

Analyzing the adverse effects of childhood stress on adult anxiety behaviors by testing on male rats and addressing it with a period of aerobic exercise during adolescence

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Abstract

Anxiety is one of the most common diagnoses in psychiatry, which is twice as common in women as in men. Anxiety and depression disorders are significant because they affect a person's social and individual functioning and lead to severe economic and social damage, so they are expected to be debilitating diseases worldwide in the coming years. Considering the importance of treating stress disorders such as anxiety, which improves patients' social and personal functioning, and taking into account the side effects of drugs, researchers proposed sports activities as a non-pharmacological and effective occupational therapy method. But it is not clear whether the effects of exercise can be related to the regulation of the serotonergic system in the brain (especially anxiety behavior). Therefore, this research was designed and carried out to answer the following question: Will doing optional exercise (Running Wheel) and not mandatory exercise (Treadmill exercise) during adolescence be able to improve the ill effects of MS? In this research, six pregnant mice were used. After giving birth, their babies experienced the stress of separation from their mother for 12 days between the 2nd and 14th days after birth, and then from the 28th day, they exercised with the Running Wheel for a month. The number of rats was 8 for behavioral tests and 4 for molecular tests. In the model implemented in this research, after birth, mouse babies are separated from their mother for 180 minutes every day for 12 days (from the second day of birth to the 14th day after birth) and placed in a separate cage. This protocol is so-called. It is called Maternal Neonatal Separation or childhood stress. The results of this research determined that childhood stress in the form of separation from the mother is capable of causing anxiety behaviors in adult male rats. On the other hand, the results of this research determined that optional exercise with running wheels during adolescence can reduce the adverse effects of stress and anxiety-related behaviors.

Keywords: Aerobic Exercise, Wheel Running exercise, Exercise Treadmill, Nervous Central System, Stress, Anxiety behavior, Adolescence, Childhood, Maternal separation stress

1.Introduction

The association between physical activity (PA), exercise, and health outcomes are well-established [1]. Those who exercise have a lower incidence of coronary events and cardiovascular disease. There is a strong inverse relationship between exercise and obesity and diabetes mellitus [2]. In addition, Recent studies provide further evidence to support the notion that regular exercise reduces the risk of insulin resistance, metabolic syndrome, and type 2 diabetes. SI improves when individuals comply with exercise guidelines in a type of diabetes called type I, and insulin secretion stops. In this case, glucose remains high in the blood, so cells can't absorb it. Therefore, the cells are exposed to glucose deficiency. And the solution is to inject insulin into the bloodstream. Indeed, this injection should be completely controlled. Otherwise, too much insulin in the blood can cause death. [3,4] Furthermore, those who exercise have fewer incidences of certain types of cancers and more robust immune responses [5]. Interventions designed to increase PA have resulted in profound reductions in physical ailments [6,7]. There is a similar picture for exercise and mental health outcomes. Those who exercise suffer from less depression [8], anxiety [9], fatigue [10, 11], and cognitive impairments [12, 13]. Early life experiences influence children's neural, behavioral, and psychological development, with long-lasting effects across various domains [1]. A meta-analysis study by North reviewed 80 studies on the impact of exercise on depression and anxiety and showed that exercise activities have antidepressant and antianxiety effects [14]. The antianxiety effects of exercise have been investigated in clinical and animal studies in different ways. The antidepressant and anxiety effects of exercise have been attributed to increasing the expression and production of neuron growth factors (Brain-Derived Neurotrophic factors or BDNF), reducing inflammatory factors, increasing the activity of the serotonergic system, and reducing oxidative stress [15-17]. Anxiety is one of the most common diagnoses in psychiatry, which is twice as common in women as in men. Anxiety and depression disorders are significant because they affect a person's social and individual functioning and lead to severe economic and social damage, so they are expected to be debilitating diseases worldwide in the coming years. In the etiological field of this mental disorder, many hypotheses have been discussed so far, and the importance of the serotonin system in the brain has been identified in this regard. Considering the importance of treating stress disorders such as anxiety, which improves patients' social and personal functioning, and taking into account the side effects of drugs, researchers proposed sports activities as a non-pharmacological and effective occupational therapy method [18]. Among the sports exercises, the running wheel and treadmill are of particular importance because they can balance stress-related mechanisms such as the strong response of stress hormones (glucocorticoids) and neurotransmitter changes [19-21]. But it is not clear whether the effects of exercise can be related to the regulation of the serotonergic system in the brain (especially anxiety behavior). Therefore, this research was designed to answer the following question: Will doing voluntary exercise (Running Wheel) and not mandatory exercise (Treadmill exercise) during adolescence improves the ill effects of MS?

1.1. Early life stress models

In mammals, including humans, monkeys and rodents, maternal input has perhaps the most significant influence on the environment experienced during development [22-25]. Developing any knowledge-based model needs at least two data collections, training and testing. Therefore, it is necessary to randomly divide the experimental data into training and testing collections. [26] Thus, most animal models of early-life stress have manipulated maternal interaction, disrupting either the quantity or quality of maternal care early in life [27-28]. For any model of early-life stress, the detection of a behavioral outcome depends on several variables. The first set of variables pertains to the stress's timing, nature, and severity. Secondly, the age at which animals are tested, whether during adolescence, adulthood, or aging, can determine the outcome. Third, the type and difficulty of the test that is used to measure behavioral outcomes are essential. For example, a rigorous test such as object location memory (OL) might uncover subtle deficits not apparent in a less challenging test, such as object recognition.[29]

Deprivation of mother (lack): Separation from the mother is the most dangerous model that is used for life stress. This model deals with the separation from the mother in early life. In this model, in the first two weeks of life, the cubs are separated from the mother for 1 to 24 hours a day. Maternal separation increases the risk and occurrence of behavioral complications and HPA responses in adulthood [30]

1.2. Open Field Maze (OFM)

The Open Field Maze (OFM) was initially developed in 1934 as a test to measure emotionality in rodents [31]. It has become one of the most widely used measures of behavior in animal psychology [32]. It provides an easy and relatively rapid assessment of well-defined behaviors requiring no training for the test subject and little to no specialized training for the human administering the test. These attributes have led to widespread use of the open field maze in research extended to other animal species such as calves, pigs, rabbits, primates, honeybees, and lobsters [33]. Part of its popularity arises from the psychological and physiological concepts underlying the tests are generally straightforward and well understood. For example, it has been postulated that evolutionary forces have been selected for a typical response in animals. Most species display anxiety-mediated fear or flight responses to specific stimuli. Rodents, for example, show distinct aversions to large, brightly lit, open, and unknown environments [34]. We can assume they have been phylogenetically conditioned to see these environments as dangerous. These features are incorporated in the open field maze and form the basis of its use in behavioral paradigm testing. An open field maze consists of a wall-enclosed area of sufficient height to prevent the subject from escaping. Typical maze shapes are circular or square with a space large enough, based on the size of the subject tested, to elicit a feeling of openness in the maze's center. A number of variables can be stored in the open field maze, with most parameters involving differing types of motor activity [35]. Ambulation is the most common behavior studied, but others such as latency or rearing, can also be measured. Most often, rodent behavior is analyzed in a bare maze. However, the addition of objects, either one or many maze floors, adds the ability to see how the subject interacts with novel additional stimuli [36]. When objects are presented, relevant parameters are typically the number of approaches to an object, preference, or aversion for one thing over another.[37]

2. Materials and Methods

Statistical population, research sample and sampling method In the present study, 10 Wistar female rats were purchased from the Pasteur Institute and placed separately in a cage (Animal Care Center, Sports Physiology Department, Tehran Branch, Islamic Azad University Center) after Pregnancy, 60 male mice were selected as research subjects and separated from their mother for 180 minutes from the 2nd to the 14th day. Then, to determine the experimental and control groups, on the 21st day, these mice were randomly divided into 3 groups and were kept in cages in groups of 4 until the 28th day. The groups included the control, with the stress of separation from the mother and the Running wheel. During the research, the animals were kept in groups of 4 mice in polyethylene cages 15x15x30 made by Razi Rad Company, with a temperature of $22 \pm 2^{\circ}\text{C}$ and humidity of $50 \pm 5\%$ under light-controlled conditions (12 hours of darkness, 12 hours of light). were kept and had free access to water and standard food (10 grams per 100 grams of body weight) manufactured by Behpur company. The experimental protocol was based on guidelines for treatment and use of the laboratory (NIH publication, No. 23-86, revised 1996)(When designing experimental procedure and numerical simulation, particular attention has been paid on the reproduction of similar in-plane loading and boundary conditions)[38]. We can use it for various forming process [39]. All the subjects were

studied in the same environmental and time conditions. The measuring tools and instruments in this research were able to measure the studied variables.[4]

2.1. Training Protocol

Before the implementation of the training protocol, on the 21st day, the special group got familiar with the running wheel for 1 week. Then, for 32 days until day 60, 2 mice were in each cage with two running wheels, each mouse only had access to one of the running wheels (to prevent social isolation, a Plexiglas glass sheet was placed between the two mice). Each running well was made of plexiglas (circumference = 105 cm, length = 10 cm, Navidan Teb company, Iran) and rotated easily with a resistance of 50 g. Each wheel was connected to a magnetic switch that was connected to a counter located outside the animal's cage and the number of rats had free access to the wheel of rodents 24 hours a day for 32 days until day 60, and the number of daily turns was kilometers. After being transferred to the laboratory environment and a week of familiarization with the new environment, the animals were randomly divided into 3 groups (each group including 12 mice, respecting the possible risk of the subjects in the research process), including control groups, with the stress of being separated from the mother and two wheels. rodents putting up. The running wheel of rodents was used as a non-pharmacological treatment in the stress group.[4]

2.1. Behavioral tests

2.2.1. Open Field

We used Open Field to examine the effects of maternal separation and exercise on locomotion. Also, this test is one of the additional tests of the research and is used to check anxiety. The experimental groups are evaluated with the open field device, a plexiglass box with dimensions of 50.50.30; the distance traveled and the animal's presence in the center of the device (space 30 x 30) by the animal is recorded for 5 minutes and is checked.

2.2.2. Elevated Plus Maze or EPM

This tool is made of dark plexiglass and has two open arms and two closed arms, located 50 cm from the ground. Each component measures 50 cm x 10 cm, and a 40 cm wall surrounds the closed arms. Four corridors lead to a 10 x 10 cm area. On the test day, the rats are placed in two central chambers toward the open arm, and their behavior is examined for 5 minutes in terms of the number of times they enter the open arms and their presence in these arms. In these results have been observed the main max strain happened in zone B because the thickness of this part was smaller than zone A.[40]

2.2.3. Statistical Analysis

Investigating childhood stress on significant anxiety behaviors in adult rats

Using EPM and Open-field behavioral tests and t-test statistical method, MS stress significantly caused animal anxiety behaviors. In the OF test, childhood stress caused significant changes in the control group regarding the time spent in the central space ($P < 0.01$, Fig. 1c). Childhood stress decreased the time the animal spent in the open arms of EPM, which means anxious behavior ($P < 0.05$, Fig. 1a). Also, MS reduced the number of adult rats entering the open arms in the EPM test. The decrease in the number of times entering the open arms in this test indicates anxiety behaviors ($P < 0.05$, Fig. 1b).



Fig. 1a

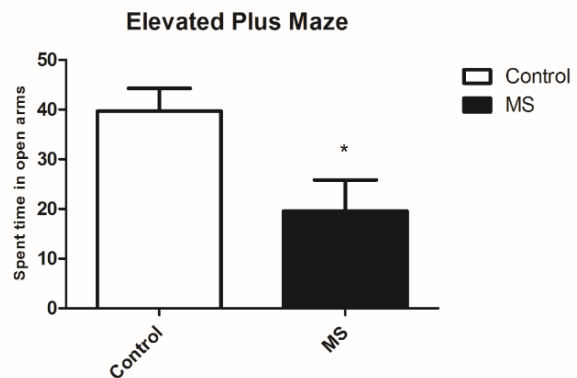


Fig.1b

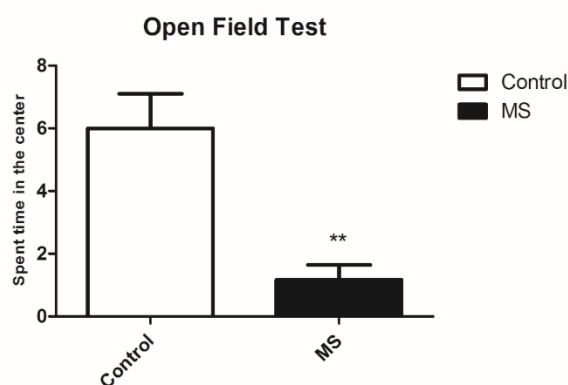


Fig.1c

Figure 1. Subjects' behavioral tests: respectively, a (EPM test of time in the open arm), b (EPM test of the number of times entering the open arm) and c are the Open-Field test.

Analyzing the effect of optional exercise during adolescence on anxiety behaviors in adulthood

This study investigated the effect of optional RW or two-wheel exercise training on the anxiety effects of childhood stress on adult mice. A significant difference between the experimental groups was observed using the ONE-WAY ANOVA statistical test. In the EPM test, voluntary exercise increased the number of animals entering the open arm compared to the stress group ($P < 0.05$, Fig2.A)). Similar results were repeated regarding the time the animal was in the open arm. ($P < 0.05$, Fig2.B). Additional statistical analysis with Tukey's posttest determined that voluntary RW exercise compared to the MS stress group caused anti-anxiety effects in the OFT test ($P < 0.01$, Fig2.C). These results are shown in the figures below.

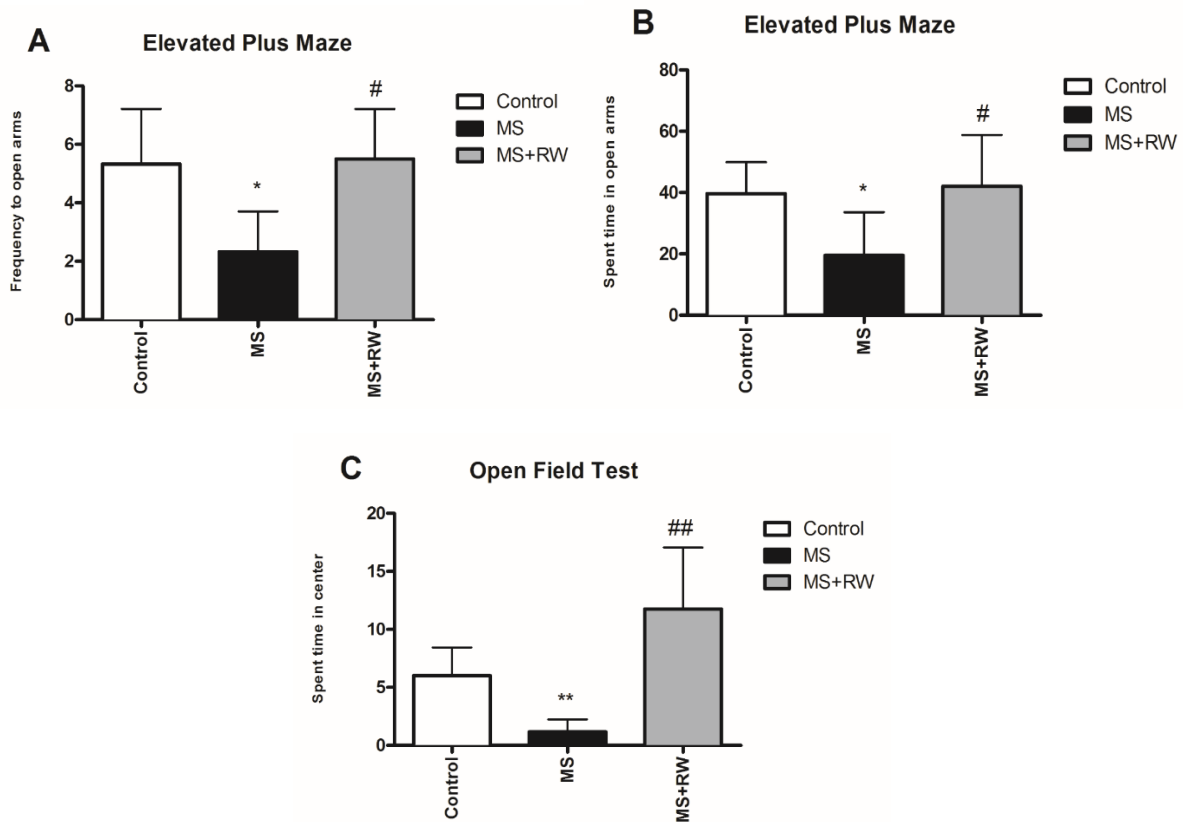


Figure 2. Subjects' behavioral tests: 2A shows the number of times entering the open arm, 2B the time spent in the open arm, and 2C the time spent in the central space of the OFT in the stress and exercise groups, respectively.

3. Results

Conclusions

The results of this research determined that childhood stress in the form of separation from the mother is capable of causing anxiety behaviors in adult male rats. On the other hand, the results of this research determined that optional exercise with well-running rodents during adolescence can reduce the adverse effects of stress. This research confirms the results of previous studies, which show that childhood stress can cause anxiety behaviors in humans and animals. The animal model of separation from the mother is a valid model for investigating the behaviors and pathophysiology of mental illnesses such as depression and anxiety. The results of the EPM test in this study showed that stressed animals have anxiety. A decrease in the number of times the animal enters the open arms in EPM indicates the presence of anxiety behaviors in the animal. An anxious animal fears being in an open space without walls and prefer to remain in closed arms.

On the other hand, the decrease in the number of times the animal entered the open arms in the stress group was accompanied by a decrease in their present time, which indicates the existence of anxiety behaviors in rats. In addition, optional exercise was able to reduce these anxiety behaviors in animals and showed a protective effect. In another test used to investigate anxiety, the Open-Field test confirmed

the results of EPM, which showed that childhood stress could cause anxiety behaviors in animals. The lack of presence in the central space of the Open-Field device indicates that the animal is anxious and afraid of being in the open space. Of course, exercise could also improve this behavior disorder.

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