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Impact of Physical Exercise on Behavioral Functions and Neurotrophic Factors on Ovariectomized Rats Exposed to SPS

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ABSTRACT

Background: Post-traumatic stress disorder (PTSD) is a severe psychiatric condition associated with anxiety, cognitive deficits, and neurobiological changes. Estrogen deficiency occurs after ovariectomy (OVX) or menopause, exacerbating PTSD symptoms and limiting treatment options. Physical exercise has emerged as a non-pharmacological intervention with neuroprotective effects, but its efficacy under estrogen-deficient conditions remains unclear. This study aimed to evaluate the effects of moderate-intensity forced running wheel (FRW) exercise on PTSD-like behaviors, recognition memory, and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1) in the hippocampus and prefrontal cortex of ovariectomized rats exposed to single prolonged stress (SPS) as a model of PTSD.

Materials and Methods: Adult female Wistar rats (n=7 per group) were allocated to eight groups in a 2×2×2 factorial design based on ovarian status (sham vs OVX), stress exposure (no stress vs SPS), and physical activity (sedentary vs FRW). FRW was performed 30 min/day, 5 days/week for 4 weeks at 10 m/min (~60% VO_2max), indicating moderate intensity. Anxiety-like behaviours (% open arm time [OAT], % open arm entry [OAE]) and recognition memory (discrimination index (DI) were assessed using standard behavioural tests. ELISA was used to measure BDNF and IGF-1 levels in the hippocampus and prefrontal cortex (PFC). Data (Mean±SEM) were analyzed using a three-way ANOVA (OVX × SPS × Exercise) with the Tukey's post-hoc test; the assumptions of normality and homogeneity were verified. Partial η^2 and Cohen's d were reported for omnibus and key pairwise comparisons, respectively ($\alpha=0.05$, two-tailed).

Results: SPS exposure significantly increased anxiety-like behaviors and impaired recognition memory in both control and ovariectomized rats. FRW exercise ameliorated anxiety and memory deficits and elevated BDNF and IGF-1 levels in control animals under both SPS and non-SPS conditions. However, in ovariectomized rats, the beneficial effects of exercise were confined mainly to non-SPS groups, with limited improvements observed in ovariectomized rats subjected to SPS. Estrogen deficiency diminished the neuroprotective and behavioral benefits of exercise under stress.

Conclusion: Moderate-intensity FRW exercise effectively mitigates PTSD-related behavioral and neurochemical deficits in rats with normal ovarian hormone levels. Still, its efficacy is substantially reduced under estrogen-deficient conditions. These findings highlight the importance of hormonal status in determining the therapeutic potential of exercise for PTSD. Although the SPS model provides valuable insights into PTSD-like symptoms, it does not fully replicate the complexity of human PTSD; therefore, extrapolation to clinical settings should be approached with caution. Combined interventions, including hormonal support, may be more effective for post-menopausal populations.

Keywords:

Brain-derived neurotrophic factor (BDNF), Cognition, Estrogen deficiency, Insulin-like growth factor I (IGF-1), Exercise, Ovariectomy (OVX), Stress disorders, Post-traumatic

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Introduction

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric condition that develops following exposure to traumatic events [1, 2]. It is characterized by persistent anxiety, impaired cognitive functions, dysregulation of HPA-axis function, and neuroinflammation [3, 4]. This disorder affects multiple brain regions, notably the hippocampus and prefrontal cortex (PFC), areas critically involved in regulating anxiety, memory processing, and stress response mechanisms [5, 6].

Animal models, such as single prolonged stress (SPS), have been widely utilized to mimic PTSD in laboratory settings [7]. The SPS model effectively reproduces behavioral symptoms such as anxiety, impaired cognition, neurochemical changes, and reduced levels of neurotrophic factors seen in PTSD patients [8-10]. Specifically, reduced levels of brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1) in the hippocampus and PFC have been consistently reported following SPS exposure, suggesting their crucial roles in the neurobiological underpinnings of PTSD [8, 11-14].

Estrogen and other ovarian hormones have a significant influence on cognitive functions, emotional responses, and neuronal plasticity [15-17]. Estrogen deprivation, as occurs naturally during menopause or artificially via ovariectomy (OVX), exacerbates anxiety-like behaviors and cognitive deficits [15, 16, 18]. Studies suggest that reduced estrogen levels might potentiate the severity of PTSD symptoms [19, 20], complicating its clinical management, particularly in postmenopausal women or ovariectomized animal models [17, 21, 22].

Physical exercise is a non-pharmacological, safe, and cost-effective intervention extensively studied for its neuroprotective effects against stress-induced psychiatric disorders, including PTSD [1, 23, 24]. Exercise promotes neurogenesis, synaptic plasticity, and cognitive resilience, partly mediated by increasing neurotrophic factors, such as BDNF and IGF-1, in brain regions associated with stress and memory [25-27]. Among various exercise paradigms, forced running wheel (FRW) exercise has emerged as an effective method for mitigating anxiety-like behaviors and cognitive impairment in rodents [8, 28-30]. While multiple studies have confirmed the neuroprotective effects of exercise in intact rodents, including improved cognition and elevated levels of BDNF and IGF-1, findings in ovariectomized models have been inconsistent [31-33]. Some studies have reported blunted or absent responses to exercise in OVX animals, especially under stress conditions [11, 34-36].

However, evidence regarding the effectiveness of FRW, specifically in ovariectomized animals under PTSD-like conditions, remains limited. These conflicting reports indicate that estrogen status may play a crucial role in determining the effectiveness of exercise interventions [8, 17]. Still, few studies have directly explored this interaction in PTSD models, leaving a significant gap in understanding how estrogen deficiency and traumatic stress together influence exercise responsiveness [3, 23].

Therefore, this study aimed to investigate the effects of moderate-intensity FRW exercise on anxiety-like behaviors, cognitive memory performance, and neurotrophic factor levels (BDNF and IGF-1) in the hippocampus and PFC of ovariectomized rats exposed to SPS-induced PTSD. Given the modulatory role of estrogen on neurotrophic signaling and emotional regulation, postmenopausal women may require tailored interventions that combine physical exercise with hormonal or pharmacological support to manage PTSD symptoms effectively [21, 37]. We hypothesized that moderate-intensity FRW exercise would attenuate behavioral impairments and enhance neurotrophic factor expression, potentially moderated by estrogen status, thus providing insights into tailored therapeutic strategies for PTSD in post-menopausal conditions.

Materials and Methods

Experimental animals

A total of 56 female Wistar rats from the animal house of Mazandaran University of Medical Science, Sari, Iran, initially weighing 200-250 g, were used in this study. Throughout the study, all rats, except for the control group rats whose ovaries were not removed, were housed individually in separate polycarbonate cages. In contrast, the control group rats, whose ovaries were not removed, were housed collectively, with eight rats per cage, in a large Plexiglas enclosure. All animals were kept in a temperature-controlled animal facility (24±1 °C) under a standard 12-hour light/dark cycle with free access to water and food. The rats were randomly allocated to one of the eight experimental groups (n=7 per group):

1. Control + None-SPS + Sedentary (CON/NSPS-SED)

2. Control + None-SPS + Exercise (CON/NSPS-EXC)

3. Control + SPS + Sedentary (CON/SPS-SED)

4. Control + SPS + Exercise (CON/SPS-EXC)

5. Ovariectomized + None-SPS + Sedentary (OVX/NSPS-SED)

6. Ovariectomized + None-SPS + Exercise (OVX/NSPS-EXC)

7. Ovariectomized + SPS + Sedentary (OVX/SPS-SED)

8. Ovariectomized + SPS + Exercise (OVX/SPS-EXC)

The experimental timeline involved OVX (for OVX groups) followed by a 20-day recovery period. Subsequently, SPS or sham procedures were administered. After 7 days post-SPS, the exercise or sedentary conditions commenced and lasted for 4 weeks. Behavioral tests were conducted after the intervention period, followed by euthanasia and tissue collection.

OVX procedure

After administering deep anesthesia with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (2.5 mg/kg) and disinfecting the site, a small incision was made in the lateral abdominal wall on both sides to remove the ovaries. The skin and muscle layers were then sutured. In the sham group, after making the incision and observing the ovaries, the skin and muscle layers were sutured without damaging the ovaries. Twenty days were allocated for surgical recovery and the clearance of endogenous ovarian hormones [11]. The sham groups underwent placebo surgery without OVX, and the OVX groups underwent OVX surgery [11].

SPS protocol

SPS, as a model of PTSD, was conducted in three stages: Restraining for two hours, forced swimming for 20 min, and anesthetizing by ether. The size of the restrainer should be appropriate for the animal that is restrained. After being restrained, animals were kept individually in Plexiglas cages. Then, the rats were forced to swim for 20 minutes in a cylindrical container (240 mm in diameter, 500 mm in height) filled to two-thirds of its height with water maintained at 23±1 ˚C. After a 15-minute recovery period, the animals were briefly exposed to diethyl ether for anesthesia, following the laboratory's approved protocol [38, 39]. Thereafter, the rats were maintained in their cages for 14 days [40]. Although diethyl ether is less commonly used in modern settings, it was applied here under controlled conditions to ensure rapid sedation. All procedures were approved by the institutional ethics committee and conducted under national guidelines to minimize animal suffering [41].

FRW exercise

Fourteen days after SPS, rats were habituated to the FRW apparatus in order to decrease stress. For habituation to the FRW, rats walked on the FRW at the lowest speed (3 m/min) for 15 min for 3 days. After habituation, moderate exercise was initiated for the exercise groups. The animals ran in aFRW at a speed of 10 m/min for 30 minutes, five days per week, for four weeks [29, 42]. Rats that refused to walk in the FRW were excluded and replaced with new animals. Rats in sedentary groups were placed in the switched-off FRW for 5 min once a day [30, 43].

Based on previous studies, a running speed of 10 m/min has been classified as moderate-intensity exercise in female Wistar rats, as it approximately corresponds to 60% of their maximal oxygen uptake (VO_2max) or maximal lactate steady state (MLSS) [29, 30].

Elevated plus maze (EPM) test

The EPM test was used to assess anxiety-like behavior in the rodent model one day after completing the treatment phase. The EPM consists of a plus-shaped apparatus elevated 46 cm above the floor with four arms (51×10×40 cm each) and a central platform (10×10 cm). Each rat was individually placed in the central zone facing an open arm and allowed to explore for 5 minutes. The rats' behavior in the EPM was recorded using a video camera (Borj Sanat Azma Co., Tehran, Iran) positioned 181 cm above the apparatus. Reduced time spent and entries into the open arms, along with increased time spent and entries into the closed arms, were analyzed as indicators of anxiety-related behaviors [1, 44].

Object recognition memory test (ORMT)

Cognitive function, specifically recognition memory, was evaluated using the ORMT. The test was conducted in an open-field arena (e.g. 50×50×40 cm). The procedure consisted of three phases: habituation, familiarization, and test.

First, in the habituation phase, each rat was allowed to explore the empty arena freely for 10 minutes. Then, during the familiarization phase, the animal was exposed to two identical objects. After a defined delay, one of the familiar objects is replaced with a novel one during the test phase. The animal's tendency to explore the novel object is interpreted as a measure of recognition memory. An increased exploration time for the novel object indicates the animal's ability to retain and recall previous information.

Due to its simplicity and high sensitivity, this test is widely used in neuroscience research to evaluate the effects of drugs, stress, or behavioral interventions on memory function [1, 45, 46].

Measurement of BDNF and IGF-1 levels in the hippocampus and PFC

After behavioral tests, animals were deeply anesthetized with ketamine and xylazine and humanely sacrificed. The brains were immediately removed, and the hippocampus and PFC were rapidly dissected on ice. Tissue samples were snap-frozen in liquid nitrogen and stored at −80 °C until analysis.

For protein extraction, the frozen tissues were homogenized in ice-cold lysis buffer (prepared according to the kit instructions) using a tissue homogenizer at temperatures below 4 °C. The homogenates were centrifuged at 2,000 rpm for 20 minutes at 4 °C, and the resulting supernatants were collected for the quantification of BDNF and IGF-1.

The concentrations of BDNF and IGF-1 in the hippocampal and PFC supernatants were determined using commercially available ELISA kits (ZellBio GmbH, Germany), following the manufacturer's protocol. Absorbance was measured at the appropriate wavelength using a microplate reader, and concentrations were expressed as ng/mg protein according to the kit's instructions. To normalize the results, the total protein content in the supernatants was determined using the Bradford assay [47].

Statistical analysis

After assessing the normality of data using the Kolmogorov-Smirnov test, descriptive data are presented as Mean±SEM. The three-way ANOVA (SPS × OVX × EXC) followed by Tukey's post-hoc test was used to determine significant differences between groups. SPSS software, version 26 was used for data analysis, and a $P<0.05$ was considered the significance level.

Result

Time spent in the open arm

Data on anxiety-like behaviors in the EPM are illustrated in Figures 1A and 1B.

A three-way ANOVA on the percentage of time spent in the open arm (Figure 1A) demonstrated significant main effects of SPS ($F_{1,\ 48}$=128.759, P=0.0001), OVX ($F_{1,\ 48}$=129.443, P=0.0001), and EXC ($F_{1,\ 48}$=78.345, P=0.0001).

Moreover, between-group comparisons indicated that, in control rats, exposure to SPS significantly reduced the percent of open arm time (%OAT) compared to the NSPS-SED group (P=0.0001). In this group, exercise significantly increased %OAT in both the NSPS (P=0.0001) and SPS (P=0.0001) subgroups compared to their respective sedentary controls. Notably, the effect of exercise was significantly greater in the NSPS group than in the SPS group (P=0.0001).

In the ovariectomized groups, a significant reduction in %OAT was observed following SPS exposure compared to the NSPS-SED group (P=0.028). Exercise significantly increased %OAT only in the NSPS subgroup (P=0.0001), and the impact of exercise was more pronounced in the NSPS group compared to the SPS group (P=0.0001). Furthermore, significant differences in %OAT were observed between all control groups and their corresponding ovariectomized groups.

Percentage of entries into the open arm

A three-way ANOVA on the percentage of open arm entries (Figure 1B) demonstrated significant main effects of SPS ($F_{1,48}$=282.735, P=0.0001), OVX ($F_{1,48}$=140.425, P=0.0001), and EXC ($F_{1,48}$=107.268, P=0.0001).

Moreover, between-group comparisons indicated that, in control rats, SPS exposure significantly reduced the percent of open arm entry (%OAE) compared to the NSPS-SED group (P=0.0001). In these groups, exercise significantly increased %OAE in both NSPS and SPS subgroups compared to their respective sedentary controls (P=0.0001). Moreover, the effect of exercise was significantly greater in the NSPS group than in the SPS group (P=0.0001). In ovariectomized rats, OVX itself caused a significant reduction in %OAE in the NSPS-SED group compared to the control group (P=0.0001). SPS exposure further decreased %OAE compared to the NSPS-SED group (P=0.0001). In these animals, exercise

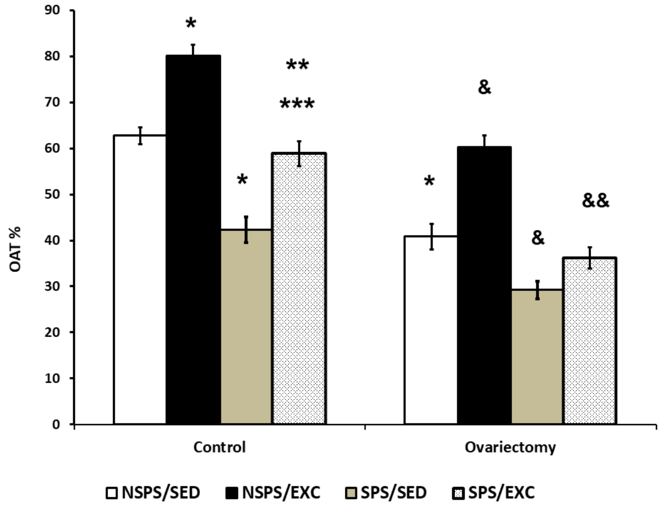
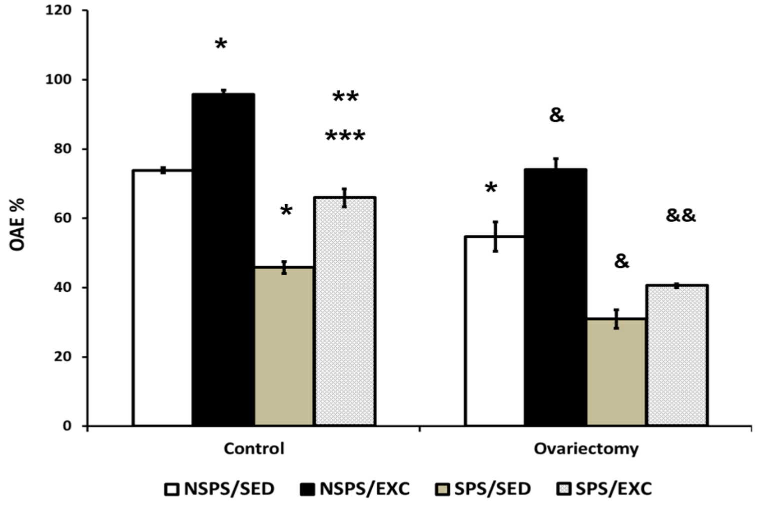


Figure 1. Effect of FRW exercise on (anxiety-like behaviors) ovariectomized SPS rats

A) Effect of FRW exercise on (anxiety-like behaviors) percentage of time spent in the open arm ovariectomized SPS rats

*Significant difference vs the CON/NSPS-SED group (P=0.0001), **Significant difference vs the CON/SPS-SED group (P=0.0001), ***Significant difference vs the CON/NSPS-EXC group (P=0.0001), & and & Significant difference vs the OVX/NSPS-SED group (P=0.0001, and P=0.028), && Significant difference vs the OVX/NSPS-EXC group (P=0.0001).



B) Effect of FRW exercise on (anxiety-like behaviors) percentage of open arm entries in ovariectomized SPS rats

*Significant difference vs the CON/NSPS-SED group (P=0.0001), **Significant difference vs the CON/SPS-SED group (P=0.0001), ***Significant difference vs the CON/NSPS-EXC group (P=0.0001), &Significant difference vs the OVX/NSPS-SED group (P=0.0001), &&Significant difference vs the OVX/NSPS-EXC group (P=0.001).

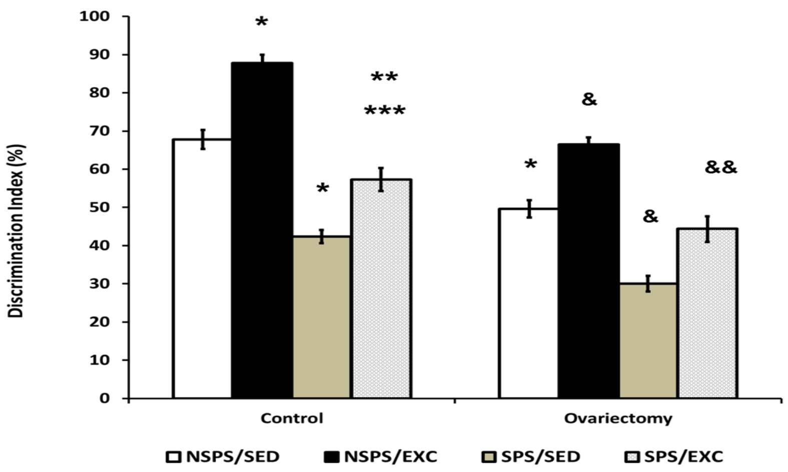


Figure 2. Effect of FRW exercise on (anxiety-like behaviors) percentage of open arm entries in ovariectomized SPS rats

*Significant difference vs the CON/NSPS-SED group (P=0.0001), **Significant difference vs the CON/SPS-SED group (P=0.0001), ***Significant difference vs the CON/NSPS-EXC group (P=0.0001), $^{\&}$Significant difference vs the OVX/NSPS-SED group (P=0.0001), $^{\&\&}$Significant difference vs the OVX/NSPS-EXC group (P=0.001).

significantly increased the %OAE only in the SPS subgroup (P=0.0001). The beneficial effect of exercise was again more pronounced in the NSPS group than in the SPS group (P=0.001). Additionally, significant differences in %OAE were found between all control groups and their corresponding ovariectomized groups.

Effect of FRW on cognitive memory in healthy and ovariectomized rats with PTSD

A three-way ANOVA on cognitive memory (Figure 2) demonstrated significant main effects of SPS ($F_{1, 48}$=203.474, P=0.0001), OVX ($F_{1, 48}$=89.093, P=0.0001), and EXC ($F_{1, 48}$=93.433, P=0.0001), as well as a significant interaction between SPS and OVX ($F_{1, 48}$=4.244, P=0.045).

Moreover, between-group comparisons indicated that, in control rats, SPS exposure significantly reduced DI compared to the NSPS-SED group (P=0.0001). Exercise significantly increased DI in both the NSPS (P=0.0001) and SPS (P=0.002) subgroups relative to their respective sedentary controls. Notably, the effect of exercise was significantly greater in the NSPS group than in the SPS group (P=0.0001).

In ovariectomized rats, OVX itself significantly reduced DI in the NSPS-SED group compared to the control group (P=0.0001). SPS exposure further decreased DI compared to the NSPS-SED group (P=0.0001). Exercise significantly increased DI in both the NSPS (P=0.0001) and SPS (P=0.003) subgroups compared to their sedentary counterparts. The positive impact of exercise was significantly more pronounced in the NSPS group than in the SPS group (P=0.0001). Additionally, significant differences in DI were observed between all control groups and their corresponding ovariectomized groups.

Effect of FRW on BDNF levels in the hippocampus and PFC regions in healthy and ovariectomized female rats with PTSD

A three-way ANOVA on hippocampal BDNF (Figure 3) demonstrated significant main effects of SPS ($F_{1, 48}$=666.881, P=0.0001), OVX ($F_{1, 48}$=186.507, P=0.0001), and EXC ($F_{1, 48}$=154.170, P=0.0001), as well as significant interactions between SPS and OVX ($F_{1, 48}$=64.030, P=0.0001) and between SPS, OVX, and EXC ($F_{1, 48}$=6.383, P=0.015). Moreover, between-group comparisons indicated that, in control rats, SPS exposure significantly reduced BDNF levels compared to the NSPS-SED group (P=0.0001). Exercise significantly increased BDNF levels in both the NSPS and SPS subgroups relative to their sedentary counterparts (P=0.0001). The exercise-induced increase in BDNF was significantly greater in the NSPS group than in the SPS group (P=0.0001). In ovariectomized rats, OVX itself led to a significant

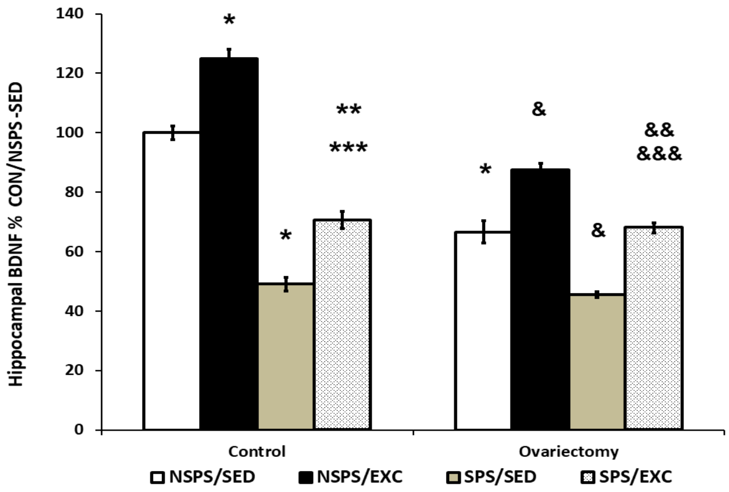


Figure 3. Effect of FRW on hippocampal BDNF levels in ovariectomized control rats with PTSD

*Significant difference vs the CON/NSPS-SED group (P=0.0001), **Significant difference vs the CON/SPS-SED group (P=0.0001), ***Significant difference vs the CON/NSPS-EXC group (P=0.0001), &Significant difference vs the OVX/NSPS-SED group (P=0.0001), &&Significant difference vs the OVX/SPS-SED group (P=0.002), &&&Significant difference vs the OVX/NSPS-EXC group (P=0.0001).

reduction in BDNF levels in the NSPS-SED group compared to the corresponding control group (P=0.0001). SPS exposure further reduced BDNF levels compared to the NSPS-SED group (P=0.0001). In these groups, exercise significantly increased BDNF levels in both the NSPS (P=0.0001) and SPS (P=0.002) subgroups compared to their non-exercising counterparts. The effect of exercise was significantly more pronounced in the NSPS group than in the SPS group (P=0.0001). Additionally, significant differences in hippocampal BDNF levels were observed between all control groups and their corresponding ovariectomized groups.

A three-way ANOVA on the BDNF levels in the PFC (Figure 4) demonstrated significant main effects of SPS ($F_{1, 48}$=318.042, P=0.0001), OVX ($F_{1, 48}$=96.501, P=0.0001), and EXC ($F_{1, 48}$=117.094, P=0.0001), as well as a significant interaction between SPS, OVX, and EXC ($F_{1, 48}$=6.319, P=0.015). Moreover, between-group comparisons indicated that, in control rats, SPS exposure led to a significant decrease in BDNF compared to the NSPS-SED group (P=0.0001). Exercise significantly increased BDNF levels in both the NSPS and SPS subgroups relative to their sedentary counterparts (P=0.0001), with the effect of exercise being significantly greater in the NSPS group than in the SPS group (P=0.0001). In ovariectomized rats, OVX itself caused a significant reduction in BDNF levels in the NSPS-SED group compared to the corresponding control group (P=0.0001). SPS exposure further decreased BDNF compared to the NSPS-SED group (P=0.0001). In these animals, exercise significantly increased BDNF in both the NSPS (P=0.0001) and SPS (P=0.030) subgroups compared to their non-exercising counterparts. The beneficial effect of exercise was significantly greater in the NSPS group than in the SPS group (P=0.0001). Additionally, significant differences in BDNF levels were observed between all control groups and their respective ovariectomized groups.

Effect of FRW on the level of IGF-1 in the hippocampus and PFC in healthy and OVX female rats with PTSD

A three-way ANOVA on hippocampal IGF-1 (Figure 5) demonstrated significant main effects of SPS ($F_{1, 48}$=234.286, P=0.0001), OVX ($F_{1, 48}$=241.006, P=0.0001), and EXC ($F_{1, 48}$=196.831, P=0.0001), as well as significant interactions between SPS and OVX ($F_{1, 48}$=13.407, P=0.001) and between SPS, OVX, and EXC ($F_{1, 48}$=7.705, P=0.008). Moreover, between-group comparisons indicated that, in the control group, SPS significantly decreased IGF-1 compared to the NSPS-SED group (P=0.0001). In this group, exercise significantly increased IGF-1 in both the NSPS and SPS groups compared to the non-exercise group (P=0.0001). The effect of exercise was significantly greater in the NSPS group than in the SPS group (P=0.0001). OVX significantly decreased IGF-1 in the NSPS-SED group compared to the control group (P=0.0001). In the ovariectomized group, SPS significantly decreased IGF-1 compared to

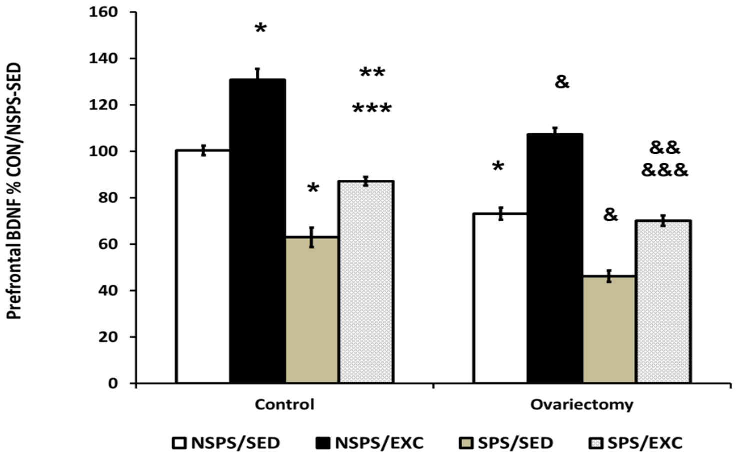


Figure 4. Effect of forced running wheel exercise on prefrontal BDNF levels in ovariectomized SPS rats

*Significant difference vs the CON/NSPS-SED group (P=0.0001), **Significant difference vs the CON/SPS-SED group (P=0.0001), ***Significant difference vs the CON/NSPS-EXC group (P=0.0001), &Significant difference vs the OVX/NSPS-SED group (P=0.0001), &&Significant difference vs the OVX/SPS-SED group (P=0.030), &&&Significant difference vs the OVX/NSPS-EXC group (P=0.0001).

the NSPS-SED group (P=0.0001). In this group, exercise significantly increased IGF-1 in both the NSPS (P=0.0001) and SPS (P=0.040) groups compared to the non-exercise group. The effect of exercise in the NSPS group was significantly greater than in the SPS group (P=0/0001). Also, a significant difference in IGF-1 was observed between all control groups and similar ovariectomized groups.

A three-way ANOVA on the level of IGF-1 in the PFC (Figure 6) demonstrated significant main effects of SPS ($F_{1, 48}$=200.359, P=0.0001), OVX ($F_{1, 48}$=29.745, P=0.0001), and EXC ($F_{1, 48}$=119.225, P=0.0001), as well as a significant interaction between SPS and OVX ($F_{1, 48}$=7.978, P=0.007). Moreover, between-group comparisons indicated that, in control rats, SPS exposure resulted in a significant decrease in IGF-1 compared to the NSPS-SED group (P=0.0001). Exercise significantly increased IGF-1 levels in both the NSPS (P=0.003) and SPS (P=0.0001) subgroups relative to their respective sedentary controls. Moreover, the effect of exercise was significantly greater in the NSPS group than in the SPS group (P=0.0001). In ovariectomized rats, OVX itself led to a significant decrease in IGF-1 levels in the NSPS-SED group compared to the corresponding control group (P=0.001). SPS exposure further reduced IGF-1 compared to the NSPS-SED group (P=0.0001). In these animals, exercise significantly increased IGF-1 levels in both the NSPS and SPS subgroups compared to their sedentary counterparts (P=0.0001). As observed in the control groups, the effect of exercise was significantly greater in the NSPS group than in the SPS group (P=0.0001). Additionally, significant differences in prefrontal IGF-1 levels were observed between all control groups and their respective ovariectomized groups.

Discussion

The present study demonstrated that the SPS model, as an established animal model of PTSD, significantly induced anxiety-like behaviors, impaired cognitive memory, and reduced neurotrophic factors, including BDNF and IGF-1, in both the hippocampus and PFC of ovariectomized and control rats. These findings are consistent with previous studies indicating the detrimental impact of SPS on behavioral and neurobiological markers associated with PTSD, such as increased anxiety behaviors, memory deficits, and reduced neurotrophic factor levels [23, 27, 48].

Interestingly, our results indicated differential effects of FRW exercise on behavioral and molecular parameters depending on the hormonal status of the rats. In control animals, FRW exercise effectively ameliorated the behavioral deficits and significantly enhanced cognitive memory, alongside increased levels of BDNF and

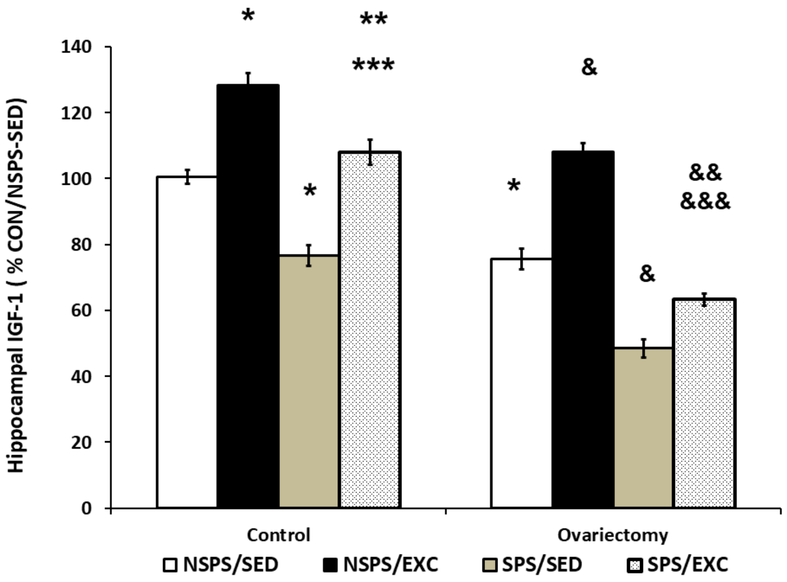


Figure 5. Effect of FRW exercise on hippocampal IGF-1 levels in ovariectomized SPS rats

*Significant difference vs the CON/NSPS-SED group (P=0.0001), **Significant difference vs the CON/SPS-SED group (P=0.0001), ***Significant difference vs the CON/NSPS-EXC group (P=0.0001), &Significant difference vs the OVX/NSPS-SED group (P=0.0001), &&Significant difference vs the OVX/SPS-SED group (P=0.040), &&&Significant difference vs the OVX/NSPS-EXC group (P=0.0001).

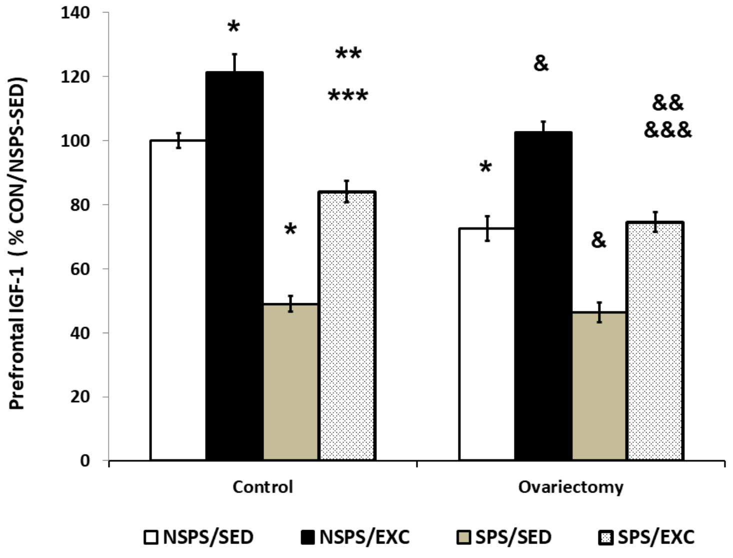


Figure 6. Effect of FRW exercise on prefrontal IGF-1 levels in ovariectomized SPS rats

*Significant difference vs the CON/NSPS-SED group (P=0.003, P=0.0001, P=0.001), **Significant difference vs the CON/SPS-SED group (P=0.0001), ***Significant difference vs the CON/NSPS-EXC group (P=0.0001), &Significant difference vs the OVX/NSPS-SED group (P=0.0001), &&Significant difference vs the OVX/SPS-SED group (P=0.0001), &&&Significant difference vs the OVX/NSPS-EXC group (P=0.0001).

IGF-1 in the hippocampus and PFC in both SPS and non-SPS groups. This aligns with previous research highlighting exercise-induced neurogenesis and synaptic plasticity as underlying mechanisms of cognitive enhancement [49-52].

Our findings support and extend previous research indicating that FRW, as used in this study, activates key neurotrophic signaling pathways underlying stress resilience and cognitive improvement [53, 54]. FRW reliably elevates hippocampal and prefrontal BDNF and IGF-1 levels, which in turn initiate downstream molecular cascades critical for neuronal survival and synaptic plasticity [55, 56]. Specifically, BDNF acts through its TrkB receptor to trigger PI3K/Akt and MAPK/ERK signaling, leading to phosphorylation of CREB and upregulation of genes associated with long-term potentiation and neuronal health [57, 58]. Compared to voluntary running, forced exercise paradigms ensure consistent intensity and duration, which may account for more robust and reproducible activation of these pathways [59, 60]. Moreover, IGF-1, upregulated both peripherally and centrally by forced exercise, synergizes with BDNF to further enhance PI3K/Akt signaling and promote neurogenesis and synaptic remodeling [61, 62].

Notably, prior studies in models of PTSD and estrogen deficiency have shown that FRW reverses stress-induced reductions in BDNF, restores downstream signaling activity, and alleviates anxiety- and depression-like behaviors, underscoring the causal relevance of these molecular adaptations [62, 63]. By demonstrating that four weeks of FRW re-engages TrkB/PI3K/Akt and MAPK/ERK pathways in OVX+SPS rats, our results provide a plausible mechanistic basis for the observed anxiolytic and cognitive benefits. Future research should directly assess the activation of these signaling pathways and further clarify the differential effects of forced versus voluntary exercise on neuroplasticity and stress adaptation.

However, in the ovariectomized groups, the beneficial effects of exercise on anxiety-like behaviors were primarily observed in the non-SPS condition, with limited and non-significant improvements seen in the SPS-exposed ovariectomized rats. This finding is supported by earlier studies suggesting that estrogen deficiency might exacerbate the neuropsychological impacts of PTSD, highlighting the importance of hormonal status in modulating responses to stress and therapeutic interventions, such as exercise [17, 22, 64]. The observed limited effectiveness of exercise in the ovariectomized SPS group suggests that estrogenic mechanisms might be essential for the full therapeutic benefits of exercise interventions [36, 65], emphasizing the necessity for combined strategies, possibly including hormonal supplementation, in managing PTSD symptoms in post-menopausal conditions [37, 66].

Regarding cognitive performance, FRW exercise significantly improved cognitive memory (DI) in control animals under both SPS and non-SPS conditions. In contrast, the cognitive enhancements in the ovariectomized group were significant only in non-SPS conditions, reflecting a complex interplay between stress exposure, hormonal status, and cognitive outcomes. This observation highlights the significance of estrogen in memory formation and cognitive resilience under stress, with several studies confirming estrogen's crucial role in hippocampal-dependent memory [15, 67].

Our biochemical analyses further reinforced these behavioral findings by showing that FRW exercise significantly elevated hippocampal and prefrontal BDNF and IGF-1 levels across all exercised groups. These neurotrophic factors are known for their crucial roles in neuronal survival, neurogenesis, synaptic plasticity, and cognitive function [15, 68-70]. Exercise-induced increases in BDNF and IGF-1 levels have been repeatedly shown to facilitate neuronal resilience against stress-induced neurodegeneration, supporting cognitive functions impaired by stress-related disorders, such as PTSD [71-74]. Nonetheless, despite the marked biochemical changes, behavioral improvements in ovariectomized SPS rats remained limited, suggesting that alterations at the molecular level might precede or require prolonged interventions to translate into observable behavioral outcomes.

The complex relationship between exercise intensity, duration, and outcomes in behavioral and neurochemical responses should also be considered. Moderate-intensity exercise, as utilized in this study, has been generally beneficial for cognitive and emotional functions [27]. Still, higher or lower intensities and prolonged durations might yield differing outcomes, [75] potentially addressing the non-significant findings observed in the ovariectomized SPS condition.

Conclusion

FRW exercise mitigates PTSD-like behavioral and neurochemical deficits in hormonally intact rats, whereas its efficacy is markedly blunted in ovariectomized animals exposed to SPS. Given this attenuated response, adjunct interventions that modulate estrogen signaling—such as selective estrogen receptor modulators or tailored hormone replacement—should be investigated to improve therapeutic outcomes in postmenopausal PTSD. Future research should further refine exercise parameters, explore optimal intensity and duration, determine the long-term durability of the observed effects, and assess combination therapies to maximize benefits in hormone-deficient populations experiencing stress-related disorders.

Limitations

This study has some limitations. The intervention lasted only four weeks, limiting insight into the long-term durability of exercise effects, as all outcomes were measured immediately post-intervention and no follow-up was conducted to assess whether the observed behavioral and neurotrophic improvements persist over time. The lack of a hormone replacement therapy group precludes the assessment of the interaction between estrogen status and exercise. Behavioral outcomes were based on single assays per domain, which may not fully capture the complexity of PTSD-like symptoms. The relatively small sample size may also restrict statistical power. Future studies should address these limitations by employing longer interventions and follow-up assessments, incorporating additional experimental arms, expanding behavioral testing, and increasing cohort sizes to enhance the understanding of these findings.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the Research Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Code: IR.MAZUMS.AEC.1402.053). All experimental trials were conducted by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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Authors contribution's

Study design and supervision: Sakineh Shafia; Laboratory experiments and data collection: Arvin Amiri, Maryam Janbazi; Investigation: Seyede Rose Jamali and Simin Ehsani Vostacolaee; Formal analysis: Sakineh Shafia; Writing: Arvin Amiri; Review and editing: Sakineh Shafia; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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