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Sleep deprivation reduces the recovery of muscle injury induced by high-intensity exercise in a mouse model

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ABSTRACT

Sleep is crucial to improve athlete performance and their circadian rhythm, but sleep patterns may be disturbed because athletes participate in several competitions. In addition, intensive training programs can cause muscle pain and psychological stress in athletes, resulting in a lack of sleep. Sleep also plays a critical role in the recovery of muscle injury induced by exercise. The current study evaluated the effect of sleep deprivation on the recovery of muscle injury induced by high-intensity exercise in a mouse model. In this study, 28 mice were randomly assigned to four groups ($N = 7$): control (Control), exercise (EX), sleep deprivation (SD), and sleep deprivation with exercise (EX + SD). The mice from the EX and EX + SD groups were subjected to high-intensity swimming. The results showed that 72-h sleep deprivation increased food intake and reduced body weight. However, the manipulation of 8-week exercise and/or 72-h sleep deprivation did not have any effect in the elevated plus maze task and tail suspension test. Interestingly, the EX + SD group exhibited improved memory performance in the Morris water maze and impaired motor activity in the open field test. According to the TNF-α level and aspartate aminotransferase (AST), and creatine phosphokinase (CK) activities, only the EX + SD group exhibited muscle impairment. Overall, high-intensity exercise may cause muscle injury, and adequate sleep can recover muscle damage. However, sleep deprivation reduces protein synthesis, which decreases the ability to restore muscle damage and aggravates the harmful effect of high-intensity exercise.

1. Introduction

The social and physical environment of athletes often causes the lack of restorative sleep [1]. The quality of an athlete's sleep may be altered due to different factors, such as a congested competition calendar, low sleep priority in relation to other training demands, and lack of knowledge about the role of sleep in optimizing athletic performance [2,3]. Although sleep is essential to athletes' performance, most of them suffer from sleep insufficiency [4]. Because of several competitions, the circadian rhythm (e.g., due to jet lag during international competitions) and sleep patterns (e.g., due to sleeping in a hotel and numbers of athletes allotted per room) of athletes are disturbed [4]. Moreover, intensive training programs result in muscle pain and psychological stress, which makes it more difficult for the athletes to have sufficient sleep [4]. Poor sleep quality can lead to fatigue and mood changes including fatigue, anxiety, and depression [5,6].

Sleep is vital to overall health and well-being. Sleep deprivation causes many aspects of damage in both animals and humans, including damage to cognitive [7], immunological [8], metabolic [9,10] and hormonal [10–13] functions. A reduction in sleep quality and quantity may result in an imbalance in the autonomic nervous system function, leading to an increase in overtraining syndrome and inflammatory markers and ultimately to immune system dysfunction [14].

After certain types of muscle injury induced by exercise or injury, sleep plays an important role in the process of muscle recovery. Muscles have high plasticity and can recover from several types of injuries [15].

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Thus, we hypothesize that high-intensity exercise can impair muscle physiology, and adequate sleep can restore muscle damage caused by exercise. However, sleep deprivation leads to reduced protein synthesis, which reduces the ability to restore muscle damage. Muscle recovery would be affected because it is regulated by anabolic and catabolic hormones that are strongly affected by sleep.

2. Materials and methods

2.1. Animals

C57BL/6J mice (aged 6 weeks) were purchased from the National Laboratory Animal Center (Taipei, Taiwan), given a standard laboratory diet and distilled water *ad libitum*, and kept on a 12-h light/dark cycle at 20 °C ± 1 °C with 40%–70% relative humidity in the Taipei Medical University Laboratory Animal Center. All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Taipei Medical University (LAC-2017-0377). The mice were housed in the animal facility for 2 weeks to adapt to the environment before study initiation. In total, 28 mice were randomly assigned to four groups ($N = 7$): control (Control), exercise (EX), sleep deprivation (SD), and sleep deprivation with exercise (EX + SD). The mice from the EX and EX + SD groups were subjected to high-intensity swimming. At the end of the experiment, each mouse was anesthetized, and the epididymal adipose tissue, perirenal adipose tissue, and muscle were weighted and stored at −80 °C.

2.2. Exercise protocol

Swimming exercise was conducted according to the method of Wasinski et al. [16] with slight modifications. The mice were individually placed in a swimming pool (47 cm × 34 cm × 26.5 cm) with a water depth of 20 cm and a water temperature of 35 °C ± 1 °C for 30 min/day and 5 days/week for 8 weeks. The protocol was swimming with a load equal to 4% of body weight gradually, which was added to the mouse's tail. The water tank was connected to an air pump system to abolish floating behavior by air bubbles [17]. To account for the stress associated with the water environment, the non-swimming mice were placed on a flat open surface in the water tank for the same length of time as the swimming mice [18]. All mice from the EX and EX + SD groups were towel-dried after the swimming exercise program and kept warm using an infrared heater.

2.3. Sleep deprivation

Animals were sleep deprived by placing them on a grid suspended over water according to the method described by Shinomiya et al. [19]. The animals were placed on a grid floor, which was made of stainless steel rods (31 cm long × 15.5 cm wide × 7 cm high), within a plastic cage (41 cm long × 21 cm wide × 20 cm high) filled with water to 1 cm below the grid surface for 72 h.

2.4. Spatial acquisition and retention

The mice were trained over 5 days, with four trials per day. The Morris water maze tank is a 122-cm-diameter tank with a circular platform (10-cm diameter), and it is hidden 1 cm below the surface of water, which is made opaque with milk. The water temperature was maintained at 22 °C ± 2 °C. The platform was located in the southwest quadrant of the water maze. The starting points were changed for every trial. Each trial lasted until the mouse had found the platform or for a maximum of 1 min. All mice were allowed to rest on the platform for 15 s at the end of each trial. In the training period, the latency to the platform of each trail was recorded manually, averaged in blocks of four and plotted as block means. After 72-h sleep deprivation (after 5 days of training), the platform was removed, and the mice were given probe trials to test retention of the task. The time spent in each quadrant was recorded using a video tracking system (ActualTrack™).

2.5. Tail suspension test

After 72-h sleep deprivation, the mice were individually suspended 35 cm above the surface of an acrylic box using an adhesive tape placed approximately 1 cm from the tip of the tail for 6 min. The immobility time (when they hung passively and were completely motionless) was measured during the 6 min of the tail suspension test (TST).

2.6. Open field test

After 72-h sleep deprivation, the mice were placed individually in an acrylic box measuring 50-cm long × 50-cm wide × 30-cm high for 5 min. Total travel distance during the 5 min was measured, and automated quantitative analysis was performed using ActualTrack™ for data acquisition. The apparatus was cleaned with 75% ethanol and dried between tests to remove any potential animal cues.

2.7. Elevated plus maze

The elevated plus maze (EPM) for the mice consists of two opposing open arms (35 cm × 5 cm) that are intersected (center platform) by two opposing closed arms (35 cm × 5 cm) with high walls (20 cm). The plus maze apparatus is elevated 40 cm above the floor. This procedure was performed after 72-h sleep deprivation. The animals were placed individually at the center of the EPM with their heads facing an open arm. During the 5-min test session, the number of entries into the open arm and the time spent in open arm of the maze were measured, and automated quantitative analysis was performed by ActualTrack™ for data acquisition. Percentage of time spent in the open arms (open/open + closed) was measured in the EPM over the 5-min test. At the end of the EPM, fecal boli and urine were removed from the apparatus, and the apparatus was cleaned with 75% ethanol to start the next session.

2.8. Measurement of TNF-α level in muscle homogenates

The soleus muscle was extracted as previously described by Dyke et al. [20] with slight modifications, and the total protein concentration of the muscle homogenates was determined colorimetrically using a commercial protein reagent kit Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). The TNF-α levels in the muscle homogenates were measured using a monoclonal antibody-based ELISA kit (Mouse TNF-α DuoSet ELISA; R&D, Minneapolis, MN, USA), as specified by the manufacturer. ELISA assay data (cytokine protein, pg) were normalized to total protein (g).

2.9. Blood biochemical assessments

At the end of the experiments, blood was withdrawn through cardiac puncture. Plasma was anticoagulated with EDTA and collected through centrifugation, and the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, lactate dehydrogenase (LDH), and creatine kinase (CK) were evaluated using a Roche Modular P800 (Roche Diagnostics, Indianapolis, IN, USA).

2.10. Statistical analysis

All results are expressed as mean ± S.E.M. ($N = 7$). The significance of the difference was examined using GraphPad Prism software package version 6.0 (GraphPad Software; San Diego, CA, USA), and results with $P < 0.05$ were considered statistically significant.

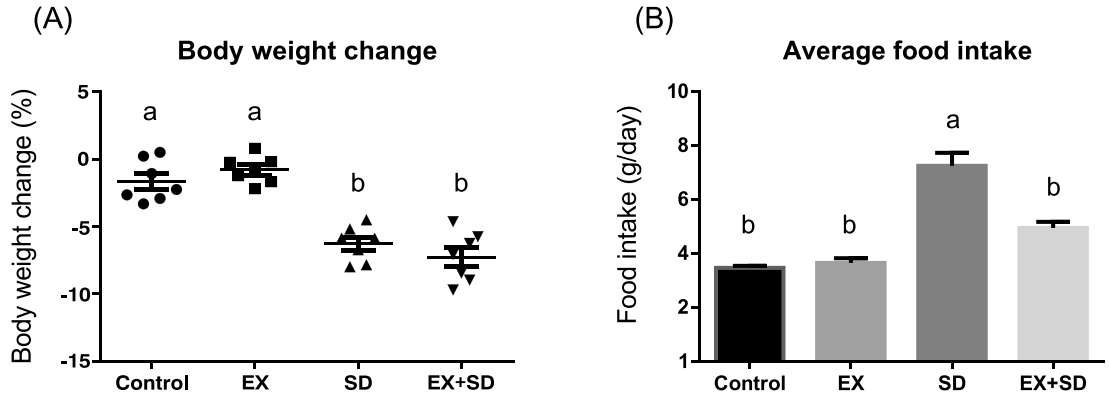

Fig. 1. Effect of 72-h sleep deprivation and high-intensity exercise on body weight and food intake. (A) Body weight change after 72-h sleep deprivation; (B) Average food intake during 72-h sleep deprivation. Control: control; EX: exercise; SD: sleep deprivation; EX + SD: exercise + sleep deprivation. Values represent means ± S.E.M. (N = 6–7). Values marked with different letters (a, b) are significantly different from each other ($P < 0.05$). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.

3. Results

3.1. Effects of exercise and sleep deprivation on the body weight and food intake

One-way ANOVA revealed the significant group effect of 72-h sleep deprivation on average body weight ($F(3, 24) = 34.6$, $P < 0.001$) and food intake ($P < 0.001$) (Fig. 1). A significant decrement in body weight was observed in the SD and EX + SD groups compared with the control and EX groups ($P < 0.001$), but no significant difference was observed between the SD and EX + SD groups ($P > 0.05$). A significant increment in food intake was observed in the SD group compared with the control, EX, and EX + SD groups ($P < 0.001$). Together, the SD group exhibited both reduced body weight and increased food intake. The aforementioned data support that sleep deprivation is a valid stress manipulation in the present study.

3.2. Effects of exercise and sleep deprivation on the behavioral tests

3.2.1. Morris water maze

Two-way mixed-design ANOVA with group as the between-subject factor and training days as the within-subject factor was used to verify the effect of exercise and sleep deprivation on spatial learning. A significant main effect of day ($F(4, 115) = 7.14$, $P < 0.001$) was observed, and no further main effect or interaction was found ($P > 0.05$). The post hoc comparison revealed a decreased latency to find the platform between day 1 and days 3, 4, and 5 ($P < 0.05$) (Fig. 2A). However, only the EX + SD group showed better memory retrieval on the test day than the control group, which was confirmed by one-way ANOVA ($F(3, 23) = 3.45$, $P < 0.05$) and subsequent post hoc comparisons ($P < 0.05$) (Fig. 2B). Taken together, all the four groups could acquire the skills for the Morris water maze within 5 training days, but only the EX + SD group memorized it better than the control group.

3.2.2. EPM and TST

To test the effect of sleep deprivation and exercise on emotions, the EPM and TST were used. However, one-way ANOVA revealed no effect of emotional fluctuations on the percentage of time spent in the open arm ($P > 0.05$) or number of entries into the open arm of the EPM ($P > 0.05$) (Fig. 2C and D). In addition, no significant difference in the immobility time was observed between the four groups in the TST ($P > 0.05$) (Fig. 2E).

3.2.3. Open field test

One-way ANOVA revealed that exercise and sleep deprivation had a significant group effect on motor performance ($F(3, 20) = 6.89$, $P < 0.05$) (Fig. 3A). Post hoc comparison confirmed that the EX + SD group traveled lesser in the test chamber than the control and SD groups ($P < 0.05$), but not the EX group ($P > 0.05$). The movement traces of each group inside the test chamber supported these results (Fig. 3B). To verify the underlying mechanism for the reduced motor performance of the EX + SD group, the muscle TNF-α level in the four groups was examined. The muscle TNF-α level was higher in the EX + SD group than in the other three groups, as detected by one-way ANOVA ($F(3, 20) = 16.45$, $P < 0.001$) and post comparisons ($P < 0.001$) (Fig. 3C). Overall, the results showed that the combination of high-intensity exercise and sleep deprivation may have caused muscle inflammation; therefore, the EX + SD group traveled less than the other groups in the open field test (OFT).

3.3. Effects of exercise and sleep deprivation on plasma biochemistry

One-way ANOVA was used to examine the effect of exercise and sleep deprivation on AST, ALT, albumin, LDH, and creatine phosphokinase (CK), and a significant group effect was observed on AST ($F(3, 25) = 18.74$, $P < 0.001$) and CK ($F(3, 25) = 8.16$, $P < 0.001$) (Table 1). However, no significant difference in ALT, albumin, and LDH was observed between the four groups ($P > 0.05$). Moreover, no liver damage was found after the exercise and sleep deprivation, as determined by the percentage of liver weight and H&E staining of the liver (data not shown). In addition, when muscles are damaged, such as in response to exercise, AST, ALT, and CK are released from the muscle and their concentration in the blood increases [21]. Overall, the results showed that the combination of exercise and sleep deprivation may cause muscle inflammation. In general, the body's growth hormone is secreted during sleep, which stimulates muscle protein synthesis to repair damaged muscles.

3.4. Effects of exercise and sleep deprivation on epididymal fat and perirenal fat

One-way ANOVA revealed the significant group effect of 72-h sleep deprivation on the weights of epididymal fat ($F(3, 24) = 6.66$, $P < 0.001$) and perirenal fat ($F(3, 24) = 4.43$, $P < 0.001$) (Fig. 4). A significant decrement in epididymal fat was observed in the EX + SD group compared with the control, SD, and EX groups ($P < 0.001$), but not between the control, SD, and EX groups (Fig. 4A). A significant decrement in perirenal fat was observed in the EX + SD group compared with the control group ($P < 0.001$), but not between the EX + SD, SD, and EX groups (Fig. 4B). Together, the EX + SD group exhibited reduced adipose tissue, which supported that both sleep deprivation and excessive exercise cause inflammation of white adipose tissue which

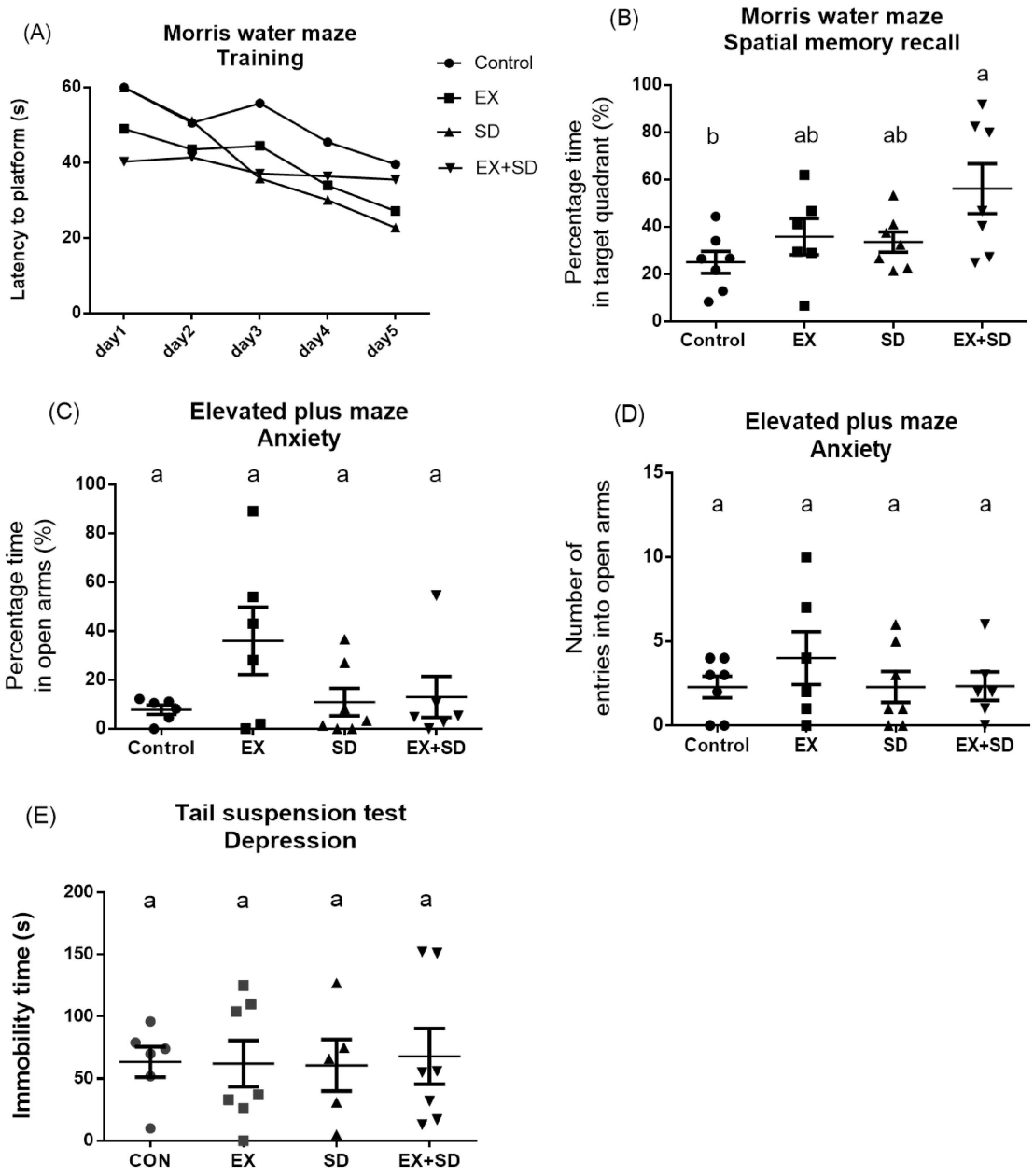

Fig. 2. Effect of 72-h sleep deprivation and high-intensity exercise on spatial learning and memory, anxiety, and depression. (A) The latency to reach the platform for each group during training days; (B) Spatial memory recall in the Morris water maze; (C) Percentage of time spent in open arms (%) of the elevated plus maze; (D) Number of entries into the open arms of the elevated plus maze; (E) Immobility time in the tail suspension test. Control: control; EX: exercise; SD: sleep deprivation; EX + SD: exercise + sleep deprivation. Values represent means ± S.E.M. (N = 6–7). Values marked with different letters (a, b) are significantly different from each other ($P < 0.05$). Data were analyzed using ANOVA followed by Tukey's multiple comparison test.

increases the secretion of the cytokine interleukin-6 (IL-6) to promote lipolysis [22], which leads to a decrease in white fat weight.

4. Discussion

The present study originally hypothesizes that 8-week exercise may alleviate the detrimental effect induced by 72-h sleep deprivation. First, 72-h sleep deprivation was an effective stress manipulation, which was supported by the increased food intake and reduced body weight in the SD group. The improvement in memory performance was only observed in the EX + SD group, and this group showed impaired motor activity in the later test. The earliest technique for sleep deprivation was proposed by Jourvet in 1964 for cats, and they took advantage of atonia, which occurs during paradoxical sleep, and animals were placed on top of a small platform surrounded by water. To avoid potential confounding factors in this method, many modifications, such as the size of the platform and nonisolated environment, were made to the task [23,24]. The grid-over-water method used in this study was a modification of the flower pot used in the original test, and the method has been proven to be a mild and effective method for sleep deprivation [19,25]. When animals were placed on the grid, the sleep latency and amount of wakefulness were significantly increased, and the amounts of non-rapid eye movement (non-REM) sleep and rapid eye movement (REM) sleep were significantly decreased [19]. Sleep loss results in deterioration of many basic functions and induces physiological changes similar to those seen after stress [26]. Rats that were sleep deprived by placing on a platform for 4 days demonstrated a loss of body weight and increase in food intake [12,27]. The present study results for the SD group are

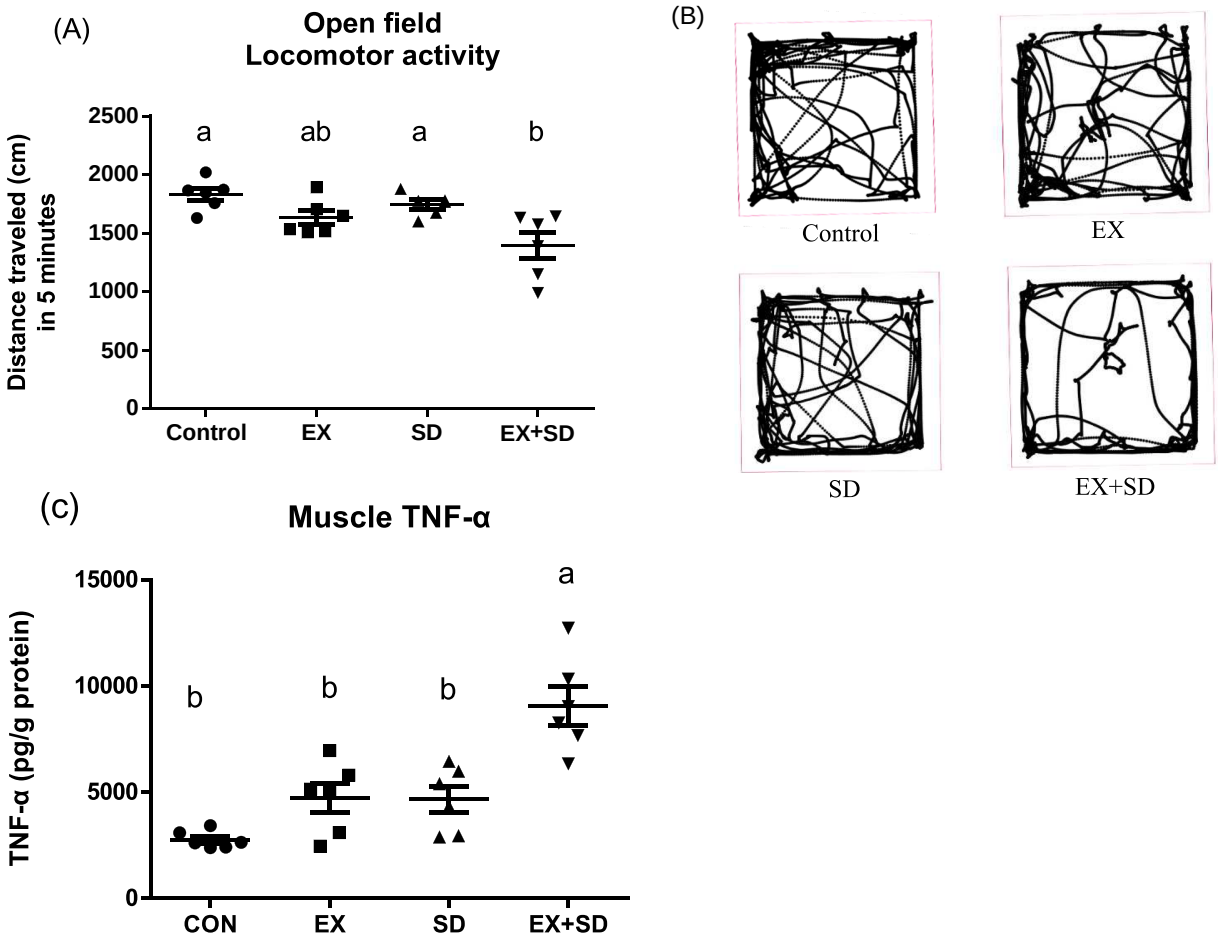

Fig. 3. Effect of 72-h sleep deprivation and high-intensity exercise on locomotor activity. (A) Locomotor activity in the open field test (in 5 min); (B) Representative movement trace of each group in the open field test; (C) The concentration of TNF-α in muscle tissue (pg/g protein). Control: control; EX: exercise; SD: sleep deprivation; EX + SD: exercise + sleep deprivation. TNF-α: tumor necrosis factor-alpha. Values represent means ± S.E.M. (N = 6–7). Values marked with different letters (a, b) are significantly different from each other ($P < 0.05$). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.

consistent with previous findings and support that the grid-over-water is a valid method for causing sleep disturbances, and this manipulation also activates stress responses [28].

Exercise is known to have beneficial effects in decreasing the risk of age-related neurodegenerative disorders, facilitating functional recovery from brain injury, and increasing resistance against stress-related psychiatric disorders [29–31]. In this study, the swimming exercise for 8 weeks was expected to alleviate the consequences of sleep deprivation, but no behavioral effect was observed in all the behavioral tests. However, 8-week swimming prior to 72-h sleep deprivation did enhance memory retrieval in the Morris water maze task and reduced motor performance in the OFT. The function of sleep remains elusive, and adequate sleep is important for learning and memory consolidation [32]. In fear conditioning, training rats to associate the tone-shock pairing during their awake phase did reduce their freezing behaviors when re-exposed to the conditioning context 12 h later, and this impairment can be attributed to the lack of sleep [33]. Sleep deprivation for 24 h after the acquisition did not enhance the animals' performance in the radial water maze [34]. Our results are consistent with previous findings that 72-h sleep deprivation in the SD group did not enhance memory retrieval on the test day. However, the EX + SD group did exhibit higher memory in the retrieval test, and this enhancement can be attributed to enhanced stress-facilitated memory consolidation [35].

Sleep problems are often paired with anxiety and chronic sleep

Table 1
Effect of 72-h sleep deprivation and high-intensity exercise on plasma biochemistry.

Parameter	Control	EX	SD	EX + SD
AST (IU/L)	64.0 ± 6.0^{b}	78.6 ± 8.4^{b}	70.4 ± 7.2^{b}	147.3 ± 11.6^{a}
ALT (IU/L)	26.4 ± 1.2^{a}	34.9 ± 6.3^{a}	30.8 ± 1.4^{a}	36.9 ± 2.9^{a}
Albumin (g/dL)	3.8 ± 0.1^{a}	4.0 ± 0.1^{a}	3.7 ± 0.1^{a}	3.9 ± 0.1^{a}
LDH (IU/L)	264.8 ± 34.8^{a}	350.7 ± 108.5^{a}	245.8 ± 25.7^{a}	322.9 ± 25.4^{a}
CK (IU/L)	173.2 ± 41.55^{b}	203.0 ± 40.9^{b}	221.3 ± 53.6^{b}	487.8 ± 55.6^{a}

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CK, creatine phosphokinase.
Control: control; EX: exercise; SD: sleep deprivation; EX + SD: exercise + sleep deprivation.
Values represent means ± S.E.M. ($N = 6–7$). Values marked with different letters (a, b) are significantly different from each other ($P < 0.05$). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.

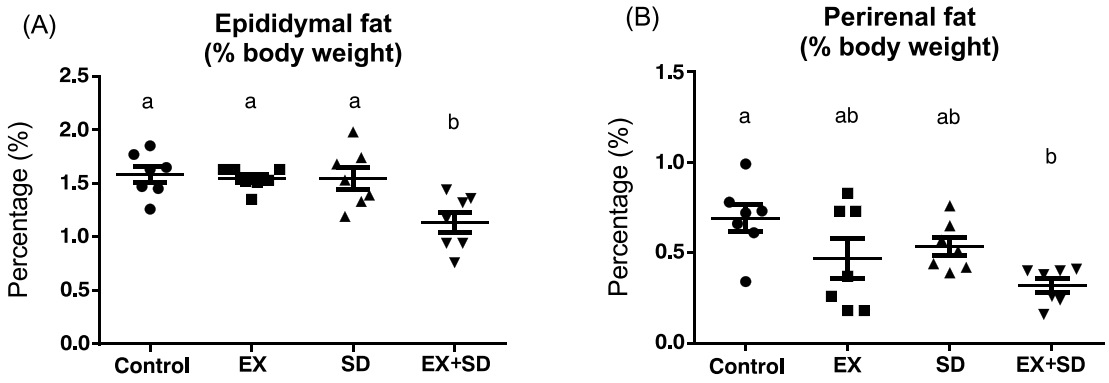

Fig. 4. Effect of 72-h sleep deprivation and high-intensity exercise on adipose tissue/body weight. (A) Epididymal fat/body weight; (B) Perirenal fat/body weight. Control: control; EX: exercise; SD: sleep deprivation; EX + SD: exercise + sleep deprivation. Values represent means ± S.E.M. (N = 6–7). Values marked with different letters (a, b) are significantly different from each other ($P < 0.05$). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.

disturbances and can lead to pathological anxiety in humans [36]. Mice were placed on multiple platforms above water for 24 h that did increase anxiety behaviors in the OFT, and the groups subjected to exercise for 4 weeks before the test ameliorated this emotional expression, whereas no effect was observed in the exercise-alone group did [37]. For the EPM, sleep deprivation for 72 h by placement on multiple platforms reduced the percentage of time lingering in the open arms but did not change the number of entries into the open arms [38]. Kumar and Singh [39] showed that mice sleep deprived for 72 h using the grid-over-water method exhibited significant anxiety-like behavior, weight loss, impaired locomotor activity, and oxidative damage compared with naïve mice. However, in this study, the animal anxiety models did not show consistent findings in terms of the effect of sleep deprivation and anxiety expressions. In the present study, the manipulation of exercise and/or sleep deprivation for 72 h did not have anxiogenic or anxiolytic effects in the EPM. Together, these discrepancies most likely resulted from variations in the mouse strain.

Disturbances in the sleep–wake rhythm can induce heterogeneous effects in animals [40]. For the motor activities in the OFT, sleep deprivation increased grooming, reduced rearing, and did not affect locomotion after 96-h sleep deprivation [41]. According to our results, the SD group traveled comparable distances to the control and EX groups, which is consistent with previous findings. The EX + SD group demonstrated traveled lesser distances in the same task, and this may be a result of anxiety or impaired locomotion. With the intact emotional changes demonstrated by all the four groups in the EPM, the potential physiological impairment requires further examination. Overtraining syndrome is seen in athletes when they become progressively fatigued and fail to recover from intensive training, and they may suffer from minor infections [42]. Chronic or acute sleep loss is directly correlated to athletic injuries, and the role of sleep in the regeneration of damaged muscle tissue has been proven [43]. Therefore, the TNF-α level was examined in this study, and our results confirmed muscle impairment only in the EX + SD group. When muscle damage has occurred or is occurring, muscle cells release CK into the blood. Thus, CK is known to be an accurate indicator of muscle damage in clinical practice [44]. In addition, AST and ALT are markers of muscle damage after strenuous exercise [21]. Thus, in this study, the results for AST and CK activities confirmed muscle impairment only in the EX + SD group. Muscle recovery in the EX + SD group may have been compromised by sleep deprivation, which strongly influences anabolic and catabolic hormones [15]. Therefore, sleep deprivation may reduce the secretion of growth hormones, thereby reducing the ability of repairing the damaged muscles after strenuous exercise training.

In conclusion, 8-week exercise and 72-h sleep deprivation do not induce anxiety-like behavior. Interestingly, they can improve memory performance and impair motor activity. In addition, 8-week high-intensity exercise and sleep deprivation lead to muscle impairment. Overall, high-intensity exercise may cause muscle injury, and adequate sleep can recover muscle damage. Taken together, 8-week swimming exercise with a load equal to 4% of their body weight can be intensive training for mice, and the sleep deprivation manipulation can be an add-on risk factor for their motor performance.

Declaration of competing interest

None of the authors has any financial or personal relationships that could inappropriately influence or act as a bias for the content of this paper.

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