The effects of moderate exercise and overtraining on learning and memory, hippocampal inflammatory cytokine levels, and brain oxidative stress markers in rats

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Abstract

To investigate the exercise intensity effects on rats' memory and learning, animals were divided into control, moderate training (MT), and overtraining (OT) groups. At training last week, learning and memory was assessed using Morris water maze (MWM) and passive avoidance (PA) tests. Finally, the rat's brains were removed for evaluating oxidative stress and inflammatory cytokines. Overtraining impaired animal's performance in MWM and PA tests. In MT group, hippocampal levels of interleukin 1 beta (IL-1β) and malondialdehyde (MDA) increased, and thiol contents in hippocampal and cortical tissues decreased compared to control. In OT group, tumor necrosis factor α, IL-1β, and C-reactive protein hippocampal levels increased, MDA and nitric oxide metabolite in hippocampal and cortical tissues increased, thiol contents, catalase and superoxide dismutase activity in hippocampal and cortical tissues decreased compared to control and MT groups. Overtraining might lead to learning and memory impairment by increasing the inflammatory cytokine and oxidative stress markers.

Keywords Moderate training · Overtraining · Learning · Memory · Inflammation · Oxidative stress

Introduction

It has been well documented that there is a relationship between neuroinflammation and neurodegenerative diseases [1]. Overproduction of inflammatory cytokines such as the tumor necrosis factor- α (TNF-α), interleukin1β (IL-1β), interleukin 6 (IL-6) in the brain [2, 3] and increased levels of C-reactive protein (CRP) in the plasma have been shown to disrupt memory and increase the risk of Alzheimer's disease (AD) [4, 5]. Also, oxidative stress status which is accompanied by increased levels of reactive oxygen species (ROS) has a detrimental role in neurodegenerative diseases and impairment of learning and memory [6].

During recent years, the incidence of many chronic non-communicable diseases, including neurodegenerative disease, chronic heart disease, cancer, metabolic syndrome, and diabetes has increased due to physical inactivity and lifestyle changes [7]. Physical exercise has advantageous effects on many physiological systems, including the central nervous system. The positive effects of exercise on cognitive function, neural health and neuronal protection from various brain insults are well reported [8, 9]. Also, it has been shown that physical exercise serves as a potent non-pharmacological treatment to improve learning and memory and

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reduce the risk of AD [10, 11]. Regular physical exercise can decrease the levels of amyloid oligomer that is very important in the pathogenesis of AD [12]. In addition, moderate exercise that does not lead to exhaustion has anti-inflammatory and antioxidant roles. It can decrease inflammatory cytokines such as TNF-α, IL-1β, IL-6 and CRP [13, 14], and oxidative damage by increasing the antioxidant activities [15, 16]. However, different types of exercise may affect brain plasticity based on the nature, severity and the time lag in between exercises as well as type and age of animal [17].

According to the hormesis theory, both lack of physical stress (inactivity) and excessive stress (overtraining) might result in decreased physiological function [18]. Excessive overloading during competitive training in combination with inadequate recovery or other lifestyle stresses can lead to overreaching and possibly overtraining syndrome. Overtraining is manifested by extreme fatigue and underperformance period, which can last several months to several years [19]. In overtraining, the homeostatic balance involving a wide range of hormonal, metabolic, and immunologic factors is altered [18, 20]. Overload exercise without adequate rest (overtraining) will lead to tissue injury, especially in muscle and joints, and release of trauma-related cytokines, which activate circulating monocytes to produce inflammatory cytokines [21]. It has been demonstrated that high-intensity exercise increases the levels of TNF-α, IL-1β, IL-6 and CRP [22, 23]. Also, some studies have shown that acute exercise and overtraining lead to the production of reactive oxygen and nitrogen species and oxidative stress, while regular exercise can stimulate the antioxidant system and protect against the side effects of oxidative damage [24]. Overtraining might cause signs and symptoms of cognitive disturbance as well as immunosuppression and inflammation [25].

Some previous studies have shown the beneficial effects of exercise on memory and cognitive processes, while a few studies highlight the effect of type and the training load of exercise on memory, and the probable related mechanisms including inflammation and imbalance of the immune system. However, the effects of overtraining in athletes on brain inflammation and cognition are still under question. Therefore, we decided to investigate the effects of overtraining and moderate exercise on learning and memory function, hippocampal cytokine levels and brain tissues oxidative damage in rats.

Materials and methods

Animals

Twenty-one male Wistar rats (6–8 weeks old and weighing 150–200 g) were obtained from the animal house of Mashhad University of Medical Sciences, Mashhad, Iran. Animals were caged in standard controlled conditions (temperature (22 ± 2 °C) and 12 h light/dark cycle) and provided food and water ad libitum throughout the experiment. All experiments were performed in accordance to the standards established by the Committee on Animal Research of Mashhad University of Medical Sciences (IR.MUMS.fm.REC.1396.468). The rats were randomly divided into three groups ($n = 7$): control, moderate trained (MT), and overtrained (OT). The chemical agents which were used for the biochemical assessments were purchased from Merck Company.

Exercise protocol and animal groups

The animals experienced 1-week familiarizing (10 min/day for 5 days at speed of 12 m/min at 0% degree inclination) on a motorized treadmill with 4 individual lanes prior to the start of the experiments to eliminate the exercise-induced stress. The animals of the control group were placed on the treadmill to acclimate to the stresses of the treadmill environment. The exercising groups undertook a progressive load of training, 6 days/week, to enhance cardiorespiratory fitness which included a 5-min warm up and a 5-min cooldown in each session. The animals of the MT group ran at a speed of 15 meters/min for 30 min, 6 days/week, for 9 weeks (Table 1) [26]. On the other hand, the animals in the OT group were submitted into a 3-phase program. In phase I, the first 4 weeks, the training speed was increased from 15 to 25 m/min and the training time from 20 min to 60 min. In phase II, the second 4 weeks, the training load was kept constant. And in phase III, which constituted the last 3 weeks, the running intensity and the training duration remained unchanged, while the recovery time between the training sessions was decreased from 24 h to 4, 3 and 2 h, and the number of exercise bouts was increased from 1 to 4 in each day (Table 2) [27]. The training programs were evaluated by a performance test at different times.

Performance test

In this study, the performance test for the animals of the MT group was performed before the start of the main exercise, at the end of the 2nd, 4th and 9th weeks, while for the animals in the OT group, it was performed before the start of the

Table 1 Exercise protocol of the moderate trained (MT) animals

Week	Training velocity (m/min)	Training time (min)	Training session number (per day)	Recovery between exercise sessions (h)
0	12	10	1	24
1	12	20	1	24
2–9	15	30	1	24

Table 2 Exercise protocol of the overtrained (OT) animals

Week	Training phase	Training velocity (m/min)	Training time (min)	Training session number (per day)	Recovery between exercise sessions (h)
0	1	12	10	1	24
1	1	15	20	1	24
2	1	20	30	1	24
3	1	22.5	45	1	24
4	1	25	60	1	24
5–8	2	25	60	1	24
9	3	25	60	2	4
10	3	25	60	3	3
11	3	25	60	4	2

main exercise, at the end of the 2nd, 4th, 8th, 9th, 10th and 11th weeks. These performance tests were performed 48 h after the last training session. The animals were running on the treadmill at a 0% inclination, at an initial speed of 12 m/min. The treadmill speed was increased 1 m/min every 2 min until it reached a speed of 20 m/min. Then, the speed was further increased to 2 m/min every 3 min until exhaustion occurred, which was defined as the time at which the animals touched the shock grid 5 times/min. The body weight of the animals was recorded before each performance test. The performance test (Pr) was calculated according to the following formula:

$$Pr = \sum_i Pr = \Sigma m V_i T_i = \Sigma m D_i = mD \,(kg/m)$$

Pr represents the rat's performance expressed in kilogram-meters (kg/m); Pr_i is the rat's performance during each stage; m is the body mass; V_i is the stage velocity; T_i is the stage running time; D_i is the stage distance; and D is the total distance covered by the rat [27].

Behavioral tests

We evaluated the learning and memory by performing the Morris water maze (MWM) and the passive avoidance test (PA) in the last week of training. All behavioral tests were conducted in a noiseless room (between 10:00 and 14:00), under moderate illumination, and the rats were kept in the room for at least 1 h before testing.

MWM test

The MWM task consisted of a black circular swimming pool filled with 23–24 °C water (pool dimensions: 60 cm deep × 136 cm diameter) that was divided into four equal quadrants with boundaries labeled north (N), east (E), south (S) and west (W). A circular platform (10 cm diameter, 28 cm high) was submerged in the central area of the southeast quadrant of the MWM pool that was hidden about 2 cm below the water surface. Fixed visual cues at several locations around the maze and on walls of the room determined the navigation path. The tests were recorded with a video camera placed above the center of the pool and connected to a recording system. The animals could swim freely to find the platform during the allotted 60 s, while allowing them to remain on the platform for 15 s. The time latency and the traveled distances to reach the platform were recorded as a measure of spatial learning. If the animals were not able to find the platform after 60 s of swimming, they were directed to the platform by the examiner and were allowed to stay on it for 15 s. The experiments were repeated with four trials each day for four consecutive days. After 24 h, the hidden platform was removed, and a probe test was carried out to assess spatial memory. The time spent and the traveled path in the target quadrant (Q_4) were compared between groups [28].

PA test

The PA task consisted of a light and a dark compartment which were connected by a guillotine door. Before starting the training sessions, the animals were placed into the apparatus and allowed to move freely between the two compartments for 300 s during two consecutive days. In the following day, the rats were placed in the light compartment, but once they had entered the dark compartment the guillotine door was shut and an electrical shock (2 mA, 2 sec) was delivered to the animal's feet. The test phase was conducted at 2, 24 and 48 h after the training sessions, then the animals were again placed into the light compartment and their latency to enter the dark compartment and the time spent in each compartment per animal subject were measured [29].

Biochemical assessments

After the behavioral tests, the rats were euthanized under deep anesthesia and their hippocampal and cortical tissues were removed, and the tissues were homogenized with

phosphate buffer solution (PBS) (pH 7.4). The homogenates were then submitted for biochemical assessments and the content of total thiol (SH), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and NO metabolite (NO^{2-}) was measured in both the hippocampal and cortical tissues, while the TNF-α, IL-1β and CRP concentrations were only determined in the hippocampal tissues.

Measurement of TNF-α, IL-1β and CRP

The hippocampal levels of TNF-α, IL-1β and CRP measurement were performed with specific rat ELISA kits (TNF-α: Diaclone Co, France) (IL-1β, CRP: Zellbio Co, Germany) while adhering to the manufacturer's instructions. The measured absorbance of the samples in a microplate reader (Biotek, USA) was compared with an established standard curve in the same measurement and the concentrations were calculated.

Measurement of MDA

MDA is an indicator of lipid peroxidation and was measured based on the MDA reaction with thiobarbituric acid (TBA), which produces a pink complex with a peak absorbance at 535 nm [30].

Measurement of total thiol content

Total thiol content was measured by the method of Ellman. SH groups produce a yellow complex which has a peak absorbance at 412 nm [31].

Measurement of SOD activity

The SOD activity was measured based on a procedure described by Madesh and Balasubramanian. The procedure involves the production of superoxide through auto-oxidation of pyrogallol and the inhibition of superoxide-dependent reduction of 3- 4, 5-dimethyl-thiazol-2-yl -2, 5-diphenyl tetrazolium bromide (MTT) conversion to formazan [32].

Measurement of CAT activity

The activity of CAT was measured according to the method of Aebi, based on the rate of decomposition of hydrogen peroxide (H_2O_2) by catalase using a spectrophotometer at 240 nm [33].

Measurement of NO metabolite

NO metabolite (nitrite) was measured based on the reaction between Griess reagent, sulfanilamide and naphthylethylenediamine (NED) solutions at 520 nm [34].

Statistical analysis

The data were presented as mean ± standard error of the mean (SEM) using the SPSS 16 software. And the data from the behavioral tests and biochemical assessments were compared using a one-way ANOVA followed by an LSD post hoc comparisons test. Statistical significance was considered at $P < 0.05$.

Results

Performance test

Our results suggest that the gradual increase in the training load improved the performance in the animals of the MT group, however, in the OT group, although the performance was initially improved, in the third stage of exercise (from the 9th week), the performance significantly declined ($P < 0.001$) (Table 3).

Behavioral results

MWM test

In this section, the results of the MWM test were presented for two parameters: the time latency and the traveled distance to reach the platform during a 4-day training, and the time spent and the traveled distance in the target quadrant (Q_4) in the probe day when the platform was removed.

The results showed that during the 4 days, the time latency and the traveled distance to reach the platform in the animals of the OT group were significantly higher than the control group ($P < 0.01$–$P < 0.001$). However, there were no significant differences between these parameters in the MT group compared to the control group. Also, the time

Table 3 Performance (Pr, Kg.m) of the MT (moderate trained) and the OT (overtrained) groups in different weeks

Week	Pr	MT	OT
0	1	65.7 ± 3.9	64.7 ± 7.9
2	2	84.7 ± 7.5[**]	122.1 ± 19.7[**]
4	3	112.2 ± 6.1[***]	175.5 ± 30.13[**]
8	4	140.4 ± 10.6[***]	210 ± 32.3[**]
9	5	138.2 ± 6.5[***]	127.6 ± 30.4[+++]
10	6	–	86 ± 16.5[+++]
11	**7**	–	62.7 ± 11.4[+++]

The data are presented as mean ± standard error of the mean ($n = 7$ in each group). The data of week zero represent the baseline measurements for each group. **$P < 0.01$ and ***$P < 0.001$ comparison of the zero week and the 2nd, 4th and 8th weeks and [+++]$P < 0.001$ comparison of the 8th week and the 9th, 10th and 11th weeks

latency and the traveled distance to reach the platform of the OT group were significantly higher than the MT group ($P < 0.01$–$P < 0.001$) (Fig. 1a, b).

In the probe trial, the animals of the OT group spent less time and traveled a shorter distance in the target quadrant (Q_4) compared to the control group ($P < 0.01$). There were no significant differences between these parameters in the MT group compared to the control group. Additionally, the time spent and the traveled distance in the Q_4 were significantly lower for the OT group compared to the MT group ($P < 0.001$) (Fig. 2a, b).

PA test

In this section, the results of the PA test including the latency to enter the dark compartment, the time spent in the dark compartment and the time spent in the light compartment at 2, 24 and 48 h after receiving the electric shock are presented.

There were no significant differences in the latency to enter the dark compartment at 2, 24 and 48 h after receiving the electric shock of the animals in the MT and OT groups compared to the control group. The latency to enter the dark
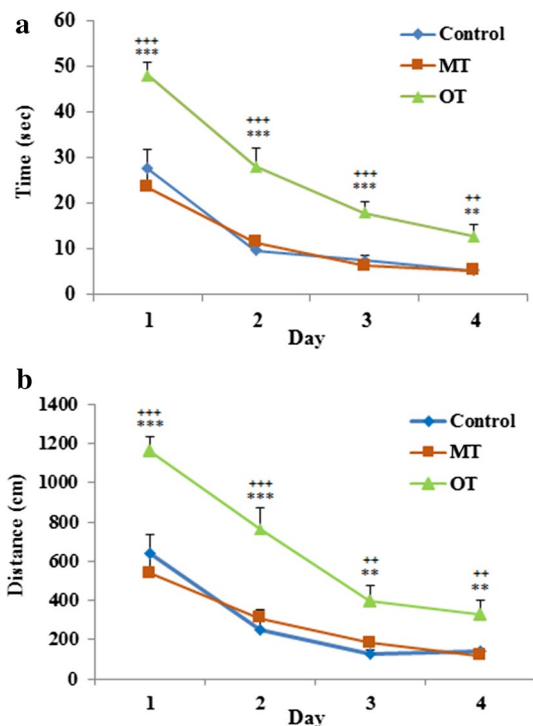
Fig. 2 Comparison of the time spent (**a**) and the traveled distance (**b**) in target quadrant between control, MT (moderate trained) and OT (overtrained) groups in probe day. The data are presented as mean ± standard error of the mean ($n = 7$ in each group). **$P < 0.01$ compared to the control group and +++$P < 0.001$ compared to the MT group

compartment at 2 and 48 h after receiving the electric shock was significantly lower in the animals in the OT group compared to the MT group ($P < 0.05$) (Fig. 3a).

The time spent in the dark compartment at 48 h after receiving the electric shock in the OT group was significantly higher than the control group ($P < 0.05$). There were no significant differences in the time spent in the dark compartment by the OT group at 2 and 24 h after receiving the electric shock and at the 2, 24 and 48 h after receiving the electric shock for the MT group compared to the control group. Also, in the OT group, the time spent in the dark compartment at 2, 24 and 48 h after receiving the electric shock was significantly higher than the MT group ($P < 0.05$–$P < 0.01$) (Fig. 3b).

The time spent in the light compartment at 48 h after receiving the electric shock of the animals in the OT group was significantly lower than the control group ($P < 0.05$). There were no significant differences between the time spent in the light compartment at 2 and 24 h after receiving the electric shock in the OT group and at 2, 24 and 48 h after receiving the electric shock in the MT group compared to the control group. Also, in the OT group, the time spent in the light compartment at 2, 24 and 48 h after receiving the electric shock was significantly lower than the MT group ($P < 0.05$–$P < 0.01$) (Fig. 3c).

Fig. 1 Comparison of the time latency (**a**) and the traveled distance (**b**) to reach the platform in Morris water maze test between the control, MT (moderate trained) and OT (overtrained) groups during the 4 days. The data are presented as mean ± standard error of the mean ($n = 7$ in each group). **$P < 0.01$ and ***$P < 0.001$ compared to the control group and ++$P < 0.01$ and +++$P < 0.001$ compared to the MT group
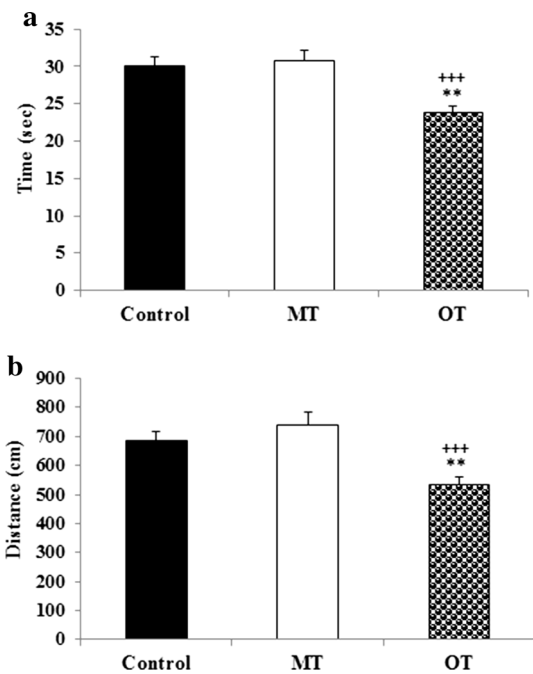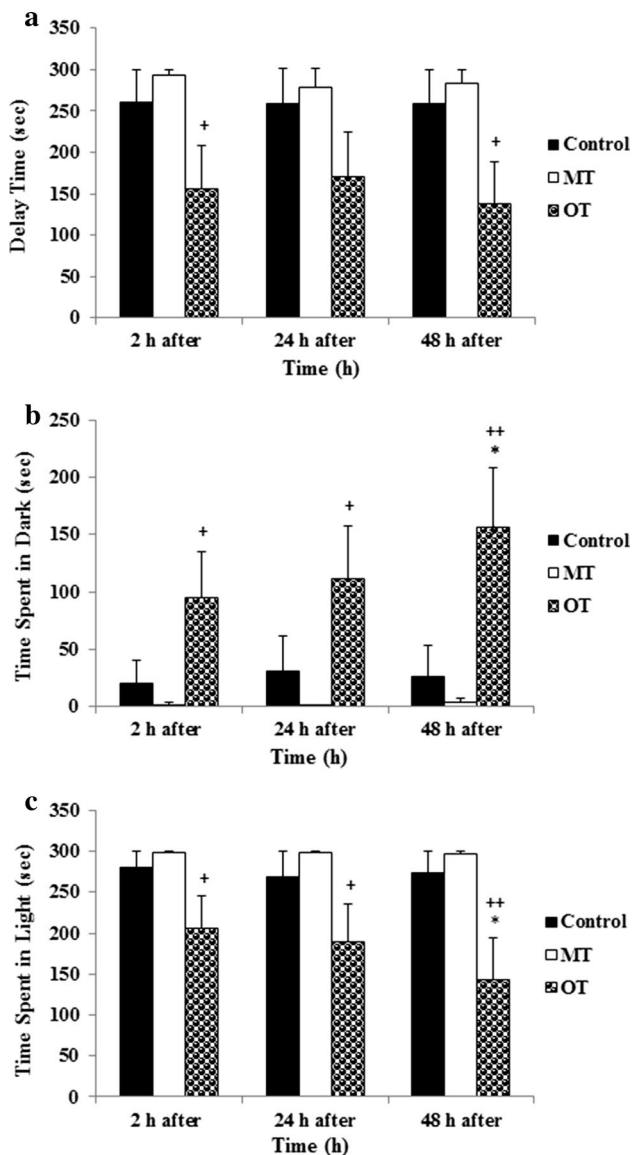
Fig. 3 Comparison of the latency to enter the dark compartment (**a**), time spent in the dark compartment (**b**) and the time spent in the light compartment (**c**) at 2, 24 and 48 h after receiving the electric shock between the control, MT (moderate trained) and OT (overtrained) groups. The data are presented as mean ± standard error of the mean ($n = 7$ in each group). *$P < 0.05$ compared to the control group and $^+P < 0.05$ and $^{++}P < 0.01$ compared to the MT group

Biochemical results

Hippocampal cytokine levels

The hippocampal levels of the TNF-α in the OT group were significantly higher than the control group ($P < 0.01$). There were no significant differences between the TNF-α levels of the MT group compared to the control group. Also, the hippocampal levels of the TNF-α in the OT group were higher than the MT group ($P < 0.01$) (Fig. 4a).
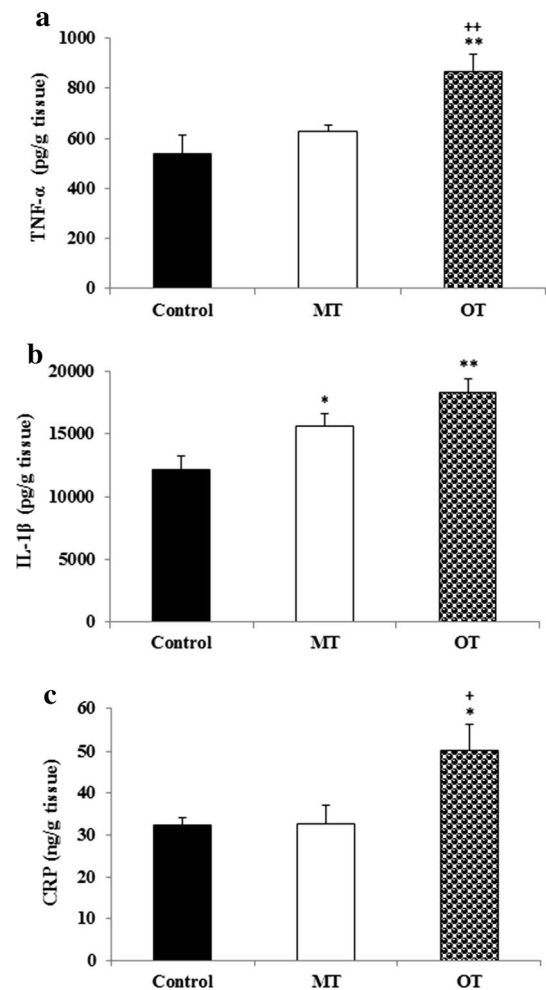
Fig. 4 Comparison of the hippocampal TNF-α (**a**), IL-1β (**b**) and CRP (**c**) levels between the control, MT (moderate trained) and OT (overtrained) groups. The data are presented as mean ± standard error of the mean ($n = 7$ in each group). *$P < 0.05$ and **$P < 0.01$ compared to the control group and $^+P < 0.05$ and $^{++}P < 0.01$ compared to the MT group

The hippocampal levels of the IL-1β in both the MT and OT groups were significantly higher than the control group ($P < 0.05$–$P < 0.01$). There were no significant differences between the IL-1β levels of the OT group compared to the MT group (Fig. 4b).

Our results showed that the hippocampal levels of the CRP in the animals in the OT group were significantly higher than the control group ($P < 0.05$). And there were no significant differences between the CRP levels of the MT group compared to the control group. Also, the hippocampal levels of the CRP in the OT group were higher than the MT group ($P < 0.05$) (Fig. 4c).

Hippocampal stress oxidative markers

The animals of the MT and OT groups had more MDA concentrations and lower thiol contents in their hippocampal tissues compared to the control group ($P < 0.001$). Additionally, in the hippocampal tissues of the OT group, MDA levels were significantly higher compared to the MT group ($P < 0.001$), however, thiol contents were lower than the MT group ($P < 0.001$) (Fig. 5a, b).

The animals in the OT group had lower CAT and SOD levels in their hippocampal tissues compared to the control group ($P < 0.001$). In the MT group, there were no significant differences between the hippocampal levels of CAT and SOD compared to the control group ($P > 0.05$). Additionally, in the hippocampal tissues of the OT group, CAT and SOD levels were significantly lower than the MT group ($P < 0.001$) (Fig. 5c, d).

Cortical stress oxidative markers

The animals in the OT group had more MDA concentrations and lower thiol contents in their cortical tissues compared to the control group ($P < 0.05$–$P < 0.001$). In the MT group, there were no significant differences between the cortical levels of MDA compared to the control group, however, the cortical thiol contents were lower than the control group ($P < 0.001$). Also, the animals of the OT group had more MDA concentrations and lower thiol contents in their cortical tissues compared to the MT group ($P < 0.05$–$P < 0.001$) (Fig. 6a, b).

The animals of the OT group had lower CAT and SOD levels in their cortical tissues compared to the control group ($P < 0.001$). In the MT group, there were no significant differences in the cortical levels of CAT and SOD compared to the control group. Additionally, CAT and SOD levels in the cortical tissues of the OT group were significantly lower than the MT group ($P < 0.001$) (Fig. 6c, d).

NO metabolite

The NO metabolite concentrations in the hippocampal tissues of the OT group were significantly higher than the control group ($P < 0.05$). However, there were no significant differences between the NO metabolite concentrations of the MT group compared to the control group. Therefore, in the hippocampal tissues of the OT group, the NO metabolite concentrations were higher than the MT group ($P < 0.01$) (Fig. 7a). Additionally, the NO metabolite concentrations in
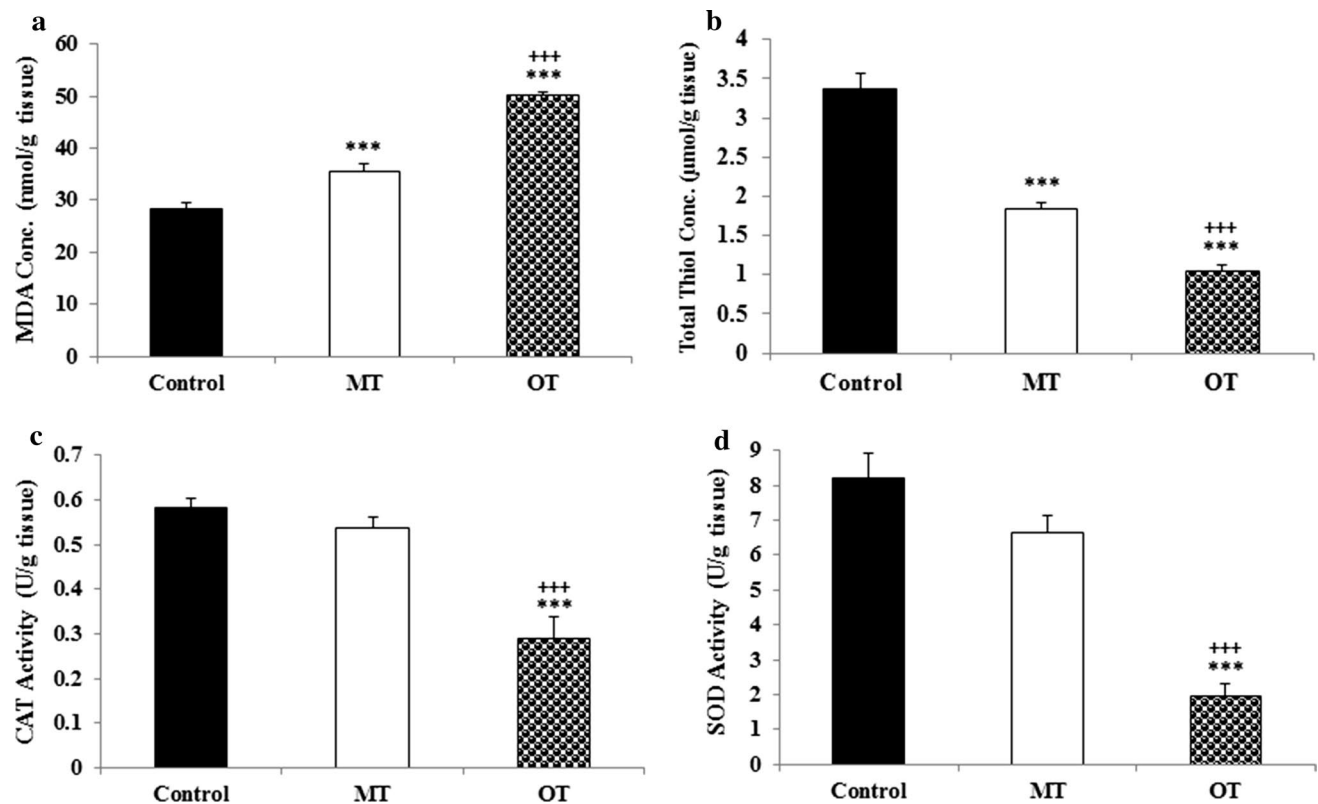

Fig. 5 Comparison of the MDA concentrations (**a**), thiol contents (**b**), CAT (**c**) and SOD (**d**) levels in the hippocampal tissues of the control, MT (moderate trained) and OT (overtrained) groups. The data are presented as mean ± standard error of the mean ($n = 7$ in each group). ***$P < 0.001$ compared to the control group and +++$P < 0.001$ compared to the MT group
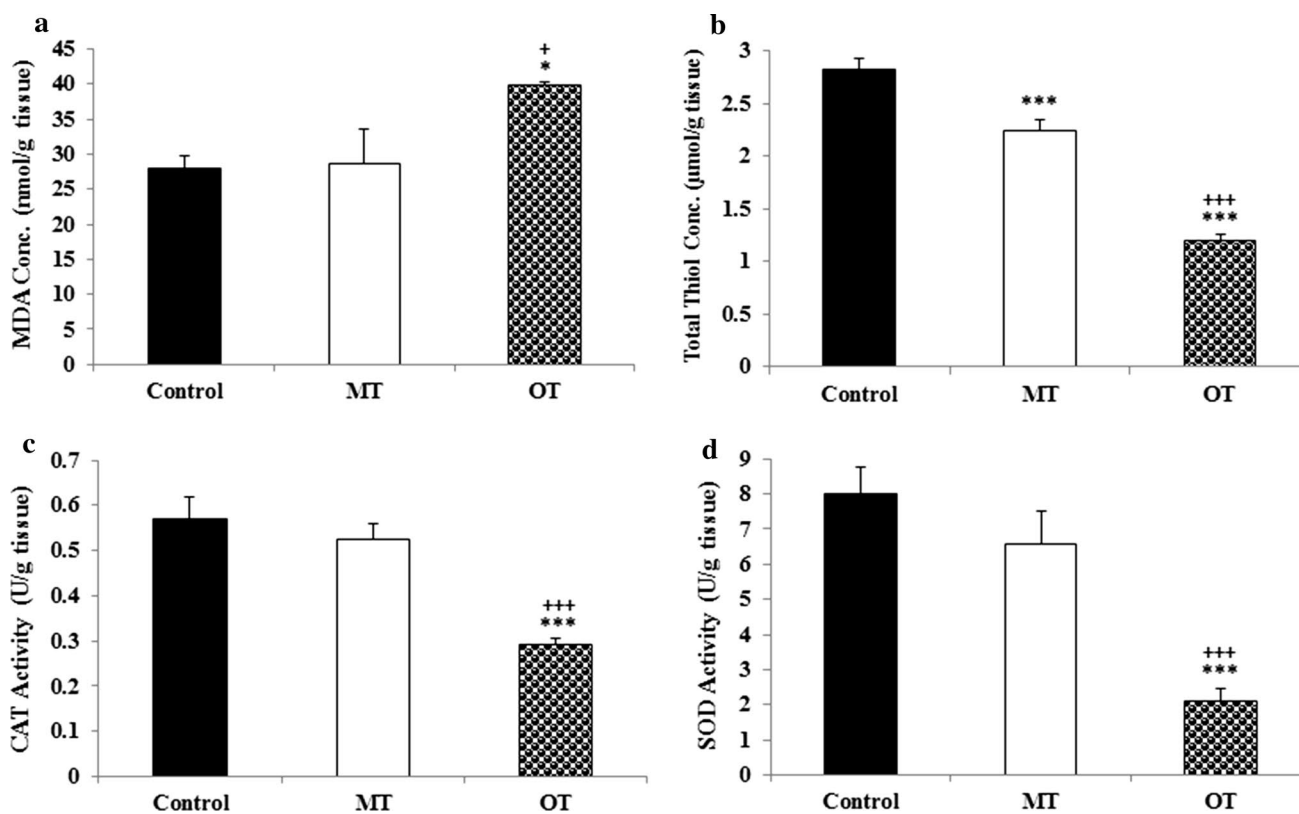
Fig. 6 Comparison of MDA concentrations (**a**), thiol contents (**b**), CAT (**c**) and SOD (**d**) levels in cortical tissues between the control, MT (moderate trained) and OT (overtrained) groups. The data are presented as mean ± standard error of the mean ($n = 7$ in each group). *$P < 0.05$ and ***$P < 0.001$ compared to the control group and +$P < 0.05$ and +++$P < 0.001$ compared to the MT group

the cortical tissues of the OT group were also higher than the control group ($P < 0.001$). While, there were no significant differences between the NO metabolite concentrations of the MT group compared to the control group. Also, in the cortical tissues of the OT group, the NO metabolite concentrations were higher than the MT group ($P < 0.001$) (Fig. 7b).

Discussion

In this study, the performance test was used as an indicator for measuring the training load. Previous studies have indicated that high-intensity training in combination with inadequate recovery leads to a reduction in performance (non-functional overreaching) and can cause an overtraining syndrome [35]. Our results showed that a gradual increase in the training load improved the performance in the animals in the MT and OT groups in the first two phases of the training (8 weeks), while in the third phase of the OT group training, signs and symptoms of overtraining syndrome were observed which included lethargy, fatigue and marked underperformance [27, 36].

The beneficial effects of regular physical activity on the brain function and central nervous system (CNS) adaptations by increasing neurogenesis, synaptic plasticity and long-term potentiation (LTP) in rodents have been previously demonstrated. These biochemical and structural effects may improve performance in behavioral tests, especially learning and memory in rodents [37]. In our study, there were no significant differences between the parameters of the MWM and PA tests of the animals in the MT group compared to the control group. Other studies have also indicated that moderate intensity treadmill exercise ($V = 17$–20 m/min, 40 min/day for 4–12 weeks) has no significant effect on spatial learning (visual and motor functions) in the MWM test [38, 39], although the spatial memory was improved in moderately trained animals [39]. Additionally, it has been reported that rats avoidance memory was unaffected by moderate treadmill activity ($V = 14$–20 m/min, 30–40 min/day for 4 weeks) [39, 40].

Our study results indicated that overtraining induced impairment of spatial learning and memory, and passive avoidance memory in rats. It was shown that that high-intensity running exercise ($V = 21$ m/min, 90 min/day for 5 weeks) has a negative effect on spatial learning compared
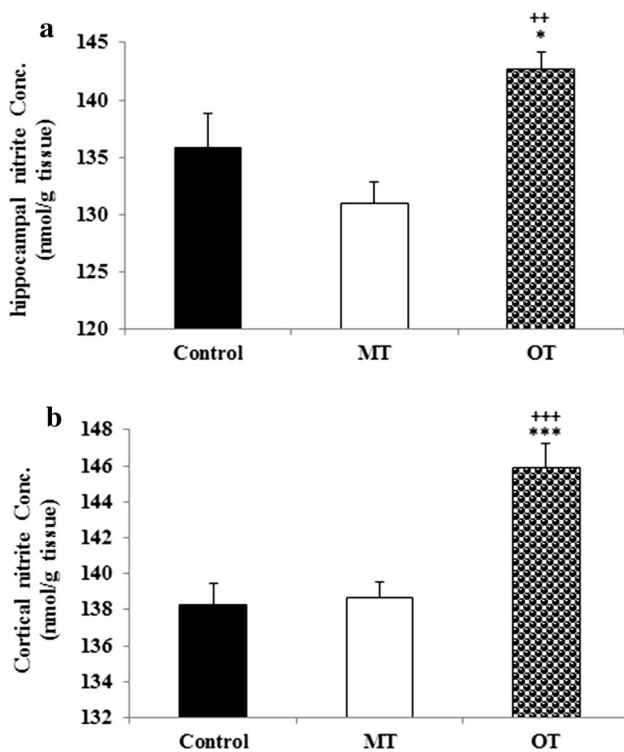
Fig. 7 Comparison of the hippocampal (**a**) and cortical (**b**) NO metabolite concentrations between the control, MT (moderate trained) and OT (overtrained) groups. The data are presented as mean ± standard error of the mean ($n = 7$ in each group). *$P < 0.05$ and ***$P < 0.001$ compared to the control group and ++$P < 0.01$ and +++$P < 0.001$ compared to the MT group

to the control and low-intensity runner mice, however, both intensities of exercise had no significant effect on spatial memory retention in the MWM test [41]. Few studies have investigated the effect of overtraining on the PA test of intact animals. While some have shown that overtraining (1 h swimming/day for 6 weeks, followed by an increase in the duration to 4.5 h during the last 2 final weeks) leads to a better performance on the PA test [42], others have reported that strenuous exercise ($V = 30$ m/min, 40 min/day for 30 days) has no effect on the outcomes of the PA test [39]. The intensity and duration of the training, as well as the age of animals are important confounding variables impacting the results of some previous studies. The probable mechanisms related to memory and learning modulation effects of these variables have been investigated in this study.

Our results showed that in the animals of the MT group, the hippocampal levels of IL-1β were higher than the control group, however, there were no significant differences between the hippocampal levels of TNF-α and CRP in the MT group compared to the control group. Several studies have reported that mild to moderate intensity of regular physical activity can suppress the expression of inflammatory cytokines and decrease the levels of TNF-α, IL-1β,

IL-6 and CRP. Balancing the inflammatory cytokine levels may be a sign of physical adaptation to exercise to create an anti-inflammatory environment [13, 14]. On the other hand, some studies have shown that moderate treadmill exercise ($V = 10–20$ m/min, 30–60 min/day for 5–8 weeks) has no effect on the expression and levels of inflammatory cytokines in the serum and hippocampus [20, 43]. Additionally, it was demonstrated that there is a correlation between the severity and duration of exercise training and the immune system's response [44]. IL-1 has a concentration-dependent effect on learning and memory consolidation. At physiologic concentration, IL-1 enhances the LTP and promotes memory, while at high levels it inhibits the LTP and synaptic strengths [45]. The ratio of the IL-1β and its antagonist receptor, the IL-1ra, in the brain has shown to be correlated with brain inflammation and memory impairment [46]. In this study, high levels of hippocampal IL-1β might explain the reason why moderate exercise did not improve spatial memory and passive avoidance results. However, it is worthy to note that in our study the IL-1β/IL-1ra ratio was not measured.

It has been suggested that an excessive training load without adequate recovery could cause microtrauma and inflammation in muscles and joints, and lead to recruitment of pro-inflammatory cytokines (IL-1β, TNFα, and IL-6) and induce systemic inflammation [20, 35]. Our results indicated that overtraining increased the levels of certain inflammatory cytokines such as TNF-α, IL-1β and CRP in the hippocampal tissues compared to the control group. The hippocampal levels of TNF-α and CRP were also higher than the MT group, while there were no significant differences between the IL-1β levels in the OT group compared to the MT group. It has been suggested that an increase in the levels of TNF-α and IL-1β can lead to the destruction of neurons, especially in the hippocampal region, and therefore, can contribute to learning and memory impairment [47]. It has also been indicated that the stress resulting from high-intensity exercise can impair brain functions [48] through an increase in the levels of hormones and cytokines, such as glucocorticoids and IL-18 which can activate glial cells, especially the microglia [49, 50]. And the activation of microglia in the hippocampus can lead to an increase in the production of inflammatory cytokines such as TNF-α, IL-1β, ROS and NO [51]. Similar to our findings, Sun et al. reported that high-intensity treadmill exercise ($V = 20$ m/min, duration of fatigue/day for 7 days) activated astrocytes and microglia and increased expression of TNF-α and IL-1β in the hippocampus of rats which led to cognitive impairment [22]. Furthermore, some studies have shown that CRP has a predisposing role in dementia and cognitive impairment, and that high levels of CRP might cause AD [5].

Several studies have indicated that regular physical activity can regulate oxidant/antioxidant balance [52], and activate the antioxidant system against free radicals [53]. In our

study, the hippocampal levels of MDA were higher in the MT group compared to the control group, while the thiol contents in both the hippocampal and cortical tissues were lower compared to the control group. There were also no significant differences between the cortical levels of MDA, SOD, CAT activities, and NO metabolite concentrations in both the hippocampal and cortical tissues of the MT group compared to the control group. The results of hippocampal and cortical oxidant/antioxidant balance in the MT group are in agreement with the results of behavioral response and increase in levels of IL-β. It had been reported that moderate treadmill exercise ($V = 15$ m/min, 50 min/day for 8 weeks) attenuates oxidative state of the hippocampus and improves cognitive impairment in the aged rats [54]. As a limitation of this study, the serum and muscle oxidant/antioxidant balance had not been evaluated. However, improvement in animal performance of the MT group despite the elevation of the hippocampal MDA level might propose the modulatory action of ROS in activating physiological response including antioxidant protection, repair of oxidative damage and muscle contraction [53]. A close relationship between ROS production, oxidative damage and overload exercise induced muscle hypoxia has been shown previously as well [18, 55]. Also, strenuous aerobic exercise causes oxidative damage and lipid peroxidation through an increase in the release of certain hormones such as epinephrine, metabolism of prostanoids, xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and macrophage activity [56]. Following high-intensity aerobic exercises, there is an increase in the levels of ROS, NO and oxidative damage, as well as a decrease in the antioxidant enzymes activity due to the activation of the antioxidant system against free radicals [51, 57]. Our results showed that overtraining led to an increase in the levels of MDA and NO metabolite, and a decline in the thiol content, SOD and CAT activities in both the cortical and hippocampal tissues compared to the control and the MT groups. These results suggest the potential role of oxidative stress in the detrimental effects of overtraining on learning and memory. Similar to our findings, Aguiar et al. also showed that high-intensity treadmill exercise ($V = 16.5$ m/min, 45 min/day for 8 weeks) which was above the anaerobic threshold in mice, increased oxidative damage and mitochondrial dysfunction as well as decreased the frontal cortex BDNF level [58]. It was reported that overtraining exercises (1–4.5 h swimming/day) did not induce oxidative damage in the brain [42], however, in this study the underperformance state of animals had not been documented. The biochemical findings of the OT group such as the hippocampal and cortical oxidant/antioxidant balance and inflammatory cytokine levels support the results of the behavioral test which indicated learning and memory impairment.

To the best of our knowledge, a few studies have evaluated the effects of moderate training in intact animals or have compared the level of exercise intensity in normal rats. The positive effects of moderate exercise on different models of learning and memory impairment (e.g., LPS, diabetic) have also been previously shown [38, 59]. While it is known that the age of animals, training duration and intensity play important roles in exercise neuroendocrine and cognitive effects, further investigations are needed to explain the probable mechanism responsible for the elevation of the IL-1β and MDA concentrations, as well as the unchanged memory and learning scores.

Conclusion

In conclusion, the result of our study showed that overtraining led to behavioral impairment, increased levels of inflammatory cytokines and oxidative stress markers, and induced learning and memory impairment.

Compliance with ethical standards

Conflict of interests The authors declare no conflicts of interest in this study.

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