Brain Research 1663 (2017) 20–28

Contents lists available at ScienceDirect

Brain Research

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

Research report

# Low- and high-intensity treadmill exercise attenuates chronic morphine-induced anxiogenesis and memory impairment but not reductions in hippocampal BDNF in female rats

Shahrbanoo Ghodrati-Jaldbakhan [a,b], Ali Ahmadalipour [a,c,1], Ali Rashidy-Pour [b,*], Abbas Ali Vafaei [b], Hossein Miladi-Gorji [b], Maryam Alizadeh [a]

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

ARTICLE INFO

*Article history:*
Received 28 October 2016
Received in revised form 21 February 2017
Accepted 22 February 2017
Available online 6 March 2017

*Keywords:*
Treadmill exercise
Chronic morphine
Memory
BDNF
Anxiety
Female rat

ABSTRACT

Previous studies from our laboratory have shown that treadmill exercise alleviates the deficits in cognitive functions and anxiety behaviors induced by chronic exposure to morphine in male rats. In this study, we investigated the effects of low and high intensities of treadmill exercise on spatial memory, anxiety-like behaviors, and biochemical changes in the hippocampus and serum of morphine-treated female rats. The adult virgin female rats were injected with bi-daily doses (10 mg/kg, at 12 h intervals) of morphine over a period of 10 days. Following these injections, the rats were exercised under low or high intensities for 30 min per session on five days a week for four weeks. After exercise training, object location memory, anxiety profile, hippocampal BDNF, and serum corticosterone and BDNF were examined. Morphine-treated animals exhibited increased anxiety levels, impaired object location memory, and reduced hippocampal BDNF. Exercise alleviated these impairing effects on anxiety profile and memory but not hippocampal BDNF. The high-intensity exercise even further reduced the hippocampal BDNF. Additionally, both exercise regimens in the morphine group and the high exercise in the saline group reduced serum BDNF. Finally, the high-intensity exercise enhanced corticosterone serum. These findings indicate that the negative cognitive and behavioral effects of chronic exposure to morphine could be relieved by forced exercise in female rats. However, the exercise intensity is an important factor to be considered during exercise training. Finally, the correlation between changes of brain and serum BDNF and cognitive functions following morphine exposure needs further research.



## 1. Introduction

Previous studies have shown that long-term exposure to opiates, including morphine, impairs cognitive functions in experimental animals and humans (Davis et al., 2002; Dougherty et al., 1996; Gu et al., 2007; Miladi Gorji et al., 2008; Robbins and Everitt, 1999; Spain and Newsom, 1991). A higher prevalence of mood disorders, such as anxiety and depression, has been clinically demonstrated among drug abusers, which may contribute to persistent use and relapse following abstinence (Davis et al., 2002; De Graaf et al., 2003). There is a growing body of knowledge about the beneficial effects of exercise on cognitive functions in humans and experimental animals (Kramer et al., 2006). Both voluntary running and forced exercise can improve learning and memory in rodents (Albeck et al., 2006; Van der Borght et al., 2007; van Praag et al., 1999; Wu et al., 2007). Exercise enhances the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, a key brain structure in the medial temporal lobe which is essential for activity-dependent learning and memory (Neeper et al., 1996). BDNF, via a TrkB-dependent mechanism, mediates exercise-induced enhancement of learning, memory, and synaptic plasticity (Vaynman et al., 2004).

*Abbreviations:* OLMT, object location memory task; EPM, elevated plus maze; BDNF, brain derived neurotropic factor; OAE, open arm entry; OAT, open arm time.

* Corresponding author at: Laboratory of Learning and Memory, Research Center and Department of Physiology, Semnan, University of Medical Sciences, 15131-38111 Semnan, Iran.
*E-mail address:* Rashidy-pour@semums.ac.ir (A. Rashidy-Pour).

[1] Equal distribution with the first author.

http://dx.doi.org/10.1016/j.brainres.2017.02.024
0006-8993/

The effectiveness of exercise in drug addiction recovery and rehabilitation is demonstrated. For instance, we recently reported that concurrent access to a running wheel blocked the impairment of learning and memory induced by chronic exposure to morphine via the BDNF-TrkB mechanism in male rats (Miladi-Gorji et al., 2011). We also have demonstrated that treadmill and running wheel exercise regimens could blunt the deleterious effects of drugs of abuse after exposure to these substances or after long periods of abstinence (Mokhtari-Zaer et al., 2014). The forced exercise, particularly at high intensity, increases the secretion of glucocorticoids, such as cortisol or corticosterone (Soya et al., 2007). Elevated levels of glucocorticoids are known to inhibit neurogenesis in the hippocampus and disrupt hippocampal synaptic plasticity, resulting in impairment of learning and memory (McEwen, 1999). For example, high impact forced exercise with speeds up to 25 m/min leads to impairment of spatial learning and memory, while low impact forced exercise with speeds up to 12 m/min, which is well below those normally considered stressful (17–30 m/min), improves learning and memory in rodents (Kennard and Woodruff-Pak, 2012).

Recent studies have demonstrated sex -dependent differences in different phases of drug addiction (Lynch et al., 2002; Lynch, 2006). Females are more susceptible than males during transition periods of drug use that are characteristic of drug addiction and relapse. Females are also more sensitive than males to the reinforcing effects of stimulants. Female sex hormones, particularly estrogen, appear to contribute to the mechanisms of action underlying these sex differences (for review see (Roth et al., 2004)). Also, the influence of gender on the physiological effects of exercise is documented. For example, it has been reported that female rats with drug addiction often run longer distances in a running wheel than male rats in the same time frame (Peterson et al., 2014). Male rats showed a smaller reduction of serum corticosteroid binding globulin than female rats in a 10-day treadmill exercise regime (Brown et al., 2007). These sex-dependent effects of drug addiction and exercise may influence differentially the effectiveness of exercise on drug rehabilitation (Fattore et al., 2008).

So far, the therapeutic effects of physical exercise on drug addiction have been mainly focused on male animals, with few studies in females (see (Fattore et al., 2008) for review). In a recent study, we have demonstrated that voluntary exercise as well as the low-intensity treadmill exercise could ameliorate the cognitive and behavioral deficits after the chronic exposure to morphine or after long periods of abstinence in male rats (Mokhtari-Zaer et al., 2014), but these effects are not known in female rats. Thus, the aim of this study was to examine the effects of treadmill exercise with low or high intensity on cognitive, behavioral, and biochemical disorders induced following chronic exposure to morphine in female rats. Similar to our recent study (Mokhtari-Zaer et al., 2014), we applied the exercise regimen after a 10 -day period of morphine administration to examine whether exercise could blunt the deleterious effects of drugs of abuse after the exposure to these substances or after long periods of abstinence. The research is of potential relevance for the determination of the therapeutic effects of different intensities of exercise on drug rehabilitation in women.

## 2. Results

### 2.1. Withdrawal signs

The overall Gellert–Holtzman scores were significantly higher in the morphine-treated female rats than the saline-treated female rats ($t_8 = 16.12$, $P = 0.0001$). Among the graded signs, abdominal contractions ($t_8 = 4.33$, $P = 0.003$) and weight loss ($t_8 = 4.32$, $P = 0.003$) were significantly higher in the morphine group than the control one (Fig. 2).

Among the checked signs (Table 1), the number of rats per group with diarrhea ($U = 0$, $P = 0.003$), writhing ($U = 0$, $P = 0.003$), genital grooming ($U = 5$, $P = 0.05$), and ptosis ($U = 0$, $P = 0.003$) was greater in the morphine-treated group than the saline one. There were no statistically significant changes in irritability or teeth chattering between the two groups.

### 2.2. EPM data

The EPM data of the experimental groups are shown in Fig. 3. A two-way analysis of variance (ANOVA) for the percent of OAT yielded a main effect of treatment ($F_{1, 48} = 19.05$, $P = 0.0001$) and a main effect of exercise ($F_{2, 48} = 4.59$, $P = 0.015$) but no interaction between group and treatment ($F_{2, 48} = 1.55$, $P = 0.223$) (Fig. 3A). Between group comparisons indicated that the percent of OAT in the MOR/SED group was significantly lower than the SAL/SED group ($P = 0.001$), demonstrating the heightened anxiety in the morphine-treated rats. The percent of OAT in the MOR/EXC-L group, but not the MOR/EXC-H group, was significantly higher than the MOR/SED group ($P = 0.01$), indicating that light treadmill exercise can decrease the heightened anxiety in the morphine -treated rats. Likewise, we found that the percent of OAT in the SAL/EXC-L group was significantly increased compared to the SAL/SED group ($P = 0.014$), indicating that light exercise can also decrease the anxiety levels in the saline -treated rats.

A two-way ANOVA for the percent of OAE revealed a main effect of treatment ($F_{1, 48} = 66.47$, $P = 0.0001$), a main effect of exercise ($F_{2, 48} = 19.74$, $P = 0.0001$), and a significant interaction between treatment and group ($F_{2, 48} = 14.92$, $P = 0.0001$) (Fig. 3B). Post-hoc comparisons indicated that the percent of OAE in the MOR/SED group was significantly lower than the SAL/SED group ($P = 0.001$), demonstrating higher levels of anxiety in the morphine-treated rats. The percent of OAE in the MOR/EXC-L and the MOR/EXC-H groups was significantly higher than the MOR/SED group (both, $P = 0.001$), indicating that low forced exercise can reduce the high levels of anxiety in morphine-exposed rats. Moreover, no significant difference was found between the SAL/SED group and the saline-treated exercising groups for OAE measurements.

A two-way ANOVA for the total arm entries revealed no significant effects of treatment ($F_{1, 48} = 1.66$, $P = 0.19$), of exercise ($F_{2, 48} = 2.63$, $P = 0.082$), and no significant interaction between treatment and group ($F_{2, 48} = 0.042$, $P = 0.96$). The total arm entries of the experimental groups were as follows: the SAL/SED group = 17.4 ± 1.36; the MOR/SED group = 21 ± 3.50; the SAL/EXC-L group = 19.2 ± 1.98; the MOR/EXC-L = 21.28 ± 1.57; the SAL/EXC-H group = 16.46 ± 2.52; and the MOR/EXC-H group = 18.85 ± 2.58.

### 2.3. OLMT

Data of OLMT are represented in Fig. 4. A two-way ANOVA for the OLMT data revealed a main effect of exercise ($F_{2,48} = 13.33$, $P = 0001$), but no significant effect of treatment ($F_{1,48} = 1.16$, $P = 0.29$) and a trend toward a significant interaction between group and treatment ($F_{2,48} = 2.56$, $P = 0.088$). Between group comparisons indicated that the discrimination index of the MOR/SED group was significantly lower than the SAL/SED group (Bonferroni *t*-test, $P = 0.044$). Also, both control and morphine exercising groups showed an increase in discrimination index (spatial memory) compared to the corresponding control sedentary groups (SAL/SED vs SAL/EXC-H, $P = 0.017$; MOR/SED vs MOR/EXC-H, $P = 0.001$), indicating that high intensity exercise increased spatial memory. No significant differences were found between groups in