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Adolescent voluntary exercise attenuated hippocampal innate immunity responses and depressive-like behaviors following maternal separation stress in male rats

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HIGHLIGHTS

- Maternal separation (MS) provoked depressive-like behaviors in adult male rats.
- Adolescent RW (and not TM) exercise attenuated the MS effects on behavior.
- MS induced overexpression of *Tlr-4* and *Myd88* genes in the hippocampus.
- Adolescent RW (and not TM) exercise mitigated the effect of MS on gene expression.
- Adolescent voluntary exercise (and not mandatory) is protective against MS stress.

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ABSTRACT

Early life stressful events have detrimental effects on the brain and behavior, which are associated with the development of depression. Immune-inflammatory responses have been reported to contribute in the pathophysiology of depression. Many studies have reported on the beneficial effects of exercise against stress. However, underlying mechanisms through which exercise exerts its effects were poorly studied. Therefore, it applied maternal separation (MS), as a valid animal model of early-life adversity, in rats from postnatal day (PND) 2 to 14 for 180 min per day. At PND 28, male Wistar albino rats were subjected to 5 experimental groups; 1) controls 2) MS rats 3) MS rats treated with fluoxetine 5 mg/kg to PND 60, 4) MS rats that were subjected to voluntary running wheel (RW) exercise and 5) MS rats that were subjected to mandatory treadmill (TM) exercise until adulthood. At PND 60, depressive-like behaviors were assessed by using forced swimming test (FST), splash test, and sucrose preference test (SPT). Our results revealed that depressive-like behaviors following MS stress were associated with an increase in expression of toll-like receptor 4 (Tlr-4) and its main signaling protein, Myd88, in the hippocampal formation. Also, we found that voluntary (and not mandatory) physical exercise during adolescence is protected against depressant effects of early-life stress at least partly through mitigating the innate immune responses in the hippocampus.

1. Introduction

Depression is one of the most prevalent psychiatric disorders and the main cause of disability in 21st century [1]. Experiencing early-life adversity plays a key role in the development of mood disorders in humans and rodents [2,3]. Postnatal period is accompanied by a significant maturation of neuronal systems. Experiencing any insult (such as stressful events) results in the majority of neurobehavioral, neurochemical and immune-inflammatory abnormalities in later life [4]. In this regard, neonatal maternal separation (MS) has been reported as a cogent and validated animal model for investigating depressive-like behaviors in rodents [5,6]. Merging lines of research indicate that innate immune system contributes to the pathophysiology of depression, namely, following early-life stressors [7,8]. Toll-like receptors (TLRs) are one of key innate immunity components and widely distributed in central nervous system (CNS) relevant to mood disorders such as hippocampus [9]. The TLR pathways, mainly TLR-4 pathway, are well-known to modulate inflammatory cascades and contribute to the development of chronic diseases such as psychiatric disorders. Evidence suggests that TLR-4 is implicated in the pathophysiology of depression and

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their activation triggers different inflammatory pathways that result in the production of pro-inflammatory cytokines and inflammatory mediators [10–12].

Although excessive researches have been performed to find effective treatments for depressive disorders, but few advances are achieved. Recently, it has been shown that non-pharmacological therapies (such as physical activities and environmental enrichment) have therapeutic potentials in the treatment of depression [13–15]. Focusing on animal studies, it has been evident that both voluntary exercise and treadmill running have antidepressant-like effects in rodents [16,17]. Interestingly, some investigations demonstrated that applying (non) pharmacological treatments during adolescence has beneficial effects in mitigation of depressive-like behaviors induced by early-life stress [18]. Adolescence correlates with developmental changes in the brain circuits in the limbic areas such as hippocampus [19]. Using of MS as a validated model system for investigating the underpinning mechanisms of depression, we applied voluntary running wheel (RW) exercise and mandatory treadmill exercise to adolescent male rats subjected to MS. We aimed to see whether; 1) applying exercise during adolescence is able to mitigate the effects of MS stress on animal behaviors; 2) depressive-like behaviors in animals are associated with alteration in the expression of *tlr2* and *tlr4* genes in the hippocampal formation of male rats and 3) the beneficial effects of exercise on animal behavior are associated with the expression of aforementioned genes.

2. Materials and methods

2.1. Animals and housing conditions

Pregnant (gestation day 1) Albino Wistar rats were purchased from the Pasteur Institute of Iran (Tehran, Iran), and housed individually under standard laboratory conditions (temperature: 22 ± 2 °C, humidity: 50 ± 10%, 12-h light–dark cycle, and free access to food and water), in the animal facility center of the Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University. Neonatal maternal separation was carried out based on previous studies [18,20]. Briefly, the day of birth was considered as postnatal day 0 (PND 0) and pups were subsequently subjected to MS paradigm. Then, litters were briefly handled and separated from their mothers for 180 min daily during PND 2 to PND 14, beginning at 09:00 a.m. At the PND 21, male offspring's were housed in groups (4 rats per cage) until the PND 28. All procedures in this study were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication # 80–23). Institutional guidelines were strictly followed for animal care and use (Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran).

2.2. Materials and treatments

Fluoxetine hydrochloride (Sigma USA) was dissolved in saline and solution was prepared instantly before injections. Fluoxetine (FLX) was administered intraperitoneally (i.p.) in a constant volume of 5 ml/kg body weight to MS rats from PND 28 to 60. We used chronic administration of FLX (5 mg/kg) to MS rats because it has been reported as an effective treatment for modifying depressive-like behaviors in rodents [21].

2.3. Postnatal exercise protocols

2.3.1. Running wheel (RW) exercise

At PND 21, animals were randomly subjected to exercise protocols, including RW exercise and treadmill exercise ($n = 12$). In case of RW exercise, MS rats were allowed to adapt to the RW apparatus for 1 week. Exercise protocol was carried out based on previous method as described by Miladi-Gorji et al. [22]. Maternally separated rats were housed in pairs in exercise cages, each having access to their own RW. To avoid of social isolation effects, two rats in a cage were kept apart with a perforated Plexiglas separator between them that facilitated communication between rats. The control group of MS rats was similarly handled, but had no access to the RW. Each activity wheel was made of Plexiglas (circumference = 105 cm, width = 10 cm, Novidan. Tab, Iran) which was freely rotated against a resistance of 50 g. Each wheel was connected to a magnetic switch that was linked to a counter placed outside of the animals' cage and monitored revolution per hour. Rats had free access to activity wheel 24 h a day for 32 days until PND 60, and daily running distance (km) was recorded.

2.3.2. Treadmill (TM) exercises

Similar to the RW exercise protocols, at PND 21, MS rats were introduced the TM in order to adapt for 1 week. At PND 28, MS rats ($n = 12$) were subjected to the TM exercise based on protocol previously published by Saadati et al. [23]. In brief, MS rats were treated to the TM exercise (0° inclination) during the day between 9:00 and 15:00, for 4 weeks, 5 days each week (they received a mild electric shock (0.25 mA) whenever they stopped running).

The TM exercise protocol carried out as follows; 30 min for first 2 weeks (10 m/min), 45 min for third week and 60 min for fourth week (both at 15 m/min speed). Every 15 min during each exercise session, rats were given a 5 min break. In the case of control groups, protocol was similar to MS rats while rats were left on the treadmill, without running (0 m/min).

2.4. Behavioral tests

2.4.1. Open-field test (OFT)

We used OFT to evaluate the effects of MS, FLX treatment and exercise conditions on locomotor activity and validating the results of forced swimming test [24,25]. The open-field apparatus was made of white opaque Plexiglas (60 cm × 60 cm × 40 cm), which was dimly illuminated. The floor of the apparatus was divided into 25 equally sized squares. Each rat was placed into a corner of the open-field and behaviors were videotaped for 5 min. The surface of the apparatus was cleaned with 70% ethanol after testing each animal. The number of squares crossed (horizontal activity) was evaluated by an experimenter blind to both housing and treatment conditions.

2.4.2. Forced swimming test (FST)

The FST was directed using the method of Cryan et al. [26]. Rats were individually forced to swim in a cylindrical glass tank (50 cm × 20 cm in diameter) containing 30 cm of water (23 ± 1 °C) and passive behavior (immobility time) was measured. Immobility was defined as a period during which the animal floats in the water, making only those movements necessary to keep its head above the water [26]. Two swimming sessions were performed with an initial 15 min 'pre-test' followed by a 5 min 'test' after 24 h.

2.4.3. Splash test

It was used to evaluate motivational deficits and self-care difficulties in rodents [27,28]. In this test, grooming behavior, which can be considered as an indirect measure of palatable solution intake, was measured. A 10% sucrose solution was squirted on the dorsal coat of animals in their home cage. First grooming latency and time spent grooming were recorded for a period of 5 min.

2.4.4. Sucrose preference test (SPT)

It applied SPT in order to evaluate the hedonic state in animals using a protocol described previously by Wallace et al. [29]. Animals were introduced to the two bottles of tap water, which were placed in the home cage of each rat in the first two days. One of the two bottles was replaced by a bottle containing 1% sucrose solution during the second two days. On the test day, animals were deprived of food and water for 8 h and sucrose preference was assessed during one hour of liquid consumption