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The effectiveness of continuous and interval exercise preconditioning against chronic unpredictable stress: Involvement of hippocampal PGC-1α/FNDC5/BDNF pathway

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ABSTRACT

Various exercise-training types are known to prevent depression, but mechanisms underlying their beneficial effects remain unknown. In the present study, the preconditioning effect of continuous and interval exercise on stress-induced depression was evaluated. Adult male Wistar rats in the exercise groups were made to run on a motorized treadmill, five sessions per week for six weeks. After that, to induce the depression model, the rats were exposed to chronic unpredictable stress for three weeks. Behavioral tests were assessed by open field, elevated plus maze, and forced swim tests. Hippocampal PGC-1α, FNDC5, and BDNF protein expression by Western blot and serum corticosterone by ELISA were detected. In the present results, after continuous and interval exercise periods, locomotor activity, the number of entries and time spent in the open arms were increased, and immobility time was significantly reduced. PGC-1α, FNDC5, and BDNF protein levels had a significant increase, and serum corticosterone did not change. Also, interval exercise training increased PGC-1α and FNDC5 more than continuous. Chronic unpredictable stress reduced the positive changes caused by exercise training, although, except FNDC5, exercise preconditioned groups experienced less significant adverse changes in most variables. These findings showed that both continuous and interval exercise preconditioning with increasing hippocampal PGC-1α, FNDC5, and BDNF proteins and improve the anxiety- and depression-like behaviors have a protective effect against chronic unpredictable stress.

1. Introduction

Depression is a common mental disorder that more than 322 million people live with this disease in the world. In 2020, depression was a significant contributor to the overall global burden and the second leading cause of disability worldwide, according to the World Health Organization (James et al., 2018). Depression causes many negative changes in the brain, especially the hippocampus, including hippocampal volume reduction (Roddy et al., 2019; Chen et al., 2020), disruption of memory function (Dillon and Pizzagalli, 2018), neuronal atrophy (Khan et al., 2019), and decreased neurotrophic factors (Gross and Seroogy, 2020) contribute to depressive behaviors. The hippocampus plays an essential role in learning and memory and is a significant site where changes occur induce by exercise. Depression can often ensue after exposure to chronic stress. Chronic unpredictable stress (CUS) is probably the most valid animal model of depression and involves the exposure of animals to a series of mild and unpredictable stressors during at least two weeks. The model has been reported to result in long-lasting changes in behavioral and neurochemical variables (Willner, 2005).

Probably performing physical exercise before exposure to stressful conditions prevents, attenuates, and possibly reverses biochemical and behavioral negative changes induced by depression (Mammen and Faulkner, 2013; Schuch et al., 2016; Stavrakakis et al., 2013). A large

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part of the positive effects of physical exercise is believed to be mediated by the increased brain-derived neurotrophic factor (BDNF), one of the most critical neurotrophic factors in the brain from the neurotrophin family (Szuhany et al., 2015; Dinoff et al., 2018). BDNF involved in neuronal differentiation, plasticity, cell survival, hippocampal function, learning, neuronal sprouting, synaptic reorganization, and neurogenesis plays a critical role in the development and maintenance of the peripheral and central nervous system (Kowiański et al., 2018; Szuhany and Otto, 2020). Reduced brain BDNF levels are also associated with CUS and depression (Murakami et al., 2005; LI et al., 2007), while, in contrast, exercise training appears to increase BDNF levels (Fang et al., 2013; Toups et al., 2011). However, the exact mechanism of exercise effects on BDNF has not been established.

There are many exercise dependent signaling pathway to activate BDNF. The newly discovered pathway is related to Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). This protein was first discovered as a transcriptional co-modulator of mitochondrial biogenesis and oxidative metabolism in brown fat (Spiegelman, 2007; Puigserver et al., 1998). It has shown that PGC-1α has a vital role in the brain. The deficiency of PGC-1α is associated with neurodegeneration (Lin et al., 2004; Ma et al., 2010). Also, chronic forced treadmill running increases Pgc1a expression in various areas of the brain (Steiner et al., 2011). During exercise and in response to contraction, PGC-1α-dependent myokine, Fibronectin type III domain-containing protein 5 (FNDC5), is cleaved and secreted from muscle induces some major metabolic benefits of exercise (Boström et al., 2012). FNDC5 is also expressed in the brain, especially the hippocampus (Wrann et al., 2013). There is a strong correlation between PGC-1a and FNDC5 gene expression as PGC-1α is a transcriptional regulator of Fndc5 gene expression. However, very little is known about the function of FNDC5 in the brain. It was shown that PGC-1α−/− mice exhibit low FNDC5 expression in the brain. FNDC5 deficiency impairs neuronal development (Hashemi et al., 2013). Recent research has shown that exercise-induced BDNF gene expression in the hippocampus is relevant to PGC1α/FNDC5/BDNF pathway, and on the other hand, BDNF through FNDC5/BDNF feedback loop negatively regulates FNDC5 (Wrann et al., 2013; Wrann, 2015; Xu, 2013). Therefore, these components form an essential and complex signaling pathway in the hippocampus.

A variety of exercise training methods for brain health against many diseases are recommended. Many recent studies have compared two types of them on treadmill, continuous, and interval exercise training. Therefore, in the present study, we tried to answer two main questions: (1) If the male Wistar rats perform two types of exercise training, continuous and interval, for six weeks and then exposure to three weeks of chronic unpredictable stress, whether PGC1α/FNDC5/BDNF protein expression will be changed? (2) Is there any difference between continuous and interval exercise training? In this study, cognitive function changes based on locomotion, anxiety- and depression-like behaviors were analyzed.

2. Materials and methods

2.1. Animals

Animals were two-month-old adult male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 175–200g. They were maintained under standard laboratory conditions on 22 ± 2 °C under 12-h light/dark cycles and 50 ± 5% humidity with food and water available ad libitum except when they were submitted to CUS. Upon entering the lab, all rats were allowed to adapt to the environment for a week. The experimental protocol was approved by the Ethical Review Board of Shahid Beheshti University of Medical Sciences, Tehran, Iran. All experimental trials were conducted in agreement with the National Institutes of Health Guide (NIH) for the Care and Use of Laboratory Animals. The weight of all animals was monitored weekly. A summary of the study design has shown in Fig. 1.

2.2. Exercise-training protocols

The experimental protocol was performed in two phases: (1) exercise phase and (2) stress phase. In the exercise phase, the rats were randomly divided into three groups: control ($n = 20$), continuous ($n = 12$), and interval ($n = 12$) groups. Rats were familiarized with treadmills for five days before the exercise began (10 min/day at a speed of 10 m/min) to minimize novelty stress. Continuous and interval exercise training was performed for six weeks, five sessions per week. Exercise training was conducted based on the overload principle by increasing time in continuous exercise training, intervals in interval exercise training, and treadmill speed in both groups (Table 1). In the first week, the interval group performed two intervals at 38 m/min. Each week, 1 m/min was added to the speed and 1 to intervals, so that in the fifth and sixth weeks, the training continued with six intervals at 42 m/min (more than 95% VO_2max). The intervals were 2 min, and active rest was performed for 2 min at 16 m/min between them. The first session of the continuous group was performed for 22 min at 23 m/min. Then in each session, 1 min was added to the training time and each week 1 m/min to the speed. The training continued for 42 min at 27 m/min (80% VO2max) in the fifth and sixth weeks. Warm-up and cool-down were performed for 2 min (16 m/min) at the beginning and end of any session that corresponds to 68% VO_2max. At the beginning of each lane, the metal bar grid delivered a mild foot shock (0.5 mA) to encourage the rats to continue training. Eventually, the rats learned to continue running to avoid this shock. After every run and before the next set, rats were put on the treadmill, it was cleaned with 70% ethanol solution, wiped, and air-dried.

2.3. Chronic unpredictable stress (CUS) procedure

After six weeks and in the stress phase, groups ($n = 7$) include Control + Stress (the control group in exercise phase that was exposed to CUS), Control + Non-Stress (the control group in exercise phase that was not exposed to CUS), Continuous + Stress (continuous group in exercise phase that was exposed to CUS) and Interval + Stress (Interval group in exercise phase that was exposed to CUS). The CUS-exposed groups were housed in separate rooms under similar conditions. The CUS procedure was modified from that previously described by Willner et al. (1987) (Willner et al., 1987). Each week of stress regime consisted of 18 h of food deprivation followed by 1 h of limited food access, 18 h of water deprivation followed by 1 h of empty bottle exposure, 21 h of the wet cage (250 ml water in sawdust bedding), two 9-h periods of 45° cage tilting, two 6-h periods of white noise (85 dB), two 6-h periods of low-intensity stroboscopic illumination (150 flashes/min), 24 h of intermittent illumination (lights on and off every 2 h), and 24 h of no stress. All stressors were applied individually and continuously, day and night. This protocol was repeated any week and continued for three weeks.

2.4. Behavioral analysis

2.4.1. Open field (OF)

General locomotor activity was measured by OF apparatus, consisted of a 1 × 1 m area surrounded by a wall 50 cm high, with a video camera (Panasonic Inc., Japan) installed 2 m above. The room that the apparatus was located in was sound-proof and illuminated with controlled light (100 lx). After acclimation of the animal to the test room for a 1-h period, the rat was placed at the center of the arena. Then, total distance traveled (cm) and velocity (cm/sec) were recorded for a 10-min period by Ethovision video tracking software (version 3.1, Noldus Information Technology, The Netherlands). The apparatus was thoroughly cleaned and dried with alcohol.

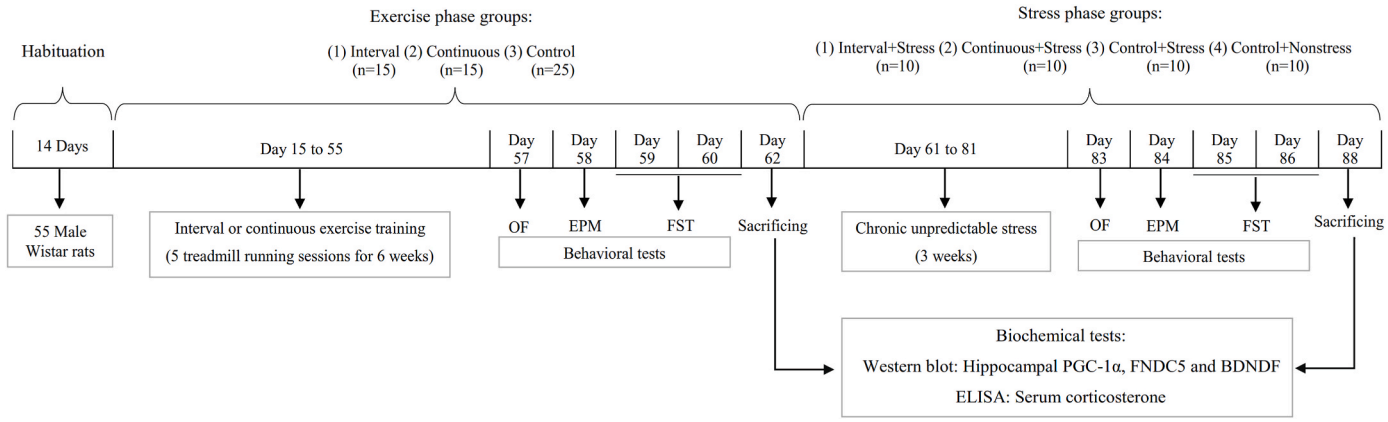


Fig. 1. Schematic time-line of the experimental protocols. The study was conducted in two phases, exercise and stress. In the exercise phase (55 animals), animals performed one of the interval and continuous treadmill running as an exercise preconditioning, and then in the stress phase (40 animals), they exposed to chronic unpredictable stress. At the end of any phase, behavioral and biochemical tests were evaluated. **OF**, open field; **EPM**, elevated plus maze; **FST**, forced swim test.

Table 1
Interval and continuous exercise trainings protocols.

Week	Continuous exercise training	Interval exercise training
1	22–26 min, 23 m/min	2 intervals, 38 m/min, 2 min
2	27–31 min, 24 m/min	3 intervals, 39 m/min, 2 min
3	32–36 min, 25 m/min	4 intervals, 40 m/min, 2 min
4	37–41 min, 26 m/min	5 intervals, 41 m/min, 2 min
5 and 6	42 min, 27 m/min	6 intervals, 42 m/min, 2 min

2.4.2. Elevated plus maze (EPM)

Anxiety-like behavior was evaluated in the EPM apparatus, which was made of two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm), surrounded by 40 cm high wooden walls and raised 50 cm above the floor. All arms were joined in the central neutral area (10 × 10 cm) of the maze so that rats could freely pass from one arm to another. On the test day, each rat was placed onto the maze facing one of the closed arms. Over 5 min, the rat's movement was tracked visually. The observer was blinded to the classification of the groups to avoid bias. Duration and number of the entries into open and close arms were recorded. Criteria for entrance were considered four paws inside of an arm. Exploration or entries in the neutral area were not counted. Following each test, the maze was wiped down with alcohol.

2.4.3. Forced swim test (FST)

The FST took place in a cylinder (50 cm height × 38 cm diameter) filled with water (24 ± 1°C) to 30 cm height. The test consisted of two trials: conditioning and test. One day before the test, during the conditioning trial, a pre-exposure of 15 min swimming was given to ensure that the rats quickly adopt an immobile posture on the test day. On the test day, each rat was placed in the cylinder for 5 min, and the total immobility time was measured. Immobile was defined as the lack of motion of the whole body except for the small movements necessary to keep the animal's head above the water. After the trials and before the rats being returned to their home cages, they were dried and placed into a warm cage with paper towels. An observer that was blind to the classification of the groups recorded the time spent in immobile condition.

2.5. Sample collection and tissue preparation

The rats were anesthetized with ketamine/xylazine, and then blood was collected by cardiac puncture 24 h after the last behavioral test to avoid the behavioral test-induced increase in the corticosterone level. Hippocampal tissue was removed immediately, frozen in liquid nitrogen, and stored at − 80 °C until Western blotting.

2.6. Biochemical analysis

Serum corticosterone: Serum corticosterone levels were determined in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit (corticosterone: Abcam, UK) following the manufacturer's instructions.

Western blot: all samples were homogenized in the appropriate lysis buffer (Niimura et al., 2006), and the total protein extract was prepared by centrifugation at 15000 rpm for 5 min. Standardized lysates equivalent to 60 μg of protein (Bradford, 1976) loaded on SDS-12.5% polyacrylamide gel electrophoresis and transferred to PVDF membrane (Chemicon Millipore Co. Temecula, USA). Then, blots blocked in 2% Electrochemiluminescence (ECL) advanced kit blocking reagent (Amersham Bioscience Co. Piscataway, USA) and probed with rabbit polyclonal primary antibodies against the PGC-1α, FNDC5 and BDNF (1/1000, Cell Signaling Technology Co. New York, USA) overnight. Membranes incubated with rabbit IgG-horseradish peroxidase-conjugated secondary antibody (1/3000, Cell Signaling Technology Co. New York, USA) which could be directly detectable by chemiluminescence kit reagent (Amersham Bioscience Co. Piscataway, USA). To detect β-actin as an internal control, blots stripped in stripping buffer (pH = 6.7) and then probed with anti β-actin antibody (1/1000, Cell Signaling Technology Co. New York, USA) (Niimura et al., 2006). The density of each band was analyzed with the Image-J software (NIH). The expression of PGC-1α, FNDC5, and BDNF was determined by calculating the density ratio of each band to β-actin protein.

2.7. Statistics

Results are expressed as mean ± SEM. Gaussian distribution for all data set was performed by the Kolmogorov-Smirnov normality test. Data were analyzed by paired and unpaired two-sample Student *t*-test and one-way analysis of variances (ANOVA), followed by post-*hoc* Bonferroni test, as needed. $P < 0.05$ were considered as statistically significant. Data were analyzed using Statistical Package for the Social Sciences (SPSS) v.19.0 software.

3. Results

In this study, by comparing Control + Non-Stress and Control + Stress groups with each other by unpaired two-sample *t*-test, it was indicated three weeks chronic unpredictable stress had significance negative effect on most behavioral variables (Table 2) include total distance traveled [$t(10) = -4.592$, $p = 0.001$] and velocity [$t(10) = 1.347$, $p = 0.208$] in OF, percent of entries to open arms [$t(10) = -4.142$, $p = 0.031$], time spent on open arms [$t(10) = -17.516$, $p =$

Table 2
The differences among the Control + Non-Stress and Control + Stress groups in behavioral variables.

	Total distance traveled in OF (cm)	Velocity in OF (m/s)	Entries to open arms in EPM (%)	Time spent on open arms in EPM (s)	Time spent on close arms in EPM (s)	Immobility time in FST (s)
Control + Non-stress	1189.8 ± 113.7	6.7 ± 1.8	63.8 ± 2.7	32.9 ± 4.8	60.3 ± 11.0	105.0 ± 5.5
Control + Stress	455.3 ± 112.5**	5.0 ± 2.8	8.0 ± 1.7*	20.6 ± 3.1***	235.5 ± 23.8***	200.3 ± 10.2***

Data are shown as mean ± SEM. **OF**, open field; **EPM**, elevated plus maze; **FST**, forced swim test.
***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$ compared to Control + Non-Stress group following *t*-test.

0.001] and time spent on close arms [t(10) = 16.380, p = 0.001] in EPM and immobility time [t(10) = 8.220, p = 0.001] in FST and also on biochemical variables (Table 3) include serum corticosterone [t(10) = 12.215, p = 0.001], hippocampal PGC-1α [t(16) = −4.016, p = 0.001], FNDC5 [t(16) = −3.641, p = 0.001] and BDNF [t(16) = −5.664, p = 0.001] protein expression. These results suggest that the CUS protocol used in the present study was effective and has properly designed.

3.1. Body weight

Changes in body weight in each group during the study period have been shown in Fig. 2A. Weight gain of any group evaluated in three periods,: the first three-week of exercise, the second three-week of exercise, and the three weeks of chronic unpredictable stress. Repeated measures ANOVA analysis followed by Bonferroni test revealed significant difference in weight gain in Control + Stress [F(2,15) = 38.634, P = 0.001], Continuous + Stress [F(2,15) = 14.024, P = 0.001] and Interval + Stress [F(2,15) = 27.814, P = 0.001] groups, however there was not any significance difference in Control + Non-Stress group [F(2,15) = 1.413, P = 0.288]. As shown in Fig. 2B, in stress-exposed groups, weight gain normally increased at the first and second three-week period of exercise phase. However, this increase was significantly slower in the three weeks of the stress phase.

3.2. Behavioral experiments

3.2.1. Open field

In this set of experiments, OF was the first behavioral test that was performed. As shown in Fig. 3A, one-way ANOVA showed a significant difference between groups in the total distance traveled during the OF in exercise [F(2,15) = 6.017; P = 0.012] and stress [F(2,15) = 19.119, P = 0.002] phases. Post-*hoc* Bonferroni analysis showed that at the end of the exercise phase, the rats of continuous and interval groups traveled more distance in the OF than the control group and also after the stress phase total distance traveled was higher in the Continuous + Stress and Interval + Stress groups than Control + Stress. However, based on one-way ANOVA results, there was not any significant difference between groups in moving velocity in exercise [F(2,15) = 0.125; P = 0.884] and stress [F(2,15) = 0.084; P = 0.920] phase (Fig. 3B). With comparing the total distance traveled between stress and exercise phases by paired two-sample *t*-test, it has been shown that the CUS reduces the positive effect of exercise training on locomotion. So, in this case there was a significant difference between control and Control + Stress [t(5) = 3.313, p = 0.021], continuous and Continuous + Stress [t(5) = 6.824, p = 0.001] and interval and Interval + Stress [t(5) = 5.766, p = 0.002], although there was no significant difference between them in moving velocity.

Table 3
The differences among the Control + Non-Stress and Control + Stress groups in biochemical variables.

	Serum corticosterone (ng/ml)	PGC-1α protein (relative)	FNDC5 protein (relative)	BDNF protein (relative)
Control + Non-Stress	106.0 ± 9.0	0.54 ± 0.01	0.65 ± 0.05	0.71 ± 0.04
Control + Stress	233.7 ± 5.3***	0.39 ± 0.03**	0.41 ± 0.03**	0.44 ± 0.01***

Data are shown as mean ± SEM. **OF**, open field; **EPM**, elevated plus maze; **FST**, forced swim test.
***$P < 0.001$ and **$P < 0.01$ compared to Control + Non-Stress group following *t*-test.

3.2.2. Elevated plus maze

The second behavioral test that was performed in this study was elevated plus maze. One-way ANOVA followed by Bonferroni test showed that the time spent on open arms (Fig. 4A) and percentage of entries to open arms (Fig. 4B) in continuous and interval groups were higher than in the control group at the end of exercise phase [F(2,15) = 7.786; P = 0.005 and F(2,15) = 10.888; P = 0.001, respectively]. Also after stress phase the above-mentioned variables were higher in the Continuous + Stress and Interval + Stress groups compared to Control + Stress group [F(2,15) = 13.068; P = 0.001 and F(2,15) = 6.420; P = 0.010, respectively]. As shown in Fig. 4C and based on one-way ANOVA analysis, there was not any significant difference between groups in time spent on close arms in exercise [F(2,15) = 1.244; P = 0.316] and stress [F(2,15) = 0.564; P = 0.581] phase. To comparing the effect of CUS on exercise-induced changes in percentage of open arms entries, time spent on open and close arms, paired two-sample *t*-test has shown there was a significant difference between control and Control + Stress [t(5) = 3.957, p = 0.011; t(5) = 8.724, p = 0.001; t(5) = −16.962, p = 0.001, respectively], continuous and Continuous + Stress [t(5) = 2.941, p = 0.032; t(5) = 12.641, p = 0.001; t(5) = −4.902, p = 0.004, respectively] and interval and Interval + Stress [t(5) = = 2.701, p = 0.043; t(5) = 8.946, p = 0.001; t(5) = −6.644, p = 0.001, respectively].

3.2.3. Forced swim test

One-way ANOVA revealed a significant difference among the groups in immobility time (Fig. 5). Results of post-*hoc* Bonferroni showed at the end of exercise phase immobility time was lower in the continuous and interval groups than in the control group [F(2,15) = 30.962; P = 0.001] and after stress phase immobility time was lower in the Continuous + Stress and Interval + Stress groups than in the Control + Stress group [F(2,15) = 7.475; P = 0.006]. By paired two-sample *t*-test analysis, changes in immobility time in the stress phase with exercise phase was compared. As a result, it was found there was a significant difference between control and Control + Stress [t(5) = −8.549, p = 0.001], continuous and Continuous + Stress [t(5) = −10.022, p = 0.001] and interval and Interval + Stress [t(5) = −9.573, p = 0.001].

3.3. Biochemical observations

3.3.1. Serum corticosterone

As shown in Fig. 6, one-way ANOVA has shown there was not any significant difference in serum corticosterone levels in exercise [F(2,15) = 0.567; P = 0.579] and stress [F(2,15) = 1.133; P = 0.348] phases. By comparing the stress phase with exercise phase, unpaired two-sample *t*-test analysis was indicated that chronic unpredictable stress increase serum corticosterone levels, so there was a significant difference between control and Control + Stress [t(10) = −10.743, p = 0.001], continuous and Continuous + Stress [t(10) = −6.671, p = 0.001] and

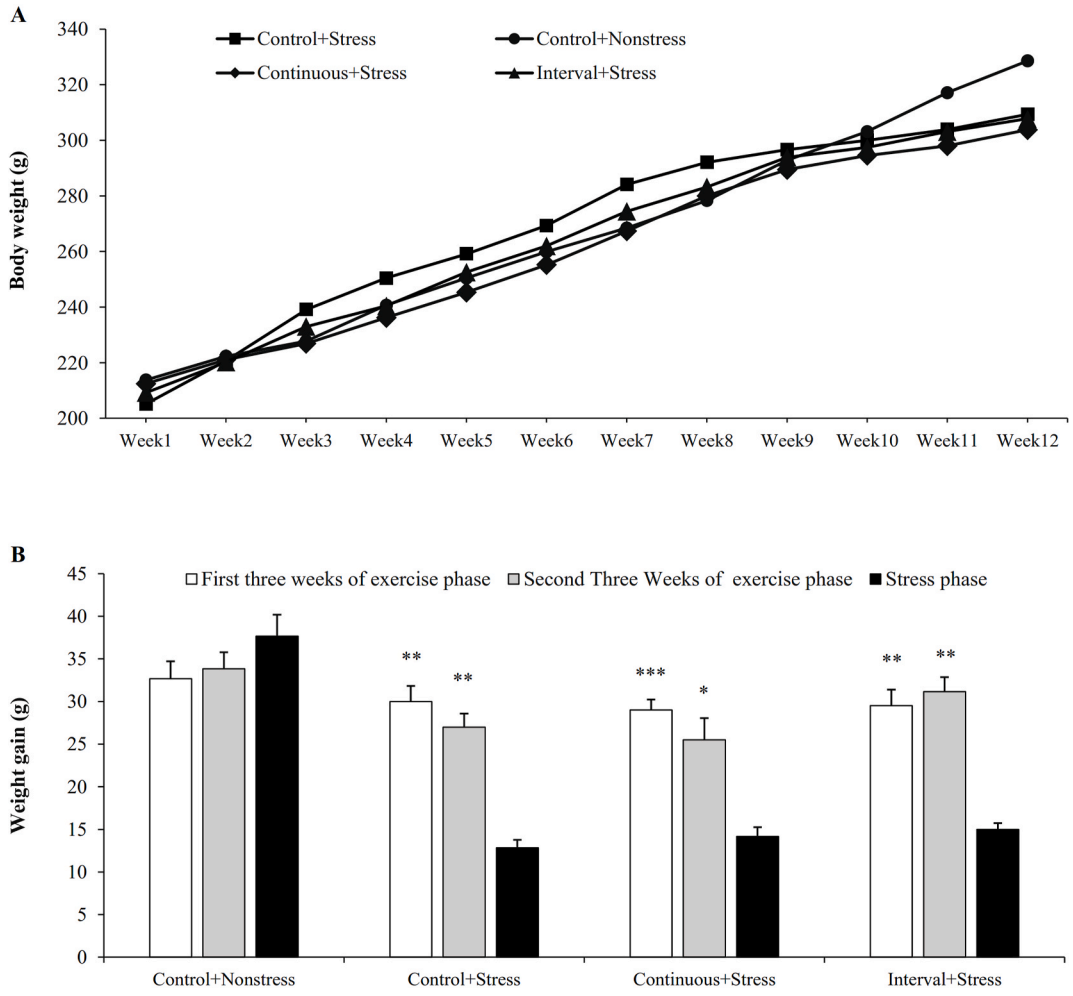


Fig. 2. ***(A)*** Rats' body weight changes during the experiment and ***(B)*** weight gain comparison within three-week periods include the first three-week of exercise, the second three-week of exercise and the three weeks of chronic unpredictable stress. Each bar represents mean ± SEM for 10–15 animals. $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ compared to the stress phase following ANOVA.

interval and Interval + Stress [t(10) = −10.509, p = 0.001].

3.3.2. *PGC-1α protein*

As shown in Fig. 7A, one-way ANOVA followed by Bonferroni test showed that exercise training enhanced expression of hippocampal PGC-1α protein in the interval and continuous groups compared to control, although this enhancement was significantly higher in interval than continuous group [F(2, 24) = 35.247; P = 0.001]. In stress phase hippocampal PGC-1α protein was higher in the Continuous + Stress and Interval + Stress groups than in the Control + Stress group [F(2, 24) = 9.373; P = 0.001]. Unpaired two-sample *t*-test analysis that was used to comparing the effect of chronic unpredictable stress on the changes caused by exercise training has shown there was a significant difference between control and Control + Stress [t(16) = 2.454, p = 0.026], continuous and Continuous + Stress [t(16) = 15.566, p = 0.001] and interval and Interval + Stress [t(16) = 15.941, p = 0.001].

3.3.3. *FNDC5 protein*

As shown in Fig. 7B, one-way ANOVA followed by Bonferroni test showed that exercise training enhanced expression of hippocampal FNDC5 protein in the interval and continuous groups compared to control, although this enhancement was significantly higher in interval than continuous group [F(2, 24) = 38.125; P = 0.001]. In stress phase and based on one-way ANOVA results, there was not any significant difference between groups [F(2, 24) = 2.357; P = 0.119]. Unpaired two-sample *t*-test analysis has shown after exercise phase, chronic stress had negative effect on hippocampal FNDC5 protein, therefore there was a significant difference between control and Control + Stress [t(16) = 6.874, p = 0.001], interval and Interval + Stress [t(16) = 10.244, p = 0.001] and continuous and Continuous + Stress [t(16) = 14.813, p = 0.001].

3.3.4. *BDNF protein*

As shown in Fig. 7C, one-way ANOVA followed by Bonferroni test showed, expression of hippocampal BDNF protein was higher in the continuous and interval groups compared to control [F(2,24) = 38.292; P = 0.001], also in stress phase hippocampal BDNF protein was higher in the Continuous + Stress and Interval + Stress groups compared to Control + Stress group [F(2, 24) = 20.062; P = 0.001]. By Comparing the stress phase with exercise through unpaired two-sample *t*-test, there was a significant difference between control and Control + Stress [t(16) = 4.721, p = 0.001], continuous and Continuous + Stress [t(16) = 6.067, p = 0.001] and interval and Interval + Stress [t(16) = 5.580, p = 0.001].

4. Discussion

The present study investigated the effect of continuous and interval exercise preconditioning against chronic unpredictable stress on hippocampal PGC-1α/FNDC5/BDNF protein expression and some male

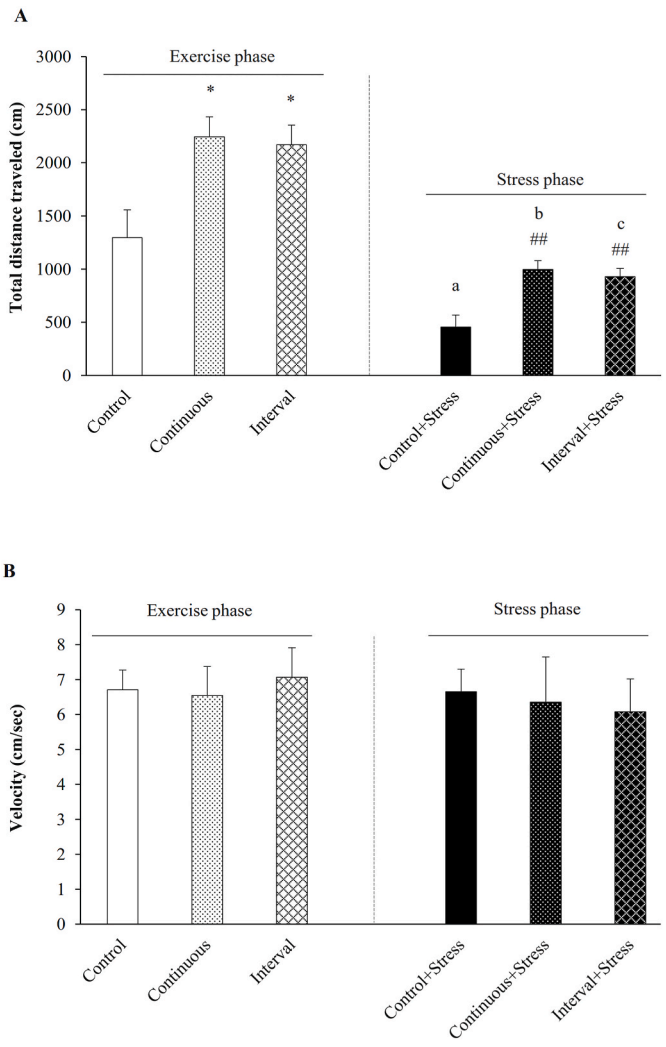
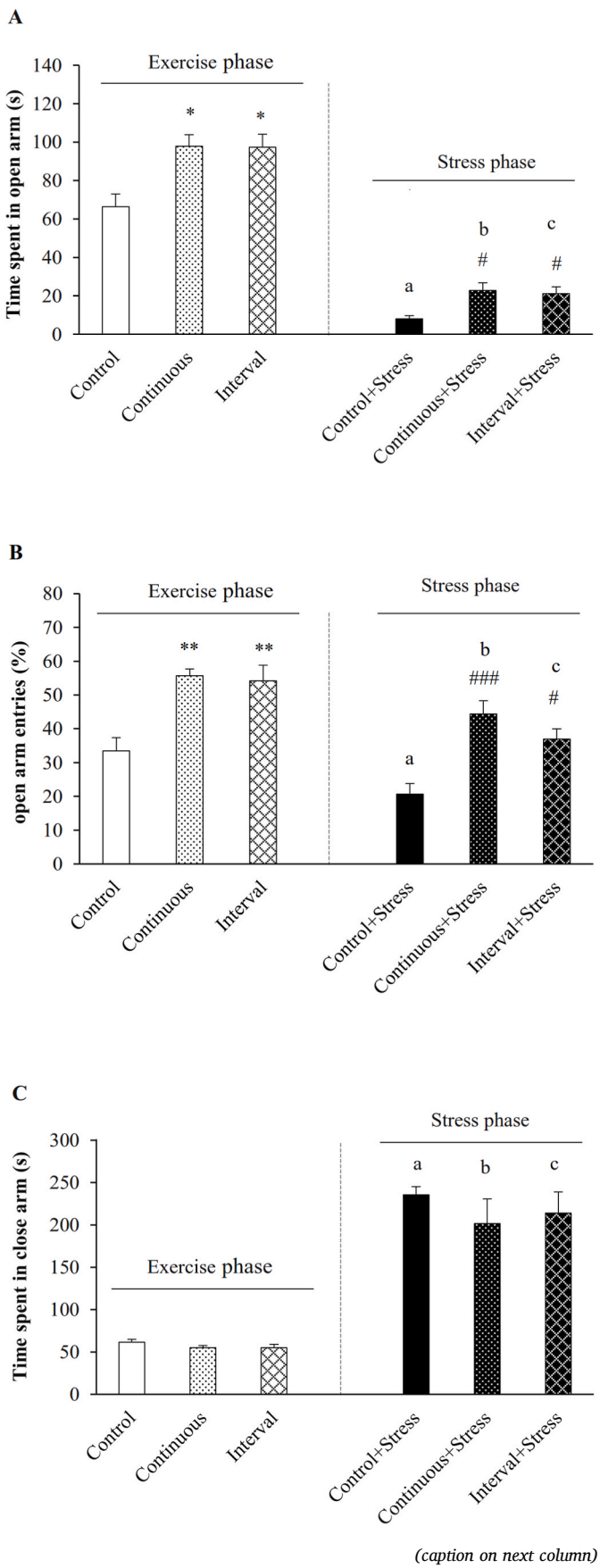


Fig. 3. Effect of exercise training and chronic stress on ***(A)*** total distance traveled and ***(B)*** moving velocity in open field test. In exercise phase, animals performed interval or continuous exercise training for six weeks and then in stress phase they were exposed to three weeks' chronic unpredictable stress. Each bar represents mean ± SEM for 6 animals. $^{*}P < 0.05$, compared to the control group and $^{\#\#}P < 0.01$ compared to the Control + Stress group following ANOVA. $^{a}P < 0.001$ compared to the control group, $^{b}P < 0.001$ compared to the continuous group and $^{c}P < 0.001$ compared to the interval group following *t*-test.

rats' behaviors. The findings have shown: (1) Both types of exercise training, interval and continuous, similarly improved locomotion, depression- and anxiety-like behaviors in male rats, (2) It was observed that after the exercise phase, hippocampal levels of PGC-1α, BDNF, and FNDC5 increased in rats. Apart from PGC-1α and FNDC5, which interval exercise training had a better effect rather than continuous, there was no difference between these two types of exercise training in the other variables and (3) Stress phase had a substantial adverse effect on all variables, however continuous and interval exercise preconditioning had a protective effect on all variables, except the FNDC5.

To the best of our knowledge, the present study is the first to compare the effect of continuous and interval exercise training on PGC-1α/FNDC5/BDNF protein expression. In the present study, continuous and interval exercise training caused the same increase in BDNF protein level in the hippocampus, while in the case of PGC-1α and FNDC5 proteins, interval exercise training was more effective than continuous. Earlier studies have examined exercise training effects on PGC-1α/FNDC5/BDNF pathway. Wrann et al. (2013) showed that there might be a link between exercise with PGC-1α, FNDC5, and BDNF expression in the hippocampus (Wrann et al., 2013). Belviranli and Okudan have shown 90 days voluntarily increase the hippocampal PGC-1α/FNDC5/BDNF pathway in both the young and aged rats (Belviranlı and Okudan, 2018). Also, up-regulation of this pathway in rats' hippocampus after four weeks of moderate treadmill exercise was reported (Azimi et al., 2018). Little researches are comparing the two types of interval and continuous exercise training on PGC-1α. Taylor et al. (2005) have shown there was not any difference between interval and continuous training in PGC-1α protein levels of different skeletal muscle fiber types (Taylor et al., 2005), whereas another study reported interval exercise training is more effective than continuous (Shirvani and Arabzadeh, 2018). It has shown that a higher concentration of systemic blood lactate generates in interval exercise as compared to continuous training. It increases NMDA activity and intracellular Ca^{2+} levels in neurons; thus, calcium/calmodulin-dependent protein kinase II (CaMKII) activates the mitogen-activated protein kinases/extracellular signal-regulated kinase/mitogen- and stress-activated kinases (MAPK/ERK/MSK) signaling. There are few comparative studies regarding the effect of two types of exercise on FNDC5 as well. Constans et al. (2020) have found an increase of FNDC5 after eight weeks of high-intensity exercise training in active fast-twitch muscle fibers, while this change was not seen in continuous training. FNDC may play a role in increasing hippocampal BDNF bypassing the blood-brain barrier (Constans et al., 2020). Therefore, it can be concluded that an increase in peripheral FNDC is probably sufficient to increase BDNF levels in the brain and is not entirely dependent on an increase in FNDC5 in the hippocampus, and this can be compared in the future research. From another look, with studying the research of Tiano et al. (2015) and comparing forced treadmill running with voluntary running, we can have a further analysis, that the high-intensity exercise, regardless of its type (continuous and interval), has a significant effect on the FNDC5 level (Tiano et al., 2015). In this study, both types of exercise training had the same effect on BDNF; however, Afzalpour et al. (2015) have shown thirty intense interval training sessions in six weeks significantly increase BDNF protein levels in the rat's brain compared with continuous training and control group. Also, BDNF protein levels were higher in continuous training compared control group. According to the authors, increased hydrogen peroxide (H2O2) and Tumor Necrosis Factor Alpha (TNF-α) can activate the BDNF protein. The mechanism that exercise increases the expression of transcriptional coactivator, PGC-1α, in hippocampal neurons is unknown. However, in the skeletal muscle, the role of exercise has been identified by increasing reactive oxygen species (ROS) and Ca^{2+} and thus activating the downstream pathways such as CaMK, AMP-activated protein kinase (AMPK), and MAPK (Lira et al., 2010; Jung and Kim, 2014). The increased PGC-1α causes the expression of its binding partner, Estrogen-related receptor alpha (ERRα). With the formation and strengthening of the PGC-1α/ERRα complex, Fndc5 gene expression enhances. FNDC5 induces BDNF gene expression in hippocampus neurons (Wrann et al., 2013; Xu, 2013). Activation of BDNF and its receptor, tropomyosin receptor kinase B (TrkB), and its underlying molecular mechanism cause improvement of depression. BDNF/TrkB stimulates downstream pathways, including MAPK and phosphatidylinositol-3 kinase (PI3K)/Akt signaling pathways, which in turn, phosphorylate cAMP response element-binding protein (CREB) as their target (Pizzorusso et al., 2000). CREB also controls PGC1a expression (Volakakis et al., 2010). These pathways have considerable influence on plasticity, neuroprotection, and especially memory (Carlezon et al., 2005; Duman and Voleti, 2012). Interestingly, elevated BDNF-TrkB signaling formed a homeostatic FNDC5/BDNF feedback loop, thus negatively regulating Fndc5 gene expression (Wrann et al., 2013; Xu, 2013). Hippocampus-related memory and learning, such as spatial memory, is one of the most important and influential factors in mental health, that exercise training through the BDNF pathway has positive effects on its formation process. In the present study, we could not analyze memory and learning, so as one of the limitations of this study can be considered



(caption on next column)

Fig. 4. Effect of exercise training and chronic stress on ***(A)*** time spent in open arms and ***(B)*** entrance percent to open arms and ***(C)*** time spent in close arms in elevated plus maze test. In exercise phase, animals performed interval or continuous exercise training for six weeks and then in stress phase they exposed to three weeks' chronic unpredictable stress. Each bar represents mean ± SEM for 6 animals. $^{**}P < 0.01$, $^{*}P < 0.05$ compared to the control group and $^{\#\#\#}P < 0.001$, $^{\#}P < 0.05$ compared to the Control + Stress group following ANOVA. $^{a}P < 0.001$ compared to the control group, $^{b}P < 0.001$ compared to the continuous group and $^{c}P < 0.001$ compared to the interval group following *t*-test.

in future researches.

Based on findings in this study, in the stress phase, PGC-1α/FNDC5/BDNF proteins have decreased significantly compared to the exercise phase; however, the rats with exercise preconditioned had more significant resistance to the CUS. Studies have shown the effect of stress on reducing BDNF (Zhu et al., 2019) and PGC-1α (Khedr et al., 2019). In line with this research, Zhan et al. (2018) have shown in chronic social defeat stress model mice, the levels of PGC-1α, FNDC5, and mature BDNF in the hippocampus are significantly reduced (Zhan et al., 2018). However, in the study of Nasrallah et al. (2019), a decrease in protein levels of PGC-1α and no change in BDNF and FNDC5 protein levels were reported following the defeat stress (Nasrallah et al., 2019). It has also reported that BDNF and PGC-1α levels were lower in patients with depression than healthy controls (Ryan et al., 2019; Molendijk et al., 2011). Stress and corticosterone are closely related. Chronic stress disrupts the physiological homeostasis of the brain.

Response to physiological stress includes neuronal and hormonal mechanisms that re-establish homeostasis. One of these mechanisms is the activity of the hypothalamic/pituitary/adrenal (HPA) axis that activates corticotropin-releasing factor/adrenocorticotropin/corticosterone. Previous researches have shown that corticosteroids reduce BDNF (Demuyser et al., 2016; Huang et al., 2011); also, dexamethasone as a glucocorticoid decreases PGC-1α protein expression and transcription (Rahnert et al., 2016). In the present study, exercise did not significantly alter serum corticosterone levels. The results of previous research in this area are very contradictory, although most have shown that intense exercise training increases corticosterone levels (Sun et al., 2020; Wu et al., 2020). In the current study, blood sampling was collected after all behavioral tests, so, sampling procedure did not change corticosterone. As in the study of Wu et al. (2020), serum corticosterone levels were higher in the 24 h after exercise training than after behavioral tests (Wu et al., 2020). Another finding of the study is a decrease in the weight gain of rats in the stress phase that is also related to the HPA axis. Weight loss is one of the significant physical signs of depression. Therefore, it is selected as one of the indicators. It has shown that high corticotropin-releasing factor (CRF) reduces appetite by suppressing it (Arase et al., 1988; Nemeroff, 1998). As mentioned, stress increased serum corticosterone levels in rats, indicating high activity of the CRF. In addition, continuous and chronic unpredictable stress during the week, as well as periods of 18 h of food deprivation, may have reduced the rats' desire to eat. As many studies have reported exposure to a variety of stress significantly reduce food intake (Santos et al., 2000; Martí et al., 1994). The exact amount of food consumed during our research period was not measured, but it was clear that food intake during the stress phase was significantly reduced.

However, exercise can provide an excellent protective effect against chronic unpredictable stress by reducing CUS's destructive effects. Liu and Zhou (2012) reported that in chronic mild unpredictable stress exposure conditions, in exercise preconditioned rats, the BDNF mRNA level of the hippocampus is higher than that of sedentary rats (Liu and Zhou, 2012). Concerning the role of exercise training in reducing the effects of chronic unpredictable mild stress on PGC-1α, it has been shown mice that have also performed aerobic exercise under stress conditions had a lower decrease in the PGC-1α than other mice. According to the authors, this may be related to the influence of the AMPK/PGC-1α pathway (Luo et al., 2020). Furthermore, overexpression

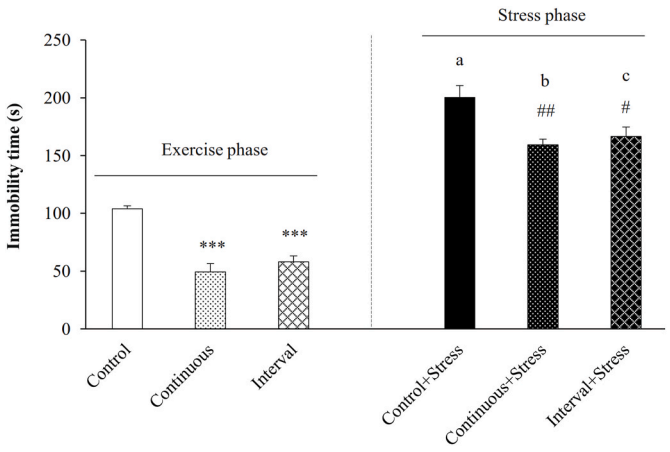


Fig. 5. Effect of exercise training and chronic stress on immobility time in forced swim test. In exercise phase, animals performed interval or continuous exercise training for six weeks and then in stress phase they exposed to three weeks' chronic unpredictable stress. Each bar represents mean ± SEM for 6 animals. $^{***}P < 0.001$ compared to the control group and $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ compared to the Control + Stress group following ANOVA. $^{a}P < 0.001$ compared to the control group, $^{b}P < 0.001$ compared to the continuous group and $^{c}P < 0.001$ compared to the interval group following *t*-test.

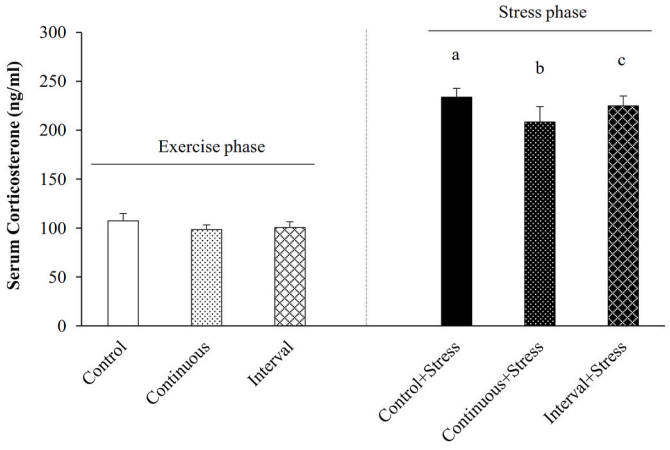


Fig. 6. Effect of exercise training and chronic stress on serum corticosterone. In exercise phase, animals performed interval or continuous exercise training for six weeks and then in stress phase they exposed to three weeks' chronic unpredictable stress. Each bar represents mean ± SEM for 6 animals. $^{a}P < 0.001$ compared to the control group, $^{b}P < 0.001$ compared to the continuous group and $^{c}P < 0.001$ compared to the interval group following *t*-test.

of PGC-1α by reducing the entry of neurotoxic kynurenine into the brain has an antidepressant-like effect. Exercise training by activation of the PGC-1α/PPARα/PPARδ pathway increases skeletal muscle kynurenine aminotransferase expression. As a result, plasma kynurenine level decreases; therefore, it protects the brain from stress-induced damage (Agudelo et al., 2014). PGC1α also activates FNDC5 that, in turn, is processed into a soluble secretory peptide termed "irisin'' which has an antidepressant-like effect in mice (Siteneski et al., 2018), as well as in rats exposed to chronic unpredictable stress (Wang and Pan, 2016). Irisin also activates uncoupling protein 2 (UCP2), which plays the neuroprotective role by increasing mitochondrial biogenesis and antioxidant effect via the Akt/ERK signaling pathway (Aguiar et al., 2019; Sumsuzzman et al., 2018). It should be noted that, in this study, the FNDC5 level in the stress phase was not different from the COD group. Probably, in addition to stress, the negative feedback effect of BDNF on FNDC5 can be mentioned as an influential factor.

In behavioral assessments of the current study, similar results were observed. In OF, distance traveled is considered as a criterion for general locomotion activity. Past studies reported that after chronic treadmill running, the locomotion increased (Pietrelli et al., 2012; Seo, 2018) and did not change (Cevik et al., 2018; Patki et al., 2014) during the OF. In this study, the rats in exercise training groups had significantly more locomotion than sedentary control rats; however, the velocity of moving was not affected by exercise training. In this study, exercise training improved anxiety-like behaviors. It was reported that following a period of exercise training, the severity of anxiety-like behavior decreased (Seo, 2018; Cevik et al., 2018) and did not change (Patki et al., 2014; Burghardt et al., 2004) during the EPM test. The most popular test to estimate the level of anxiety in rodents is elevated plus-maze. Some components of this test, such as the percent of the entrance to open arms and time spent in open arms, were used to assess rats' anxiety. Rats in exercise training groups had a better function in EPM, indicating the anti-anxiety effect of intense and continuous exercise training. To determine the depression-like behavior, including the desire to move, curiosity, and despair behaviors, a forced swimming test can be used. It was reported that the severity of depression-like behavior decreased (Luo et al., 2020; Marais et al., 2009) and did not change (Qu et al., 2020) during the FST. In the present study, immobility time, which showed despair behavior, decreased in both exercise training groups. Like other variables in this study, CUS had a significantly destructive effect on behavioral test results and cause decreases locomotion and increased anxiety- and depression-like behavior. These changes were expected because they have been observed in many previous studies (Luo et al., 2020; Qu et al., 2020; Hei et al., 2019; Zorkina et al., 2019). In the complex effects of exercise training to improve stress, anxiety, and depression, many factor play roles include endorphins, mitochondrial function, mammalian target of rapamycin (mTOR), neurotransmitters, cytokines, toll-like receptors (TLR), vagal tone (Mikkelsen et al., 2017). Each of these factors can be examined in future research.

5. Conclusion

Generally, the six-week continuous and interval exercise training increases PGC-1α/FNDC5/BDNF protein expression of male rat's hippocampus and improves anxiety- and depression-like behaviors, although the interval training increases the PGC-1α and FNDC5 more than continuous training. After exercise training, the three weeks' chronic unpredictable stress reduced PGC-1α, FNDC5, and BDNF protein levels and behavioral scores. However, exercise preconditioning, except the FNDC5, has a significant protective effect against CUS in other variables.

CRediT authorship contribution statement

Ayyub Babaei: Funding acquisition, contributed to the acquisition of behavioral and molecular data, Formal analysis, assisted with data analysis and interpretation of findings, Writing - original draft. **Maryam Nourshahi:** Conceptualization, were responsible for the study concept and design, Formal analysis, assisted with data analysis and interpretation of findings, provided critical revision of the manuscript for important intellectual content. **Maryam Fani:** Funding acquisition, contributed to the acquisition of behavioral and molecular data. **Zahra Entezari:** Funding acquisition, contributed to the acquisition of behavioral and molecular data. **Seyed Behnamedin Jameie:** provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication. **Abbas Haghparast:** Conceptualization, were responsible for the study concept and design, Formal analysis, assisted with data analysis and interpretation of findings, provided critical revision of the manuscript for important intellectual content.

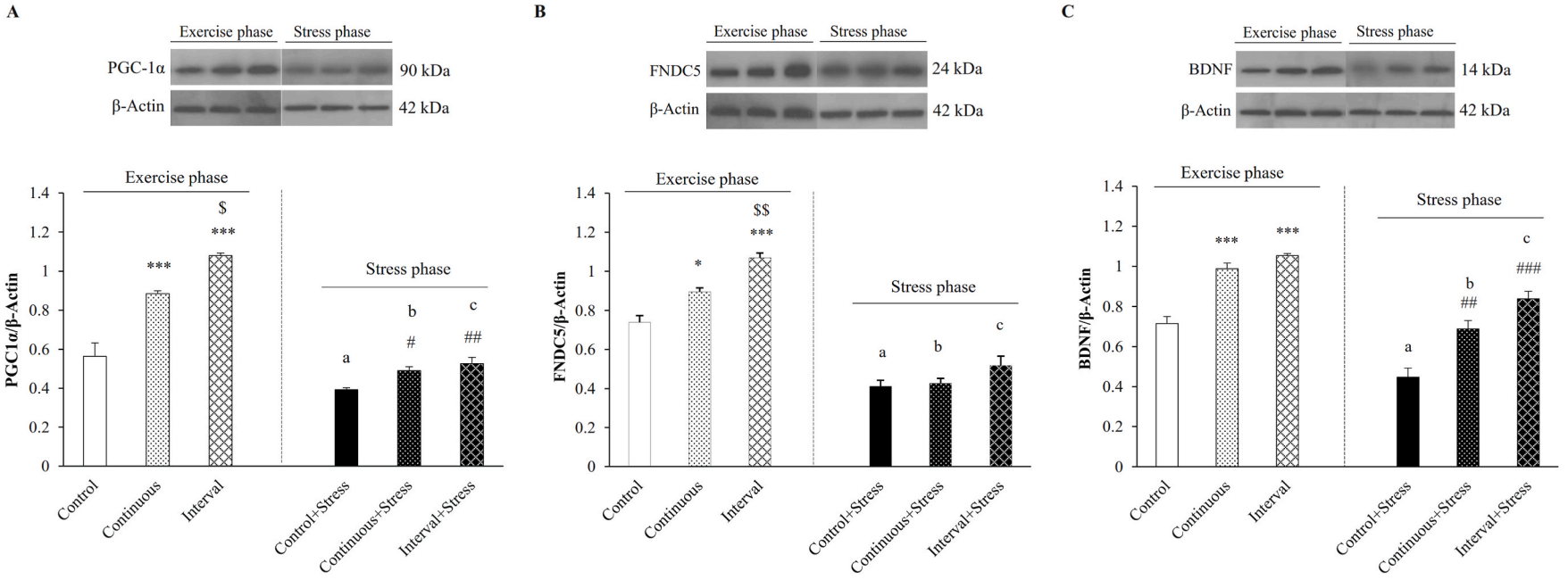


Fig. 7. Effect of exercise training and chronic stress on hippocampal protein expression of ***(A)*** PGC-1α, ***(B)*** FNDC5 and ***(C)*** BDNF. In exercise phase, animals performed interval or continuous exercise training for six weeks and then in stress phase they exposed to three weeks' chronic unpredictable stress. Each bar represents mean ± SEM for 5 animals $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared to the control group, $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ compared to the Control + Stress group and $^{\$\$}P < 0.05$, $^{\$}P < 0.05$ compared to the continuous group following ANOVA. $^{a}P < 0.001$ compared to the control group, $^{b}P < 0.001$ compared to the continuous group and $^{c}P < 0.001$ compared to the interval group following *t*-test.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2021.02.006.

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