.210) (Fig. 3C). However, significant differences were found for center duration ($F_{(2, 21)}$=8.85, η^2_p=0.180, p=0.002) and center entries ($F_{(2, 21)}$=5.54, η^2_p=0.136, p=0.012). *Post hoc* comparisons showed that REM-SD rats spent less time in the center compared to the control group (p=0.001) and made fewer crossings into the center compared to the control group (p=0.010) (Fig. 3A, Fig. 3B).

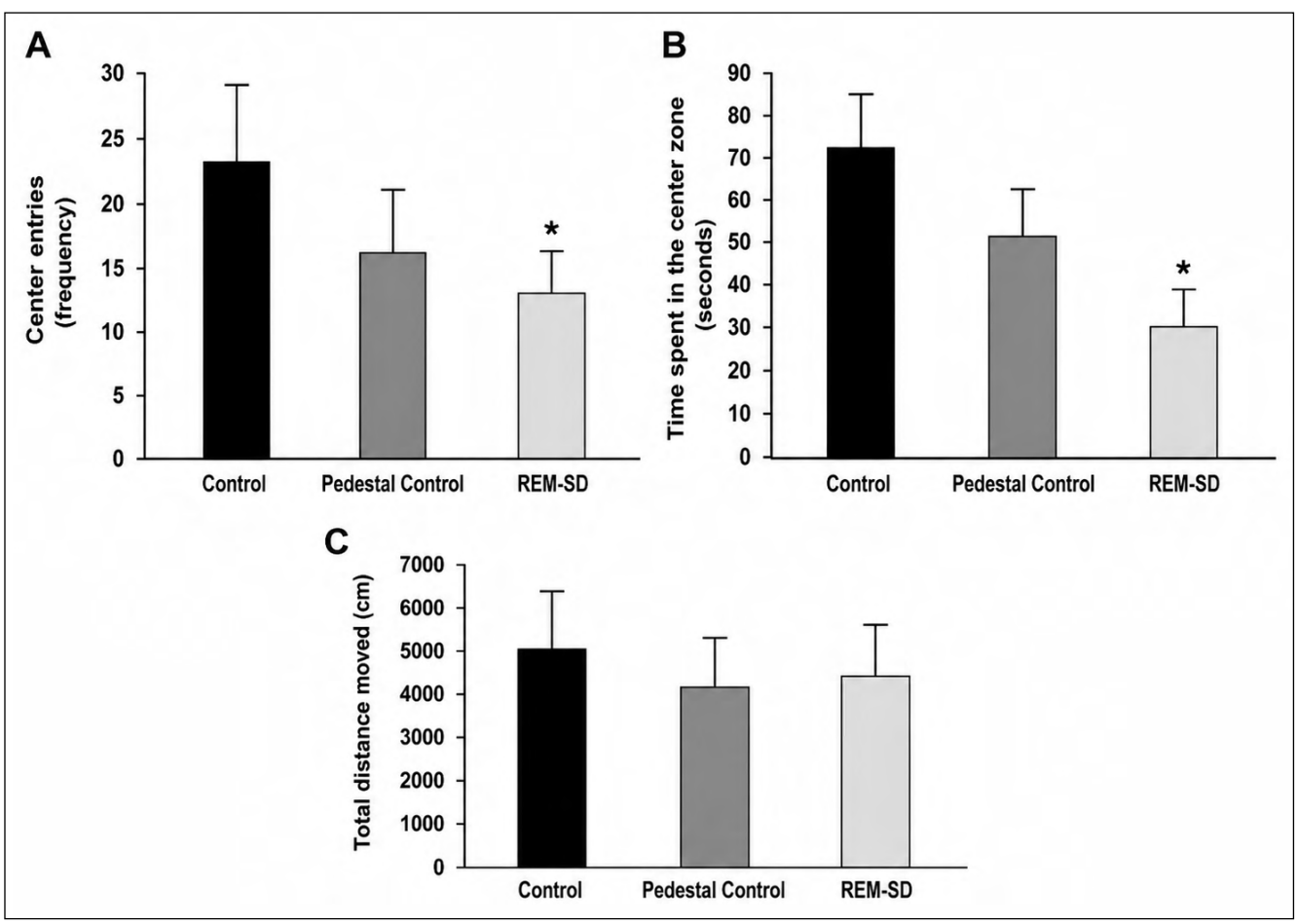

Fig. 3. Open field test parameters assessing anxiety-like behavior and locomotor activity. (A) Frequency of center entries. *p<0.05 *vs.* Control (C) group. (B) Time spent in the center zone. *p<0.05 *vs.* Control (C) group. (C) Total distance moved. No statistically significant differences were observed (p>0.05). Data are presented as mean ± SD.

Behavioral assessment of anxiety-like and risk-taking behavior in EPM

A one-way analysis of variance (ANOVA) revealed a significant difference between groups in the time in open arms ($F_{(2, 21)}$=4.84, η^2_p=0.315, p=0.018). Subsequent *post hoc* comparisons indicated that the REM-SD group spent a significantly higher proportion of time in open arms compared to the control group (p=0.030, Cohen's d=1.19), and the PC group spent a greater proportion of time compared to the control group (p=0.014, Cohen's d=1.88). No significant difference was observed between the REM-SD and PC groups (p>0.05) (Fig. 4A). However, the frequency of open arms entries did not demonstrate a statistically significant difference among the groups ($F_{(2, 21)}$=2.81, η^2_p=0.211, p=0.083) (Fig. 4B), suggesting that the increased time in open arms was not accompanied by an increase in the number of entries.

For closed arm measures, ANOVA revealed a significant difference among groups in the time in closed arms ($F_{(2, 21)}$=7.08, η^2_p=0.403, p=0.004). Subsequent *post hoc* comparisons (Tukey's HSD) indicated that both the REM-SD group (mean difference=38.1 s, p=0.004, Cohen's d=1.87) and the PC group (mean difference=28.7 s, p=0.008, Cohen's d=1.39) (Fig. 4C) demonstrated a significant reduction in time in closed arms compared to the control group. No significant difference was observed between the PC and REM-SD groups (mean difference=9.4 s, p=0.800, Cohen's d=0.40). No significant differences were observed in closed arm entries ($F_{(2, 21)}$=0.19, η^2_p=0.018, p=0.830) (Fig. 4D), total arm entries ($F_{(2, 21)}$=0.64, η^2_p=0.058, p=0.539) (Fig. 4E), percentage of open arm entries ($F_{(2, 21)}$=2.02, η^2_p=0.162, p=0.158) (Fig. 4F), distance moved ($F_{(2, 21)}$=0.12, η^2_p=0.011, p=0.887) (Fig. 4G), or velocity ($F_{(2, 21)}$=1.01, η^2_p=0.088, p=0.381) (Fig. 4H), suggesting that the observed differences were not accompanied by detectable changes in locomotor activity measures.

Evaluation of the corticosterone level

A one-way analysis of variance (ANOVA) revealed a significant difference in serum corticosterone levels among groups ($F_{(2, 21)}$=7.57, η^2_p=0.419, p=0.003). Sub-

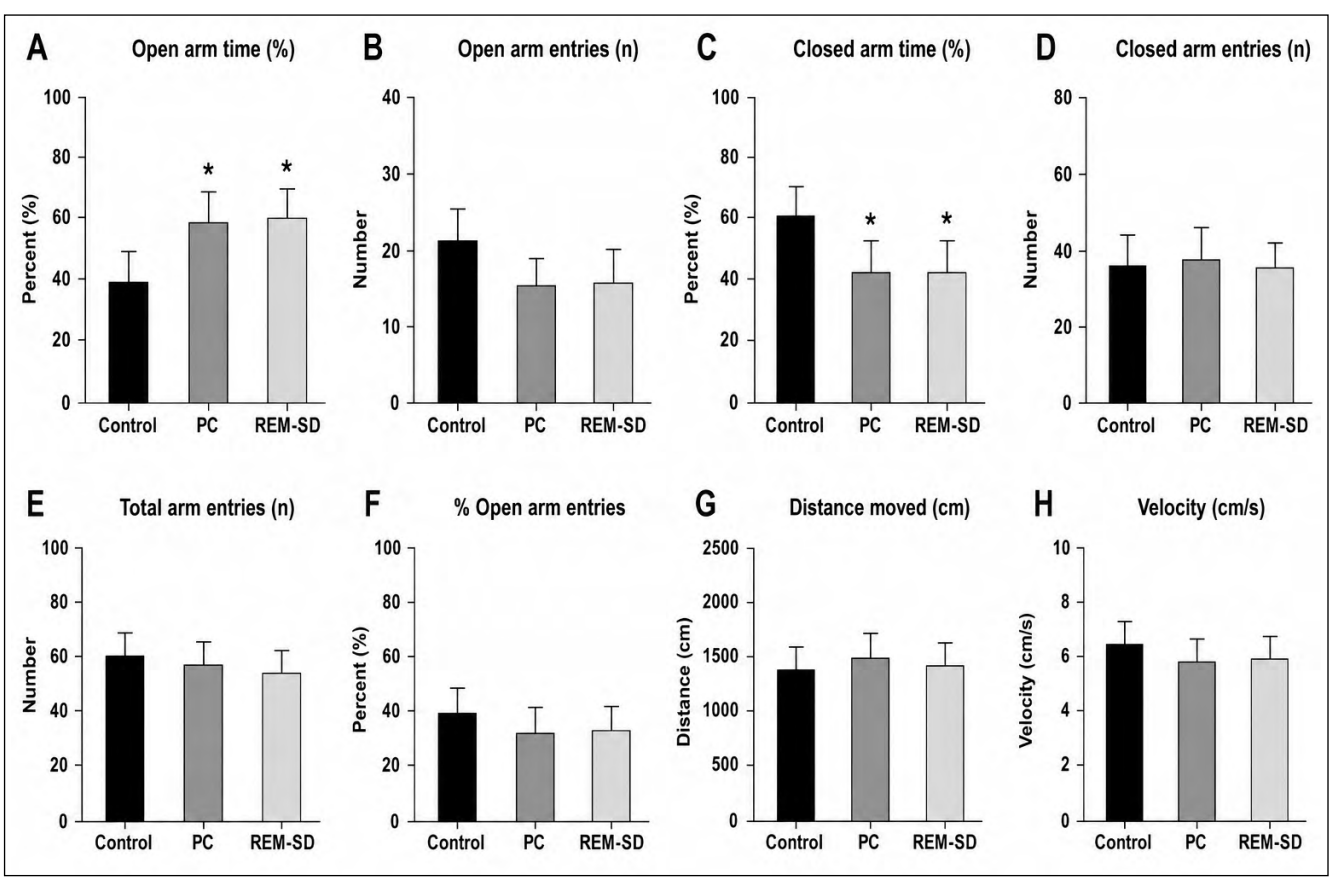

Fig. 4. Elevated plus maze (EPM) behavioral parameters. (A) Time spent in the open arms. *p<0.05 *vs.* control. (B) Frequency of open arm entries. (C) Time spent in closed arms (%) *p<0.05 *vs.* control (D) Closed arm entries, (E) Total arm entries, (F) % open arm entries, (G) Distance moved, (H) Velocity. No statistically significant differences were observed (p>0.05). Data are presented as mean ± SD.

sequent *post hoc* comparisons (Tukey's HSD) revealed that the REM-SD group had significantly higher corticosterone levels compared to the PC group (mean difference = 4.97 ng/mL, p=0.003, Cohen's d=1.64) (Fig. 5) and compared to the control group (mean difference = 3.37 ng/mL, p=0.044, Cohen's d=1.21). No significant difference was observed between the control and PC groups (mean difference = 1.61 ng/mL, p=0.449, Cohen's d=0.87).

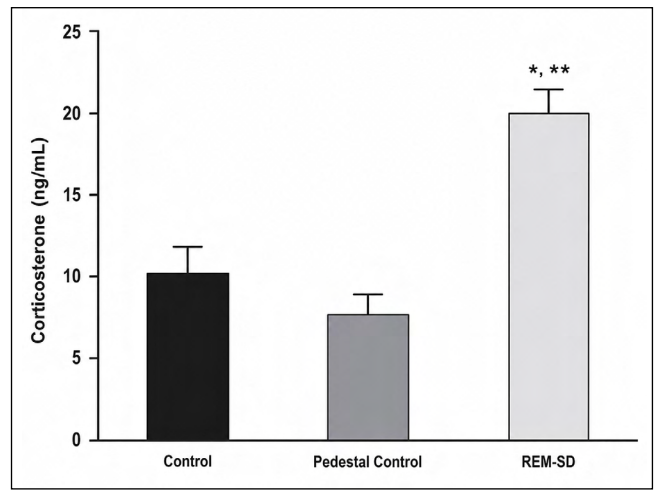

Fig. 5. Serum corticosterone levels (ng/ml). Data are shown as mean ± SD. *p<0.05 compared to Control; **p<0.05 compared to PC. No significant difference was observed between Control and PC (p>0.05).

Hippocampal Neurotrophic Factors

A one-way analysis of variance (ANOVA) revealed a significant difference among groups for NGF levels ($F_{(2, 21)}$=6.23, η^2_p=0.372, p=0.008). A significant reduction in NGF was observed in the REM-SD group compared to the control group (p=0.010, Cohen's d=1.31) (Fig. 6A). No significant group differences were observed in BDNF levels ($F_{(2, 21)}$=0.75, η^2_p=0.067, p=0.484) (Fig. 6B).

The differences in TAS among the groups were statistically significant ($F_{(2, 21)=}$4.96, η^2_p=0.321, p=0.017). *Post hoc* comparisons showed that TAS was significantly lower in the REM-SD group compared to the control group (p=0.015, Cohen's d=1.28) (Fig. 6C). No significant group differences were found in MDA levels ($F_{(2, 21)}$=0.04, η^2_p=0.004, p=0.958) (Fig. 6D).

A one-way analysis of variance (ANOVA) revealed a significant difference SOD activity among groups ($F_{(2, 21)}$=7.14, η^2_p=0.405, p=0.004). SOD levels were significantly lower in the REM-SD group compared to the control group (p=0.003, Cohen's d=1.50) (Fig. 6E). A one-way analysis of variance (ANOVA) revealed a significant difference in TOS among the groups ($F_{(2, 21)}$=4.54, η^2_p=0.302, p=0.023). *Post hoc* comparisons (Tukey's HSD) showed that the REM-SD group had significantly higher TOS levels compared to the control group (p=0.026, Cohen's d=1.12) (Fig. 6F).

DISCUSSION

The present study examined the effects of exposure to the REM sleep deprivation (REM-SD) paradigm during the acquisition phase of learning on spatial memory performance and neurobiological markers in male adolescent rats. REM sleep deprivation did not completely impair acquisition performance, as all groups exhibited comparable improvements in escape latency, distance traveled, and swimming velocity across training days. These findings indicate that REM-SD did not fully block the ability to learn the platform location. Our acquisition trial data showed that REM-SD particularly affected performance on the final acquisition day (Day 4), during which REM-SD rats exhibited significantly higher escape latencies compared to controls. This finding is consistent with those reported by Yang et al. (2019) who reported that sleep deprivation impairs spatial learning during the later stages of MWM acquisition training. Importantly, REM-SD did not completely prevent acquisition learning, as animals improved across training days; however, the increased escape latency observed on Day 4 suggests altered learning efficiency during the late acquisition phase. These changes may be associated with the impaired memory retention observed during the probe trial. Previous studies have similarly reported that REM sleep deprivation paradigms may differentially affect acquisition and retention processes depending on deprivation duration and timing. For instance, Walsh et al. (2011) reported that short-term REM sleep deprivation following training did not significantly impair MWM acquisition performance. Our findings indicated that the REM-SD group demonstrated impaired memory retention during the probe trial when compared to the control group. In support of this, Soto-Rodríguez et al. (2016) reported that a single 72-hour REM-SD episode triggers deleterious effects that persist for at least 21 days, characterized by impaired spatial memory (Barnes maze) and a sustained increase in hippocampal apoptosis in mice. Together, these findings suggest that REM sleep deprivation during development may have persistent effects on memory-related processes across different behavioral paradigms. The significant group × day interaction observed in the latency data suggests that the observed differences are unlikely to be fully explained by detectable locomotor alterations and may instead be related to changes in learning performance across training days.

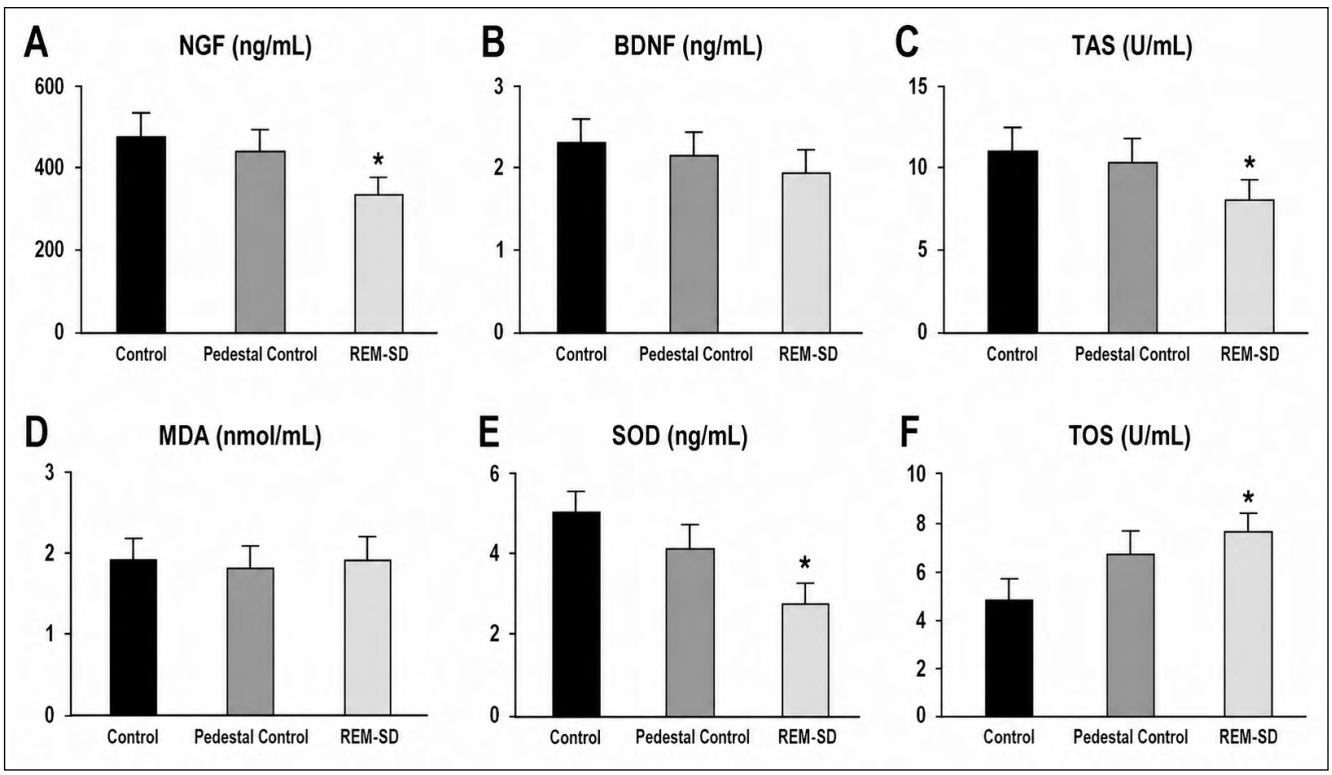

Fig. 6. Alterations in hippocampal oxidative stress markers and neurotrophic factors. (A) Nerve Growth Factor (NGF). *p<0.05 *vs.* Control (C). (B) Brain-Derived Neurotrophic Factor (BDNF). No statistically significant differences were observed (p>0.05). (C) Total Antioxidant Status (TAS). *p<0.05 *vs.* Control (C) group. (D) Malondialdehyde (MDA). No statistically significant differences were observed (p>0.05). (E) Superoxide Dismutase (SOD). *p<0.05 *vs.* Control (C) group. (F) Total Oxidant Status (TOS). *p<0.05 *vs.* Control (C). Data are presented as mean ± SD.

We observed no substantial differences in locomotor-related parameters across both the OFT and the EPM, including total distance traveled, velocity, and total arm entries. These findings suggest that overall locomotor alterations were unlikely to account for the observed impairments in MWM performance, although subtle motor or motivational influences cannot be completely excluded. The OFT revealed that the subjects exhibited a marked reduction in time spent in the central area and a concomitant decrease in center entries. These changes suggest increased anxiety-like behavior following REM-SD. The EPM results demonstrated an augmented time spent in the open arms in the REM-SD group, which may suggest an alteration in anxiety-related exploratory behavior.

From a translational perspective, the increased exploration of the open arms observed in REM sleep-deprived rats may be relevant to the growing clinical evidence linking insufficient sleep with greater risk-taking propensity. Although the elevated plus maze is primarily used to assess anxiety-like behavior, increased open-arm exploration may also reflect reduced avoidance of potentially threatening environments and a greater tendency to approach risky or aversive contexts, rather than anxiolysis alone. This interpretation is particularly noteworthy in light of the systematic review and meta-analysis by Short and Weber (2018), which included 26 studies comprising 579,380 adolescents and demonstrated that insufficient sleep was associated with 1.43-fold greater odds of risk-taking. Moreover, Gulia et al. (2015) reported increased open-arm exploration and greater mobility in the EPM in weanling rats born to sleep-deprived dams, interpreting these changes as indicative of reduced risk assessment and increased risk-taking behavior. Similarly, Cortese et al. (2010) reported increased open-arm exploration following sleep deprivation in rats, suggesting reduced fearfulness and increased risk-taking behavior. Thus, the increased open-arm time observed in the present study may not necessarily reflect reduced anxiety alone, but may also indicate altered risk assessment or behavioral disinhibition. The similar EPM pattern observed in the pedestal control group further suggests that procedural or stress-related factors associated with the MMPM may have contributed to this response. Furthermore, Suchecki et al. (2002) discovered that paradoxical sleep (PS) deprivation could result in a reduction of anxiety-like behavior in the plus maze. As indicated by

the existing body of literature, there is a prevalence of inconsistencies in anxiety-related behavioral assays. These inconsistencies underscore the notion that distinct tests may be more appropriate for the assessment of specific aspects of emotional behavior (Cevik et al., 2025). Furthermore, experimentally induced REM sleep deprivation in rodents does not fully reproduce the complexity of adolescent sleep disturbances in humans. Therefore, these findings should be considered as providing mechanistic insight rather than direct evidence of equivalent effects in adolescents.

The neuroendocrine findings provide evidence to support the hypothesis that exposure to the REM-SD paradigm is associated with elevated corticosterone levels in the REM-SD group. An increase in corticosterone may be indicative of an activation of stress-related physiological responses, which have been demonstrated to be sensitive to both sleep disruption and procedural stressors such as immobilization and isolation. An increase in corticosterone may be indicative of an activation of stress-related physiological responses, which have been demonstrated to be sensitive to sleep disturbances (Haack et al., 2007; Tufik et al., 2009). In the present study, elevated corticosterone levels were observed alongside reduced hippocampal NGF levels and impaired memory performance. An important consideration is the potential contribution of nonspecific stress associated with the MMPM procedure. Although the pedestal control group did not differ from controls in the OFT or MWM, both the PC and REM-SD groups showed increased open-arm and reduced closed-arm time in the EPM, suggesting that procedural factors associated with the paradigm may have contributed to this behavioral response. In contrast, corticosterone levels were significantly higher in REM-SD rats than in both control and PC rats, indicating a more pronounced HPA-axis response under the REM-SD condition. Thus, although the overall findings support an effect of REM-SD, the contribution of procedure-related stress, particularly to the EPM findings, cannot be excluded completely.

At the molecular level, exposure to the REM-SD paradigm was associated with a significant oxidative imbalance in the hippocampus, as indicated by increased TOS and decreased TAS and SOD activity. Qualitatively similar oxidative changes have been reported following various sleep deprivation paradigms, including studies showing that sleep loss can enhance reactive oxygen species production (Villafuerte et al., 2015). In the present study, while significant alterations were observed in TAS, TOS, and SOD levels, MDA levels did not differ significantly among groups. Furthermore, our results indicate that exposure to the REM-SD paradigm is associated with alterations in hippocampal neurotrophic markers, particularly NGF levels. Previous studies have shown that stress-induced HPA axis activation can decrease NGF levels *via* mineralocorticoid and glucocorticoid receptor signaling (Smith et al., 1995). In the present study, the most pronounced reduction in NGF levels was observed in the REM-SD group compared with the PC group. Sleep deprivation paradigms have previously been linked to reductions in neurotrophins expression, which may impair neuronal resilience and plasticity (Meerlo et al., 2009). The observed dissociation between NGF and BDNF levels—with only NGF showing a significant reduction—suggests that the present paradigm may differentially affect neurotrophic factors in the hippocampus. However, the functional implications of this dissociation remain unclear and require further investigation in paradigms that can isolate REM-specific effects.

LIMITATIONS

The MMPM, while widely used to induce REM sleep deprivation, may simultaneously trigger multiple physiological and stress-related responses beyond selective REM sleep loss itself. Although the MMPM predominantly suppresses REM sleep, it is not completely selective for this sleep stage. Electrophysiological studies have demonstrated marked REM sleep suppression and a pronounced REM rebound following deprivation, while alterations in slow-wave sleep may also occur (Suchecki et al., 2000). Therefore, in the absence of EEG/EMG monitoring in the present study, the observed effects cannot be attributed exclusively to REM sleep loss, and a contribution of alterations in other sleep stages cannot be excluded. The study was conducted exclusively in male adolescent rats, limiting the generalizability of the findings across sexes and developmental stages. Future studies should explore sex differences and the involvement of additional molecular pathways, including synaptic proteins.

CONCLUSIONS

The present findings indicate that exposure to the REM-SD paradigm during the acquisition phase was associated with behavioral and molecular alterations related to memory performance. By applying the REM-SD procedure during the learning period, the present study examined the effects of sleep disruption in conjunction with the associated procedural and physiological factors of the modified multiple platform method during memory acquisition. This design also minimized the potential confounding influence of REM rebound.

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