Pharmacology, Biochemistry and Behavior 187 (2019) 172819

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Voluntary exercise and estradiol reverse ovariectomy-induced spatial learning and memory deficits and reduction in hippocampal brain-derived neurotrophic factor in rats

Ali Rashidy-Pour[a,b], Kowsar Bavarsad[c,d,1], Hossein Miladi-Gorji[a,b], Zahra Seraj[c], Abbas Ali Vafaei[a,b,*]

[a] Laboratory of Learning and Memory, Research Center of Physiology, Semnan University of Medical Sciences, Semnan, Iran
[b] Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[c] Student Research Committee, Semnan University of Medical Sciences, Semnan, Iran
[d] Department of Physiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

ARTICLE INFO

Keywords:
Exercise
Learning
Memory
Estrogens
Ovariectomy
BDNF

ABSTRACT

Ample evidences have demonstrated the beneficial effects of physical exercise on cognitive functions such as learning and memory. It is well established that female sex hormones have an important role in regulating learning and memory. This study was designed to investigate the effects of voluntary exercise and estrogen replacement on learning and memory deficits and reduction in hippocampal brain derived neurotrophic factor (BDNF) levels induced by ovariectomy. Ovariectomized rats were given daily vehicle or 17 β-estradiol (20 μg/kg) and allowed to freely exercise in a running wheel over the course of 2 weeks. After this period, they were trained and tested on a water-maze spatial task for 5 consecutive days, followed by a probe test one day later. At the end of the behavioral tests, all animals were decapitated and their hippocampal levels of BDNF were measured. Ovariectomy impaired spatial learning and memory and reduced hippocampal BDNF levels. Exercise significantly improved performance during both training and the retention of the water-maze task and increased hippocampal BDNF. Exercise, 17 β-estradiol and their combination recovered the impairing effects of ovariectomy on learning and memory performance. The combined treatment did not produce stronger effect than either exercise or 17 β-estradiol alone. Our findings provide an important evidence about positive influences of regular exercise and estrogen treatment against cognitive and BDNF deficits induced in ovariectomized rats, an experimental model of menopause.

1. Introduction

Previous studies have shown that the brain functions as a target for several hormones including sex hormones, which influence behavior, sexual orientation, and cognition (Sandstrom and Williams, 2001). Experiment on animals have also shown that sex hormones, especially estrogen, prevent the onset of the cerebrovascular event-induced decline of neuronal function (Suzuki et al., 2006), enhance neuronal connectivity (Spencer et al., 2008b) and cognition (Kim and Casadesus, 2010), and delay senile dementia (Frick, 2009). Remarkable evidence in laboratory animals has showed that estrogen has a significant impact on the brain, specifically on the hippocampus and basal forebrain, which are involved in memory-related processes. Estrogen was found to increase the number of CA1 dendritic spines in the hippocampus of female animals (Frick et al., 2004). A previous study has reported that ovariectomized mice undergoing estrogen treatment displayed better performance in spatial reference memory testing in the Morris water maze (Heikkinen et al., 2002). Estrogen replacement therapy has shown promising results in controlling the cognitive decline because of estrogen deficiency (Genazzani et al., 2007).

Cognitive function varies with estrogens levels throughout the lifespan of the female. High levels of estrogens are associated with an alteration in the density of synaptic spines in pyramidal neurons in the prefrontal cortex and hippocampus and improved cognitive function (Luine and Frankfurt, 2013). The exact mechanism (s) for enhancement of cognitive function and dendritic spines are not well understood.

Abbreviations: OVX, ovariectomy; BDNF, brain derived neurotrophic factor; VE, voluntary exercise; WM, water maze; EST, estrogen

* Corresponding author at: Laboratory of Learning and Memory, Research Center of Physiology, Semnan University of Medical Sciences, Semnan, Iran.
E-mail address: aavafaei@semums.ac.ir (A.A. Vafaei).

[1] Equal with the first author.

https://doi.org/10.1016/j.pbb.2019.172819
Received 28 May 2019; Received in revised form 28 October 2019; Accepted 3 November 2019
Available online 05 November 2019
0091-3057/

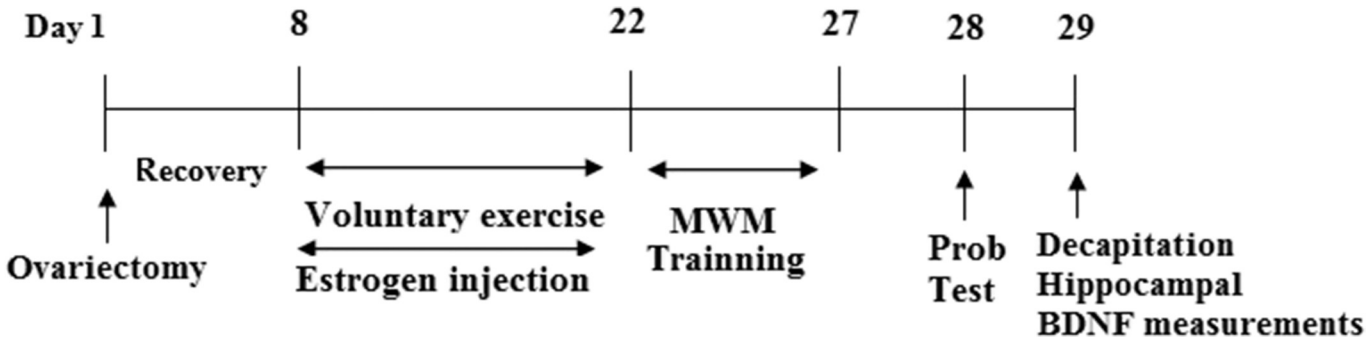

Fig. 1. Time line of experimental procedures (see Methods for details).

Brain derived neurotrophic factor (BDNF) is an important brain neurotrophin, which increases the survival and growth of neurons and enhances memory function. Estrogens increase BDNF levels and its receptors (TrkA and TrkB) in the hippocampus (Berchtold et al., 2001; Pan et al., 2010; Spencer et al., 2008a). Thus, estrogens could enhance brain morphology and cognitive function via BDNF. Recent evidences also indicate that estrogens and BDNF may work in concert to improve learning and memory (Luine and Frankfurt, 2013).

Recently, research has focused on finding optimal ways, including exercise, to control the memory impairment associated with the loss of post-menopausal ovarian steroid secretion (Sherwin, 2005). Many studies have shown that voluntary exercise enhances learning and memory in hippocampal dependent tasks such as the water maze, the radial-arm maze, contextual fear conditioning, and passive avoidance in rats (Baruch et al., 2004). Voluntary exercise may facilitate rehabilitation of the central nervous system when there is a loss of function caused by damage (Grealy et al., 1999) and reduce the progress of senile dementia (Laurin et al., 2001). Physical exercise increases the rate of cell proliferation in the dentate gyrus and improves animals' performance in spatial learning tests (van Praag et al., 1999). Exercise also increases hippocampal BDNF expression and this neurotrophin mediates the efficacy of exercise on synaptic plasticity and cognition and exerts its effect through TrkB receptors (Vaynman et al., 2004).

Physical exercise is typically prescribed for postmenopausal symptoms in women (Shangold, 1990), and it increases the concentration of serotonin in the brain, preventing depression (Brown et al., 1979). A clinical study has reported that exercise may recover memory impairment in the postmenopausal woman (Aiello et al., 2004). A recent animal study has demonstrated that ovariectomy caused an impairment in spatial navigation and avoidance memory in rats, and these harmful effects on cognition could be prevented by voluntary or forced running exercise (Ben et al., 2010). A recent study suggested a central role for BDNF in the neuroprotection mediated by running wheel exercise therapy against ovariectomy–induced apathy and memory in the triple transgenic mouse model Alzheimer's disease (García-Mesa et al., 2014). However, the effects of voluntary exercise, estrogen replacement, and their combination on learning and memory deficits induced by ovariectomy and also the possible role of BDNF on this process have not been well defined. Thus, the present study investigated the effects of voluntary exercise, estrogen replacement, and their combination on cognitive performance and hippocampal BDNF in ovariectomized rats. We hypothesize that physical activity and estrogen replacement can improve ovariectomy-induced impairments in spatial learning and memory and hippocampal BDNF in rats.

2. Methods

2.1. Animals and experimental groups

Adult female Wistar rats (210 ± 10 g) were housed in cages (n = 4–5 per cage) in a 12-h light/dark cycle at 22–24 °C, with food and water ad libitum. All procedures were conducted in agreement with the National Institutes of Health Guide for the care and use of laboratory animals. Also, in each experiment care was taken to use the minimum number of animals.

Rats were randomly assigned to 6 groups (n = 10 animals per group): Sedentary-Vehicle-Sham (SED/VEH-Sham), Sedentary-Vehicle-Ovariectomy (SED/VEH-OVX), Voluntary Exercise-Vehicle-Sham (VE/VEH-Sham), Voluntary Exercise-Vehicle-Ovariectomy (VE/VEH-OVX), Sedentary-17 β-Estradiol-Ovariectomy (SED/EST-OVX), and Voluntary Exercise-17 β-Estradiol-Ovariectomy (VE/EST-OVX). The exercising rats were given the 14 days of voluntary exercise according to the procedure described the below. Vehicle or 17 β-Estradiol was injected once per day (at 6 pm) for 14 days. One day after exercise, the animals in all groups were subjected to learning and memory tests using the water maze (WM) as described the below. One day after the WM test, all animals in each group were decapitated under ketamine anesthesia (100 mg/kg) and their hippocampi were dissected over wet ice and were then immediately frozen at −70 °C until used for assessment of protein BDNF levels (Fig. 1). We used this paradigm to examine the therapeutic effects of exercise and estrogen therapy on OVX-induced behavioral and biochemical deficits. To prevent any possible interference of these treatments with behavioral and biochemical changes during training and testing, exercise and drug injection was terminated before the starting the study.

2.2. Drugs

17 β-Estradiol (Sigma Co.) was injected subcutaneously at a dose of 20 μg/kg in a volume of 2 ml/kg on the dorsal surface of the neck. The drug was dissolved in 96% ethanol and diluted with a 0.9% physiological saline solution to reach the appropriate concentration. The final concentration of ethanol was 4%. The vehicle contained the same ethanol concentration. This dose was selected based on other studies (Inagaki et al., 2010; Luine, 2016).

2.3. Ovariectomy procedure

The rats were anesthetized by intra-peritoneal injections of 70 mg/kg ketamine and 10 mg/kg xylazine and underwent either bilateral OVX or sham surgery. The flank midline skin was cut and retracted so that small incisions (1 cm) could be cut bilaterally in the abdominal wall below the 12th rib. OVX was performed by carefully removing both ovaries and the surrounding fat with forceps. Control rats underwent sham surgery, which involved locating the ovary in question and lightly touching it with a sterile surgical instrument. Immediately following surgery, all rats were injected with penicillin (0.2 ml, 30,000 IU) and were monitored daily.

2.4. Exercise paradigm

Each of the exercising rats were given access to a running wheel

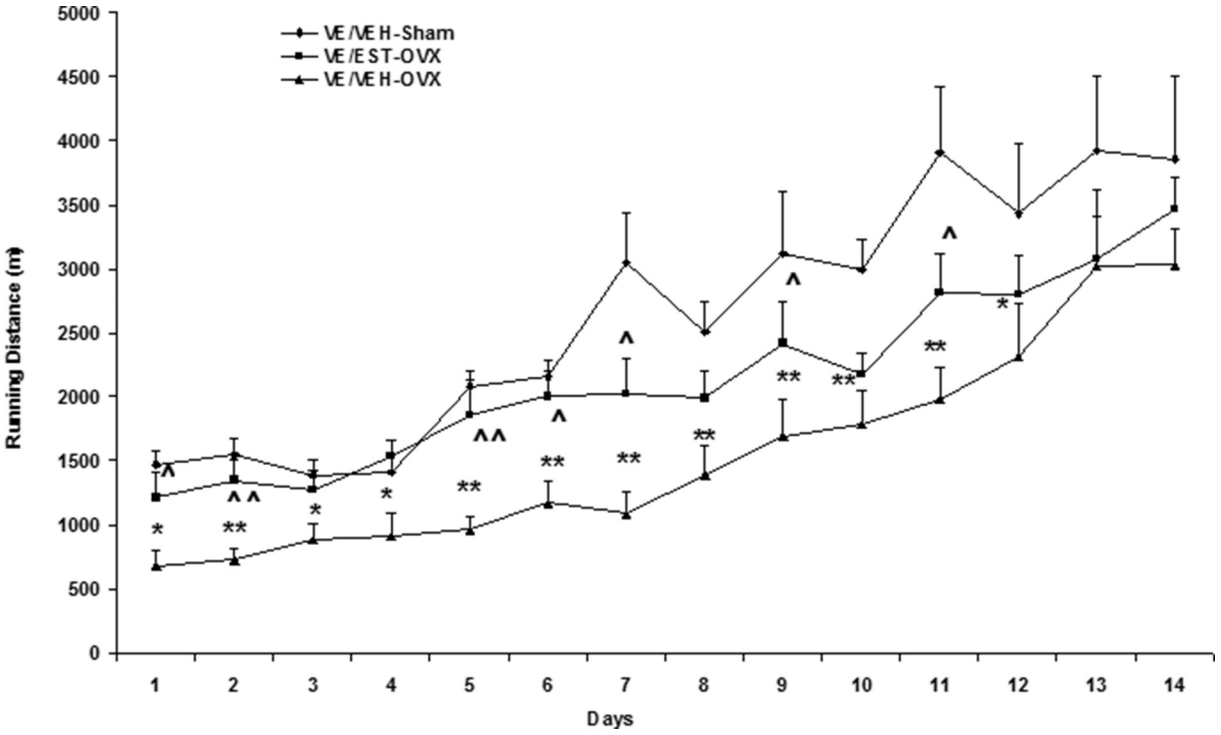

Fig. 2. Effect of ovariectomy (OVX) and 17 β-estradiol administration on the total running distance in the exercising groups. The data are expressed as the mean ± SEM of the total running distance in meters per day for each exercise group. The running distance was monitored by counter devices attached to each cage. *P < 0.05 compared the VE/EST/OVX group with VE/VEH/OVX group. VEH: vehicle; EST: 17 β-estradiol; VE: voluntary exercise; OVX: ovariectomy. *P < 0.05, and **P < 0.01 significant difference between the VE/VEH-Sham and VE/VEH-OVX; ^P < 0.05, ^^P < 0.01 significant difference between the VE/EST-OVX and VE/VEH-OVX groups.

(diameter = 34.5 cm, width = 9.5 cm) that was freely rotated against a resistance of 100 g for 14 days. Each wheel was equipped with a magnetic switch, which was connected to a separate counter located outside of the animal house, so it was possible to check the number of revolutions without disturbing the exercising animals. The revolutions of each wheel were recorded every day at 6 AM. The distance (in meters) of each animal ran was then calculated. The sedentary rats were confined to similar cages with no access to running wheels.

2.5. Testing of spatial learning and memory in the WM

For the WM test, we used a swimming pool (140 cm diameter, 60 cm height) that was divided into four quadrants. The quadrant housing the escape platform (11 cm diameter) was designated as the target zone, such that the escape platform was fixed in a permanent position 2 cm under the water surface during the WM procedure. The other three quadrants were designated as left, right, and opposite to the target zone. The water was kept at a steady 22 °C ± 2 °C. Spatial reference cues around the pool were maintained in fixed positions throughout the duration of the WM experiments. A camera suspended above the maze was wired to an automated tracking system (EthoVision, Version 3.1, the Netherlands). The WM protocol which was used in this report has been described elsewhere (Ebrahimi et al., 2010). Twenty-four hours prior to the start of training, rats were allowed swimming 3 min in the pool containing no platform for habituation. In the WM training, each rat was given 4 trials per day for 5 consecutive days. Each trial lasted until the rat found the platform or for a maximum duration of 60 s. Animals who failed to find the platform within the allocated time were gently guided to the platform. At the end of each trial, the animals were allowed to stay on the platform for 20 s. The escape latency (platform search time) for each trial was recorded. After the last trial, each animal was towel dried and returned to its home cage with no access to a running wheel. A spatial probe test was performed 2 d after the last acquisition trial, during which the platform was removed. The rats were allowed to swim for 60 s during which the time spent swimming within each zone, the platform location latency, proximity (the average distance from center of the platform across the probe test), and velocity were recorded.

2.6. BDNF measurements

BDNF protein levels were assessed using rat BDNF ELISA kits (Booster Biological Technology, Wuhan, China) according to the manufacturer's recommendations. The hippocampal extracts were prepared in lysis buffer, and the homogenates were centrifuged to remove insoluble materials (12,500 × *g* for 20 min at 4 °C). The total protein concentration was determined according to the Micro BCA procedure (Pierce, Rockford, IL, USA). After the antibody incubation, the plates were washed three times with TBS and incubated for 1 h (at room temperature) with avidin–biotin–peroxidase complex (ABC). After the incubation, an ABC working solution was added to each well, which was then incubated at room temperature for 30 min and washed 5 times with TBS. Then, TMB color developing agent was added to each well followed by incubation for 30 min at room temperature. After the samples turned blue, the reaction was stopped by the addition of the TMP stop solution, and the absorbance was measured at 450 nm using an automated ELISA plate reader. The sensitivity of the assay was > 2 pg/ml.

2.7. Statistical analysis

Acquisition data were analyzed by three-way analysis of variance (ANOVA) with repeated measures. The three factors were: sedentary or exercise (two levels), sham group receiving vehicle or estrogen, and ovariectomized group receiving vehicle or estrogen (4 levels) and days (5 levels). Other data were analyzed by two-way ANOVA. ANOVAs

were followed by Tukey's test for multiple comparisons. The Pearson correlation test was used to examine the association between BDNF levels and memory retention (percentage of time spent in the target zone) in exercising OVX rats. Sample sized was determined based on previous studies and the statistical power rangers were from 50 to 90%. Statistical differences were considered significant when $P < 0.05$.

3. Results

3.1. Running distance

A two-way ANOVA for the average distance run (m) at 14 days voluntary exercise revealed significant group ($F_{2, 252} = 9.12$, $P = 0.0001$), and day effects ($F_{13, 252} = 9.15$, $P < 0.0001$), and a significant interaction between both factors ($F_{26, 126} = 2.26$, $P = 0.003$) (Fig. 2). The running distance in the VE/VEH-OVX group was significantly lower than the VE/VEH-Sham group in days 1–12 running (all, Ps < 0.05 to < 0.01). The running distance in the VE/EST-OVX group was significantly higher than the VE/VEH-OVX group in days 1–2, 5–7, 9 and 11 (Ps < 0.05 to < 0.01).

3.2. Acquisition

Acquisition data of the experimental groups during 5 days training are illustrated in Fig. 3. A three-way ANOVA on escape latencies data (exercise × groups × day) with repeated measures on days showed significant effects of exercise ($F_{1, 54} = 13.43$, $P = 0.0006$), groups ($F_{2, 54} = 5.11$, $P = 0.009$) and days ($F_{4, 216} = 66.71$, $P < 0.0001$) and a significant interaction between exercise × groups ($F_{2, 54} = 3.19$, $P = 0.048$). No significant interactions between exercise × groups ($F_{4, 216} = 0.61$, $P = 0.65$), between groups × days ($F_{8, 216} = 0.55$, $P = 0.81$) and between exercise × groups × days ($F_{8, 216} = 0.25$, $P = 0.97$) were found (Fig. 3). Between-group comparisons showed that escape latency of the SED/VEH-OVX group was significantly higher than the SED/VEH-Sham group in days 4 and 5 ($P < 0.05$). The escape latency of the VE/VEH-Sham group was significantly shorter than the SED/VEH-Sham group in all tested days (all, $P < 0.05$). Escape latency of the VE/VEH-OVX group was significantly shorter than the SED/VEH-OVX group in days 3–5 ($P < 0.05$). The escape latency of the SED/EST-OVX and VE/EST-OVX groups was significantly shorter than the SED/VEH-OVX group on days 3–5 (all, $P < 0.05$). These findings show that exercise and estrogen restored the impairing effects of OVX on learning acquisition replacement (Fig. 3).

3.3. Retention

The data from the memory retention test are shown in Fig. 4. A two-way ANOVA (exercise × groups) for platform location latency showed a significant effect of exercise ($F_{1, 54} = 41.84$, $P = 0.0001$), no significant of groups ($F_{2, 54} = 2.48$, $P < 0.09$) and no significant interaction between both factors ($F_{2, 54} = 0.99$, $P = 0.37$). Between-group comparisons showed that the SED/VEH-OVX group showed higher platform location latency than the SED/VEH-Sham group ($P < 0.05$). The SED/EST-OVX and VE/EST-OVX groups showed the lower platform location latency than the SED/VEH-OVX group ($P < 0.05$ and $P < 0.01$, respectively). The VE/VEH-OVX group showed the lower platform location latency than the SED/VEH-OVX group ($P < 0.05$). The VE/VEH-Sham group showed the lower platform location latency than the SED/VEH-Sham group ($P < 0.01$) (Fig. 4A).

A two-way ANOVA (exercise × groups) for time spent in the target zone showed a significant effect of exercise ($F_{1, 54} = 7.06$, $P = 0.01$), no significant effect of groups ($F_{2, 54} = 1.98$, $P = 0.14$) and no significant interaction between both factors ($F_{2, 54} = 0.75$, $P = 0.47$). Between-group comparisons indicated that the SED/VEH-OVX group spent

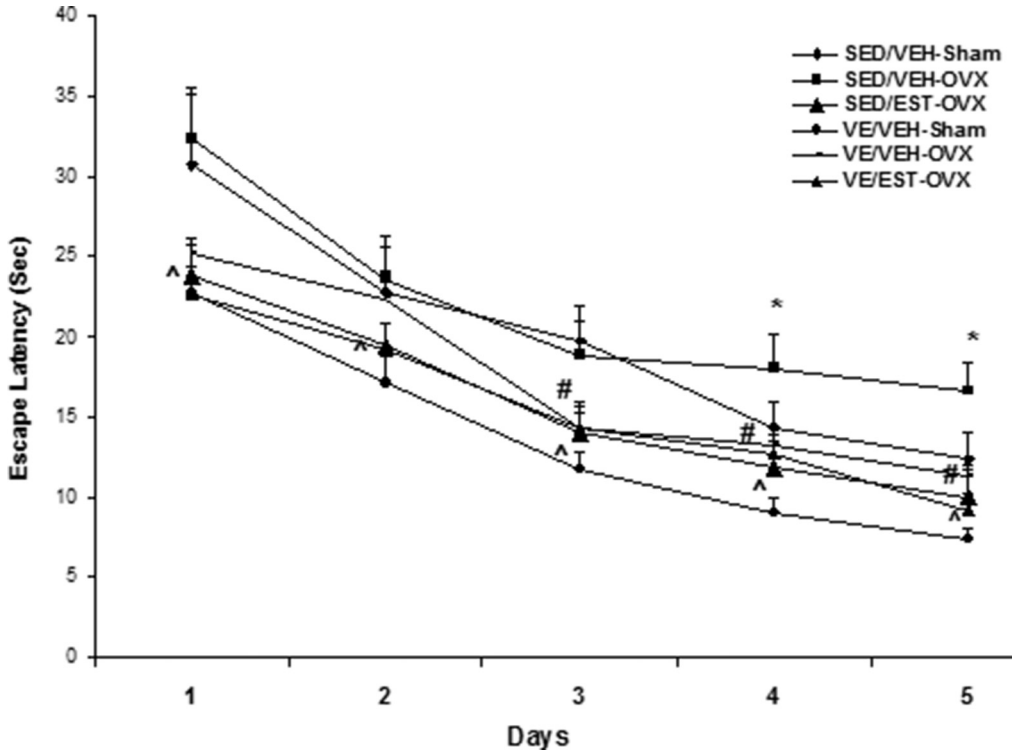

Fig. 3. Effect of voluntary exercise and 17 β-estradiol administration on ovariectomy (OVX)-induced impairment of spatial learning in the water maze task. Exercise improved learning ability (i.e., the exercising animals took significantly less time to learn the location of the platform than their sedentary control counterparts). OVX impaired spatial learning, and this effect was rescued by exercise, and 17 β-estradiol. ^P < 0.05 compared the VE/VEH-Sham group with the SED/VEH-Sham group on the same day. #P < 0.01 compared the SED/EST-OVX, and VE/EST-OVX groups with the SED/VEH-OVX group on the same day. *P < 0.05 compared the SED/VEH-OVX group with the SED/VEH-Sham group on the same day. The data are expressed as the mean ± S.E.M. SED: Sedentary. Other legends are the same as Fig. 2.

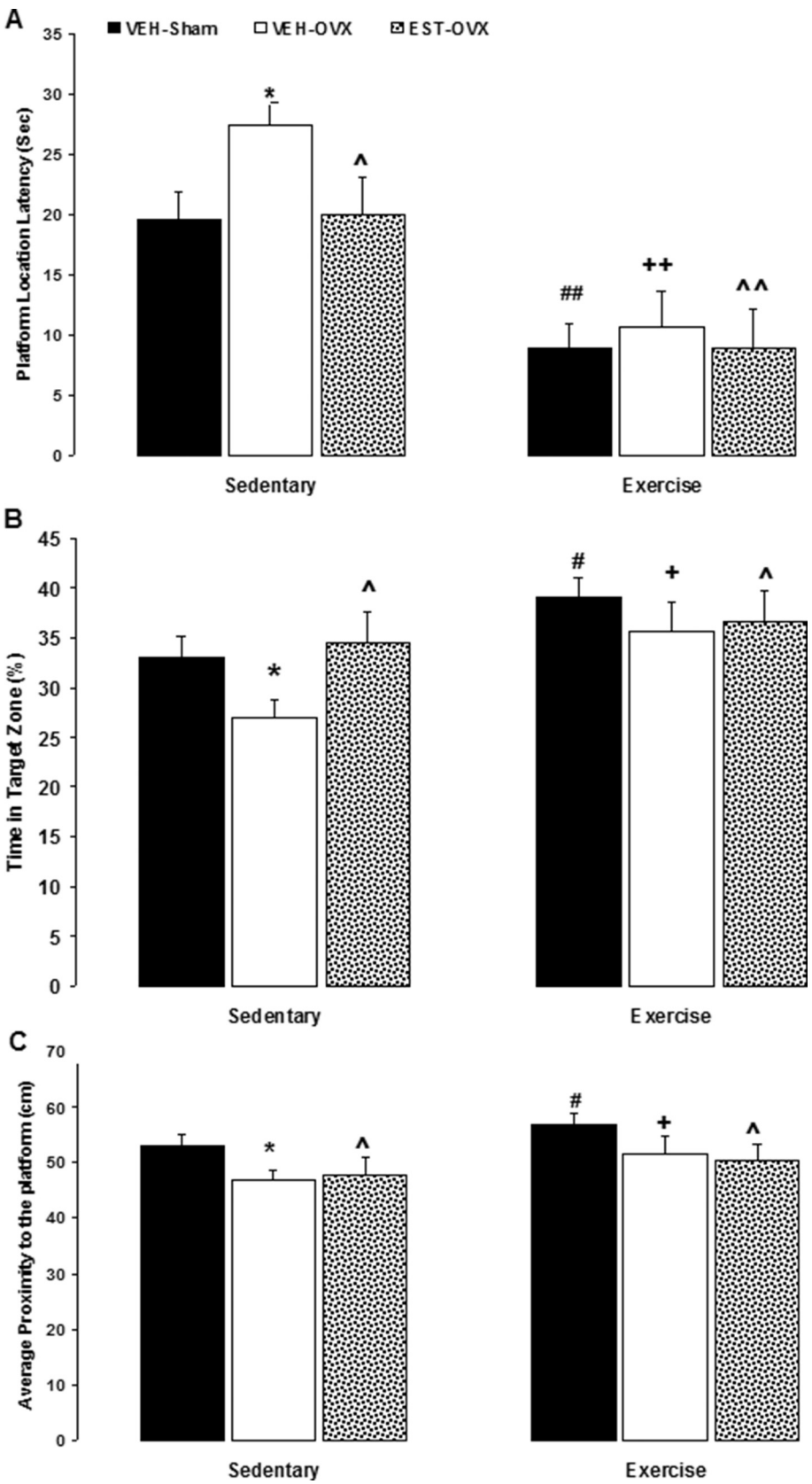

Fig. 4. Effect of voluntary exercise and 17 β-estradiol administration on ovariectomy (OVX)-induced impairment of spatial memory retention using the probe test in the water maze task. (A) Mean latency to reach the platform location, (B) mean time spent in target zone and (C) the average proximity to the platform. Exercise improved memory retention in vehicle-treated rats (i.e., exercising animals spent significantly more time in the target quadrant and had smaller proximity than their sedentary control counterparts). OVX disrupted memory retention, and this effect was reversed by exercise, and 17 β-estradiol. $^{*}P < 0.05$ compared with the SED/VEH-Sham group, $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ compared with the SED/VEH-OVX, $^{+}P < 0.05$ and $^{++}P < 0.01$ compared with the SED/VEH-OVX, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the SED/VEH-Sham. The data are expressed as the mean ± S.E.M.

significantly lower time in the target zone than the SED/VEH-Sham group ($P < 0.05$). The SED/EST-OVX and VE/EST-OVX groups spent more time in the target zone than SED/VEH-OVX group (both, $P < 0.05$). The VE/VEH-OVX group spent significantly more time in this zone than the SED/VEH-OVX group ($P < 0.05$). The VE/VEH-Sham group spent more time in the target zone than the SED/VEH-Sham group ($P < 0.01$) (Fig. 4B).

The average proximity to the platform is shown in Fig. 4C. A two-

way ANOVA showed a significant effect of exercise ($F_{1, 54} = 41.84$, $P = 0.0001$), no significant of groups ($F_{2, 54} = 2.48$, $P < 0.09$) and no significant interaction between both factors ($F_{2, 54} = 0.99$, $P = 0.37$). Between-group comparisons showed that the SED/VEH-OVX group had significantly higher proximity than the SED/VEH-Sham group ($P < 0.05$). The SED/EST-OVX and VE/EST-OVX groups had lower proximity than the SED/VEH-OVX group (both, $P < 0.05$). The VE/VEH-OVX group had significantly lower proximity than the SED/VEH-OVX group ($P < 0.05$). The VE/VEH-Sham group had lower proximity than the SED/VEH-Sham group ($P < 0.05$) (Fig. 4C). Together, these findings show that both exercise and administration of EST improved the impairing effects of OVX on memory retention.

To control for differences in the WM performance, we also recorded each rat's swimming speed during probe test. We found none differences ($F_{5, 54} = 1.78$, $P = 0.13$) in the swimming speeds between the six groups: SED/VEH-Sham (23.9 ± 3.63 cm/s), SED/VEH-OVX (24.78 ± 4.03 cm/s), SED/EST-OVX (24.9 ± 3.34 cm/s), VE/VEH-Sham (27.7 ± 2.43 cm/s), VE/VEH-OVX (24.4 ± 3.67 cm/s) and VE-EST-OVX (27.4 ± 5.2 cm/s).

3.4. BDNF data

The data on the effect of exercise and estrogen administration on BDNF protein levels in the hippocampus are shown in Fig. 5. Total BDNF of the hippocampus was expressed as % of SED/VEH-Sham group. A two-way ANOVA for BDNF levels showed significant effects of exercise ($F_{1, 42} = 4.88$, $P = 0.03$), of groups ($F_{2, 42} = 4.6$, $P = 0.01$), and significant interaction between the both factors ($F_{2, 42} = 4.9$, $P = 0.01$). Between-group comparisons indicated that the SED/VEH-OVX group had significantly lower hippocampal BDNF levels than the SED/VEH-Sham ($P < 0.05$). The SED/EST-OVX and VE/EST-OVX groups had higher BDNF levels than SED/VEH-OVX ($P < 0.05$, $P < 0.01$, respectively). The VE/VEH-OVX group had significantly lower BNDF levels than the SED/VEH-OVX group ($P < 0.05$). The VE/VEH-Sham group had higher BDNF levels than the SED/VEH-Sham ($P < 0.01$).

We found that exercise led to a significantly greater increase in the levels of BDNF in the hippocampus of the VE/VEH-Sham group compared with the SED/VEH-Sham group ($P = 0.01$). In addition, we found that EST administration enhanced the levels of BDNF in the SED/EST-OVX group compared with the SED/VEH-OVX group ($P < 0.03$). The levels of BDNF in the VE/VEH-NOVX group were significantly higher than the BDNF levels in the VE/VEH-OVX group ($P < 0.01$). These findings indicate that exercise and estrogen could improve the impairing effects of OVX on BDNF levels.

To correlate between behavioral (memory retention) and BDNF levels, we analyzed the correlation between time spent in the target zone and hippocampal BDNF levels in OVX exercising rats. We found a significant positive correlation in individual rats, between the BDNF levels and memory performance (time in target zone) in both VE/VEH-OVX ($r = 0.982$, $P < 0.01$), and VE/EST-OVX ($r = 0.935$, $P < 0.01$) groups (Fig. 6). These data show a positive correlation between the amount of hippocampal BDNF and memory performance.

4. Discussion

The present study showed that ovariectomy causes impairment in spatial learning and memory and hippocampal BDNF. Exercise, 17 β-estradiol and their combination improved the impairing effects of ovariectomy on learning and memory performance and hippocampal BDNF levels. The combined treatment did not produce stronger effect than either voluntary exercise or 17 β-estradiol alone. These findings indicate that voluntary exercise and 17 β-estradiol could restore the detrimental effects of ovariectomy on cognitive function and hippocampal BDNF levels in an experimental menopause rat model.

In this study, we investigated the effects of ovariectomy on spatial learning and memory and hippocampal BDNF. We examined adult female rats, which are commonly used animal models in experiments

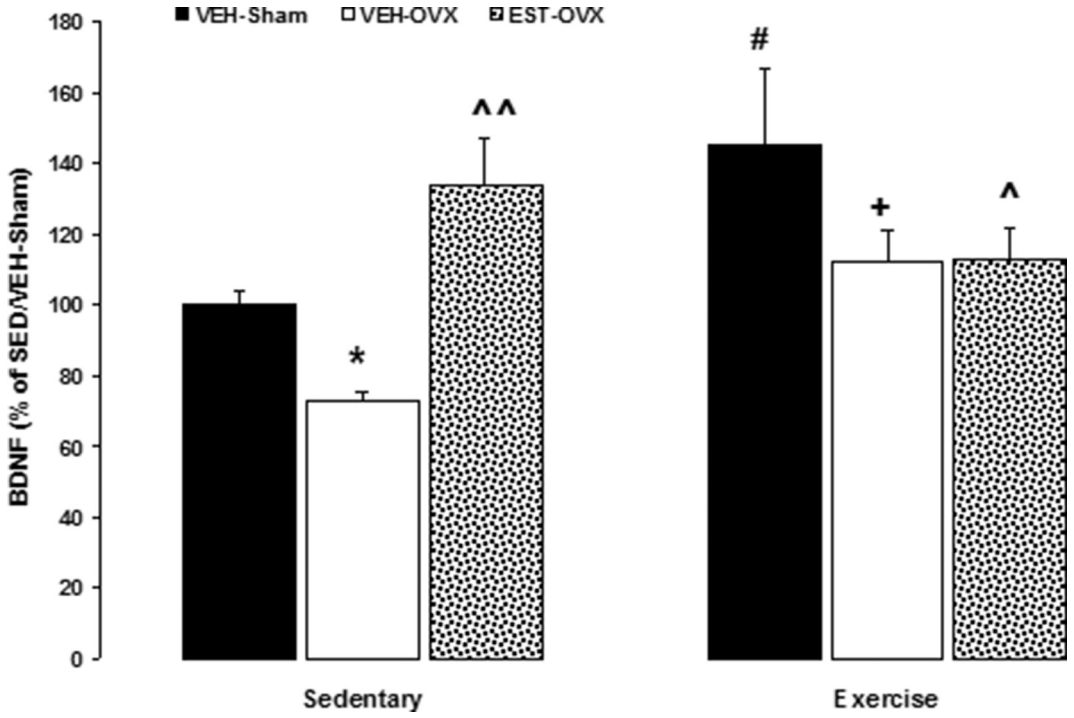

Fig. 5. Effect of voluntary exercise and 17 β-estradiol administration on ovariectomy (OVX)-induced deficit of the BDNF levels in the hippocampus. The data are expressed as the mean ± S.E.M of % SED/VEH-Sham group. The BDNF levels are displayed as the percentages of the SED/VEH-Sham group control levels. Exercise significantly increased BDNF protein. OVX deficit of BDNF in hippocampus, and this effect was rescued by exercise, and 17 β-estradiol. *$P < 0.05$ compared with the SED/VEH-Sham group, ^$P < 0.05$ and ^^$P < 0.01$ compared the SED/VEH-OVX group, $^{+}P < 0.05$ compared with SED/VEH-OVX group, the $^{\#}P < 0.01$ compared with the SED/VEH-Sham group.

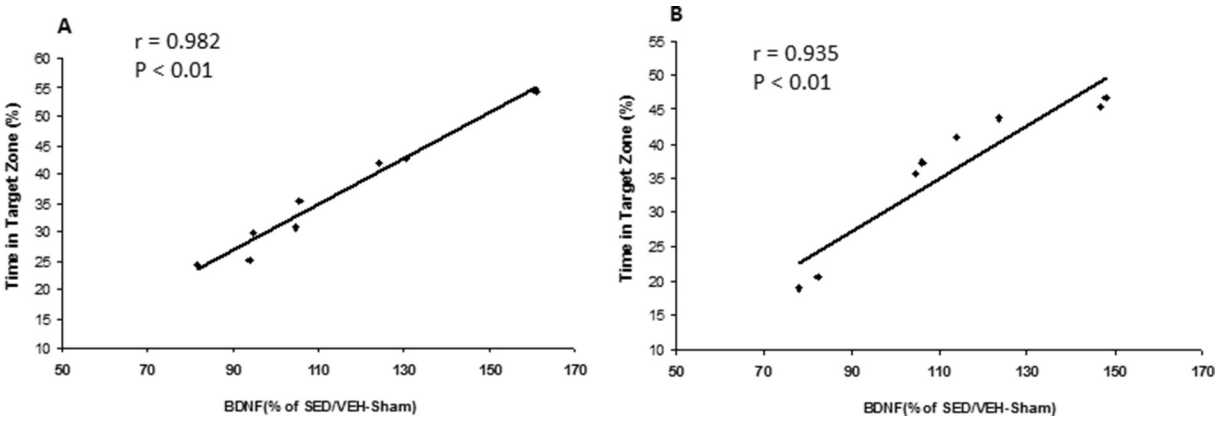

Fig. 6. The correlation between the amount of hippocampal BDNF and memory performance in exercising ovariectomized rats. A significant positive correlation was found between the amount of hippocampal BDNF levels and memory performance in the VE/VEH-OVX (A) and VE/EST-OVX (B) groups.

involving hormones (Savonenko and Markowska, 2003) in spatial navigation tasks, such as the Morris water maze. Based on our results, ovariectomy impaired spatial learning and memory in the water maze. The differences in the water maze performance between ovariectomized and control rats were not because of motor impairment as ovariectomy not influenced their swimming speeds. Voluntary running wheel exercise for 14 days improved the performance of rats and prevented cognitive impairment caused by ovariectomy, suggesting that voluntary running exercise reverses memory impairment caused by ovariectomy in rats. Our results are consistent with previous studies on ovariectomized rats and spatial memory impairment (Monteiro et al., 2005), which showed voluntary or forced exercise improves either passive avoidance or Morris water maze performance in rats (Alaei et al., 2006). A clinical study found a relationship between exercise and the improvement of memory in menopausal women (Aiello et al., 2004). Also, based on a large prospective clinical study carried out in old women, long-term regular physical activity led to higher levels of cognitive function and prevented cognitive decline (Weuve et al., 2004).

Several mechanisms may mediate the beneficial effects of exercise against learning and memory loss in ovariectomized rats. A recent study has that physical exercise may exert its beneficial effects on memory performance in ovariectomized via increasing hippocampal estrogen and estrogen receptor-β levels (KAIDAH, 2016). Exercise also ameliorated depression-like behavior in ovariectomized rats via increasing serum estrogen levels (Lu et al., 2014). Also, it has been shown that combined exercise ameliorates ovariectomy-induced cognitive impairment by enhancing cell proliferation and suppressing apoptosis (Kim et al., 2016). Exercise causes a variety of morphological changes, most likely leading to changes in brain structure and function (Booth and Lees, 2006). Running exercise leads to the release of trophic factors such as BDNF, which stimulates neurogenesis and control brain neuronal function (Wu et al., 2007). It is reported the greater cell proliferation and survival in the dentate gyrus (DG) of the hippocampus in rats undergoing exercise training (Trejo et al., 2001), and these proliferated cells are recently considered facilitating learning and memory (Hillman et al., 2008). Other studies also support that exercise increases neuronal proliferation in the hippocampus under low estrogen conditions (Jin et al., 2008). Although the exact mechanism is not clear, it can be assumed that exercise during periods of low estrogen levels or during menopause may increase cell proliferation in the hippocampus. There is a high correlation between neurogenesis and the improvement of learning and memory. For instance, exercise-induced neuronal proliferation has been shown in the DG of young and aged rats (Kim et al., 2004). The cognitive effects of physical activity might be mediated via an increase in hippocampal neurogenesis, and BDNF levels (Huang et al., 2006), and the modulation of acetylcholinesterase activity in ovariectomized rats (Ben et al., 2009).

We found that systemic injection of 17 β-estradiol recovered learning and memory impairment induced by ovariectomy. A recent study on young female animals showed that systemic or dorsal hippocampal estradiol administration when accompanied with an inhibitor of the enzyme that phosphorylates extracellular signal-regulated kinase (ERK) showed the beneficial effects of estradiol on memory. These data suggest that estradiol facilitates memory consolidation in young female animals through the ERK pathway in the dorsal hippocampus (Fernandez et al., 2008), a mechanism that might also be involved in the results of our study. Some studies have showed that synaptic proteins increase during estrogen administration (Stone et al., 1998) and either long-term potentiation (LTP) or neurogenesis were also elevated (Tanapat et al., 1999). Estrogens improve the function of cholinergic neurons, which originate from the basal forebrain and innervate the hippocampus (Gibbs and Gabor, 2003). Similarly, studies have pointed out that estradiol improves learning and memory performance in aged rats (Markham et al., 2002). Therefore, it is probable that age and the duration of hormone deprivation may have a combined influence on the beneficial effects of estrogen therapy on cognition.

Many studies have shown that hormonal status influences the expression of BDNF and/or its receptor, therefore, suggesting a biological substrate for regulating this neurotrophin by a gonadal hormone (Spencer et al., 2008b). Systemic administration of estrogens enhances BDNF levels and its receptors in the hippocampus (Berchtold et al., 2001; Pan et al., 2010; Spencer et al., 2008a). Both BDNF and estrogens enhance cognitive function and dendritic spines in the hippocampus and prefrontal cortex (Luine and Frankfurt, 2013). Thus, estrogens could enhance brain morphology and cognitive function via BDNF. Recent evidences also show that estrogens and BDNF may work in concert to improve learning and memory (Luine and Frankfurt, 2013). Estrogens replacement in adult ovariectomized female rats increased BDNF expression in many brain regions, including the hippocampus (Engler-Chiurazzi et al., 2011). However, high endogenous estrogens levels were associated with decreased BDNF mRNA in the hippocampus and prefrontal cortex. In our study, 17 β-estradiol treatment was associated with increased BDNF levels in the hippocampus in ovariectomized animals. Since the administration of 17 β-estradiol to ovariectomized animals produces hormone levels similar to those observed in gonadally intact young animals, our results are in accordance with data from the literature showing BDNF protein levels are physiologically elevated during high-estradiol phases, proestrus and estrous (Scharfman et al., 2003), and that in these same phases the BDNF receptor TrkB was activated throughout dorsal hippocampal formation. Adding to that study, circulating BDNF, and the expression of its receptor TrkB in the hippocampus, decreased not only with increasing age but also during prolonged periods of low estrogen levels (Kramar et al.,

2014). These findings corroborate the low BDNF levels found in control adult and middle-aged animals, and in ovariectomized animals. Hippocampal BDNF is of importance because of its role in the process of learning and memory, and especially in spatial memory (Macbeth et al., 2008) and improvement of cognitive function (Komulainen et al., 2008). As hippocampus is implicated in spatial memory performance, we suppose that the elevated BDNF levels found in the hippocampus may underlie the effects of exercise and estrogen treatment on spatial learning and memory in ovariectomized rats. Interestingly, we found a positive correlation between the hippocampal BDNF levels and memory performance in ovariectomized exercising animals.

One limitation of the present study was single housing of the animals during exposure to voluntary exercise, which can be stressful for rodents. However, to reduce the potential confounding effects of a single housing stress during 14 days physical activity on the results of experiments, the corresponding sedentary rats were confined to similar cages with no access to a wheel. Moreover, the lack of the determination of the stage of ovarian cycle in the sham animals is another limitation of the present study.

Menopause-related cognitive impairment is a common complaint. The findings of the present are of clinical significance for improving cognitive functions in menopausal women. Appropriate voluntary exercise, estrogen therapy and their combination therapy can improve cognitive abilities such as learning and memory in menopause woman.

In conclusion, ovariectomy impairs spatial navigation and decreases hippocampal BDNF level in rats. However, these deficits can be recovered by voluntary running exercise, replacement of estrogen, and the combined treatment. Thus, this study provides important evidences about positive influences of regular exercise, estrogen treatment or their combination against learning and memory and hippocampal BDNF in an experimental model of menopause.

Funding

This research was financially supported by a grant to A.A.V and A.R.P. from the Semnan University of Medical Sciences (Semnan, Iran, 311).

Declaration of competing interest

We attest that we have disclosed all financial or other relationships that could be construed as a conflict of interest and that all sources of financial support for this study have been disclosed.

Acknowledgments

We appreciate all of our colleagues for their kind help during the practical work. This work was supported by a grant from Semnan University of Medical Sciences (Semnan, Iran, 311). In addition, Ms Kowsar Bavarsad carried out this work in project fulfillment of the requirements to get the MSc degree in Physiology.

Authors' contributions

A.A.V. and A.R.P. designed the experiment. A.A.V., K.B., H.M.G and ZS. conducted the research, collected data and carried out the lab work. A.A.V. and A.R.P. carried out the statistical analysis and mostly drafted the manuscript. A.A.V. and A.R.P. coordinated and supervised the study. All authors approved the manuscript.

References

Aiello, E.J., Yasui, Y., Tworoger, S.S., Ulrich, C.M., Irwin, M.L., Bowen, D., et al., 2004. Effect of a yearlong, moderate-intensity exercise intervention on the occurrence and severity of menopause symptoms in postmenopausal women. Menopause 11, 382–388.

Alaei, H., Borjeian, L., Azizi, M., Orian, S., Pourshanazari, A., Hanninen, O., 2006. Treadmill running reverses retention deficit induced by morphine. Eur. J. Pharmacol. 536, 138–141.

Baruch, D.E., Swain, R.A., Helmstetter, F.J., 2004. Effects of exercise on Pavlovian fear conditioning. Behav. Neurosci. 118, 1123–1127.

Ben, J., Soares, F.M., Cechetti, F., Vuaden, F.C., Bonan, C.D., Netto, C.A., et al., 2009. Exercise effects on activities of Na(+),K(+)-ATPase, acetylcholinesterase and adenine nucleotides hydrolysis in ovariectomized rats. Brain Res. 1302, 248–255.

Ben, J., Soares, F.M., Scherer, E.B., Cechetti, F., Netto, C.A., Wyse, A.T., 2010. Running exercise effects on spatial and avoidance tasks in ovariectomized rats. Neurobiol. Learn. Mem. 94, 312–317.

Berchtold, N.C., Kesslak, J.P., Pike, C.J., Adlard, P.A., Cotman, C.W., 2001. Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. Eur. J. Neurosci. 14, 1992–2002.

Booth, F.W., Lees, S.J., 2006. Physically active subjects should be the control group. Med. Sci. Sports Exerc. 38, 405–406.

Brown, B.S., Payne, T., Kim, C., Moore, G., Krebs, P., Martin, W., 1979. Chronic response of rat brain norepinephrine and serotonin levels to endurance training. J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 46, 19–23.

Ebrahimi, S., Rashidy-Pour, A., Vafaei, A.A., Akhavan, M.M., 2010. Central beta-adrenergic receptors play an important role in the enhancing effect of voluntary exercise on learning and memory in rat. Behav. Brain Res. 208, 189–193.

Engler-Chiurazzi, E., Tsang, C., Nonnenmacher, S., Liang, W.S., Corneveaux, J.J., Prokai, L., et al., 2011. Tonic Premarin dose-dependently enhances memory, affects neurotrophin protein levels and alters gene expression in middle-aged rats. Neurobiol. Aging 32, 680–697.

Fernandez, S.M., Lewis, M.C., Pechenino, A.S., Harburger, L.L., Orr, P.T., Gresack, J.E., et al., 2008. Estradiol-induced enhancement of object memory consolidation involves hippocampal extracellular signal-regulated kinase activation and membrane-bound estrogen receptors. J. Neurosci. 28, 8660–8667.

Frick, K.M., 2009. Estrogens and age-related memory decline in rodents: what have we learned and where do we go from here? Horm. Behav. 55, 2–23.

Frick, K.M., Fernandez, S.M., Bennett, J.C., Prange-Kiel, J., MacLusky, N.J., Leranth, C., 2004. Behavioral training interferes with the ability of gonadal hormones to increase CA1 spine synapse density in ovariectomized female rats. Eur. J. Neurosci. 19, 3026–3032.

García-Mesa, Y., Pareja-Galeano, H., Bonet-Costa, V., Revilla, S., Gómez-Cabrera, M.C., Gambini, J., et al., 2014. Physical exercise neuroprotects ovariectomized 3xTg-AD mice through BDNF mechanisms. Psychoneuroendocrinology 45, 154–166.

Genazzani, A.R., Pluchino, N., Luisi, S., Luisi, M., 2007. Estrogen, cognition and female ageing. Hum. Reprod. Update 13, 175–187.

Gibbs, R.B., Gabor, R., 2003. Estrogen and cognition: applying preclinical findings to clinical perspectives. J. Neurosci. Res. 74, 637–643.

Grealy, M.A., Johnson, D.A., Rushton, S.K., 1999. Improving cognitive function after brain injury: the use of exercise and virtual reality. Arch. Phys. Med. Rehabil. 80, 661–667.

Heikkinen, T., Puoliväli, J., Liu, L., Rissanen, A., Tanila, H., 2002. Effects of ovariectomy and estrogen treatment on learning and hippocampal neurotransmitters in mice. Horm. Behav. 41, 22–32.

Hillman, C.H., Erickson, K.I., Kramer, A.F., 2008. Be smart, exercise your heart: exercise effects on brain and cognition. Nat. Rev. Neurosci. 9, 58–65.

Huang, A.M., Jen, C.J., Chen, H.F., Yu, L., Kuo, Y.M., Chen, H.I., 2006. Compulsive exercise acutely upregulates rat hippocampal brain-derived neurotrophic factor. J. Neural Transm. 113, 803–811.

Inagaki, T., Gautreaux, C., Luine, V., 2010. Acute estrogen treatment facilitates recognition memory consolidation and alters monoamine levels in memory-related brain areas. Horm. Behav. 58, 415–426.

Jin, J., Jing, H., Choi, G., Oh, M.S., Ryu, J.H., Jeong, J.W., et al., 2008. Voluntary exercise increases the new cell formation in the hippocampus of ovariectomized mice. Neurosci. Lett. 439, 260–263.

Kaidah, S., 2016. Exercise improves hippocampal estrogen and spatial memory of ovariectomized rats. Bratisl. Med. J. 116 (2), 94–99.

Kim, H.J., Casadesus, G., 2010. Estrogen-mediated effects on cognition and synaptic plasticity: what do estrogen receptor knockout models tell us? Biochim. Biophys. Acta 1800, 1090–1093.

Kim, Y.P., Kim, H., Shin, M.S., Chang, H.K., Jang, M.H., Shin, M.C., et al., 2004. Age-dependence of the effect of treadmill exercise on cell proliferation in the dentate gyrus of rats. Neurosci. Lett. 355, 152–154.

Kim, T.-W., Kim, C.-S., Kim, J.-Y., Kim, C.-J., Seo, J.-H., 2016. Combined exercise ameliorates ovariectomy-induced cognitive impairment by enhancing cell proliferation and suppressing apoptosis. Menopause 23, 18–26.

Komulainen, P., Pedersen, M., Hänninen, T., Bruunsgaard, H., Lakka, T.A., Kivipelto, M., et al., 2008. BDNF is a novel marker of cognitive function in ageing women: the DR's EXTRA Study. Neurobiol. Learn. Mem. 90, 596–603.

Kramar, C.P., Chefer, V.I., Wise, R.A., Medina, J.H., Barbano, M.F., 2014. Dopamine in the dorsal hippocampus impairs the late consolidation of cocaine-associated memory. Neuropsychopharmacology 39, 1645–1653.

Laurin, D., Verreault, R., Lindsay, J., MacPherson, K., Rockwood, K., 2001. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch. Neurol. 58, 498–504.

Lu, J., Xu, Y., Hu, W., Gao, Y., Ni, X., Sheng, H., et al., 2014. Exercise ameliorates depression-like behavior and increases hippocampal BDNF level in ovariectomized rats. Neurosci. Lett. 573, 13–18.

Luine, V., 2016. Estradiol: mediator of memories, spine density and cognitive resilience to stress in female rodents. J. Steroid Biochem. Mol. Biol. 160, 189–195.

Luine, V., Frankfurt, M., 2013. Interactions between estradiol, BDNF and dendritic spines

in promoting memory. Neuroscience 239, 34–45.
Macbeth, A.H., Scharfman, H.E., Maclusky, N.J., Gautreaux, C., Luine, V.N., 2008. Effects of multiparity on recognition memory, monoaminergic neurotransmitters, and brain-derived neurotrophic factor (BDNF). Horm. Behav. 54, 7–17.
Markham, J.A., Pych, J.C., Juraska, J.M., 2002. Ovarian hormone replacement to aged ovariectomized female rats benefits acquisition of the Morris water maze. Horm. Behav. 42, 284–293.
Monteiro, S.C., Matté, C., Bavaresco, C.S., Netto, C.A., Wyse, A.T., 2005. Vitamins E and C pretreatment prevents ovariectomy-induced memory deficits in water maze. Neurobiol. Learn. Mem. 84, 192–199.
Pan, M., Li, Z., Yeung, V., Xu, R.-J., 2010. Dietary supplementation of soy germ phytoestrogens or estradiol improves spatial memory performance and increases gene expression of BDNF, TrkB receptor and synaptic factors in ovariectomized rats. Nutr. Metab. 7, 75.
Sandstrom, N.J., Williams, C.L., 2001. Memory retention is modulated by acute estradiol and progesterone replacement. Behav. Neurosci. 115, 384–393.
Savonenko, A.V., Markowska, A.L., 2003. The cognitive effects of ovariectomy and estrogen replacement are modulated by aging. Neuroscience 119, 821–830.
Scharfman, H.E., Mercurio, T.C., Goodman, J.H., Wilson, M.A., MacLusky, N.J., 2003. Hippocampal excitability increases during the estrous cycle in the rat: a potential role for brain-derived neurotrophic factor. J. Neurosci. 23, 11641–11652.
Shangold, M.M., 1990. Exercise in the menopausal woman. Obstet. Gynecol. 75, 53S–58S (discussion 81S-3S).
Sherwin, B.B., 2005. Estrogen and memory in women: how can we reconcile the findings? Horm. Behav. 47, 371–375.
Spencer, J.L., Waters, E.M., Milner, T.A., McEwen, B.S., 2008a. Estrous cycle regulates activation of hippocampal Akt, LIM kinase, and neurotrophin receptors in C57BL/6 mice. Neuroscience 155, 1106–1119.
Spencer, J.L., Waters, E.M., Romeo, R.D., Wood, G.E., Milner, T.A., McEwen, B.S., 2008b. Uncovering the mechanisms of estrogen effects on hippocampal function. Front. Neuroendocrinol. 29, 219–237.
Stone, D.J., Rozovsky, I., Morgan, T.E., Anderson, C.P., Finch, C.E., 1998. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease. J. Neurosci. 18, 3180–3185.
Suzuki, S., Brown, C.M., Wise, P.M., 2006. Mechanisms of neuroprotection by estrogen. Endocrine 29, 209–215.
Tanapat, P., Hastings, N.B., Reeves, A.J., Gould, E., 1999. Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J. Neurosci. 19, 5792–5801.
Trejo, J.L., Carro, E., Torres-Aleman, I., 2001. Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. J. Neurosci. 21, 1628–1634.
van Praag, H., Kempermann, G., Gage, F.H., 1999. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat. Neurosci. 2, 266–270.
Vaynman, S., Ying, Z., Gomez-Pinilla, F., 2004. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur. J. Neurosci. 20, 2580–2590.
Weuve, J., Kang, J.H., Manson, J.E., Breteler, M.M., Ware, J.H., Grodstein, F., 2004. Physical activity, including walking, and cognitive function in older women. JAMA 292, 1454–1461.
Wu, C.W., Chen, Y.C., Yu, L., Chen, H.I., Jen, C.J., Huang, A.M., et al., 2007. Treadmill exercise counteracts the suppressive effects of peripheral lipopolysaccharide on hippocampal neurogenesis and learning and memory. J. Neurochem. 103, 2471–2481.