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Treadmill Exercise Improves Stereotypical Behaviors in Autistic Rats

Vahide Alipour¹, Ramin Shabani¹ ✉, Mohammad-Reza Zarrindast², Farhad Rahmani-Nia³, Mohammad Nasehi⁴

¹ Department of Physical Education and Sport Sciences, Faculty of Humanities, Rasht Branch, Islamic Azad University, Rasht, Iran

² Department of Pharmacology School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

³ Department of Physical Education and Sport Sciences, University of Guilan, Rasht, Iran

⁴ Cognitive and Neuroscience Research Center (CNRC), Amir-Almomenin Hospital, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Abstract

Background: Autism spectrum disorder (ASD) is identified by developmental deficits that lead to repetitive/stereotypic patterns of behavior and impaired social interactions. Studies have been indicated that exercise can decrease stereotypic behaviors in animal models of ASD. This research was designed to discover the effects of different models of forced exercise on stereotypical behaviors in a rat model of ASD induced by thimerosal (THIM). **Materials and Methods:** Fifty-six male Wistar rats were divided into eight groups. The rats were received saline (1 ml/kg) or THIM (300 µg Hg/kg) by four intramuscular injections on 7, 9, 11, and 15 postnatal days. The rats were also treated by several protocols of treadmill exercise, including non-sedentary, sedentary, protocol 1, protocol 2, and a combination of protocols 1 and 2. **Results:** Our study showed that THIM decreased the grooming time compared to the control group. Moreover, protocol 2 exercise significantly decreased grooming time in stranger zone 2 compared to the THIM group. **Conclusions:** Our results showed that stereotypical behaviors exaggerated by THIM and moderate exercise could improve ASD-associated behaviors in the THIM-treated rats. Hence, moderate exercise may be a useful protocol for the treatment of ASD. [GMJ.2022;11:e1990] DOI:[10.31661/gmj.v11i10.1990](https://doi.org/10.31661/gmj.v11i10.1990)

Keywords: Autism; Thimerosal; Grooming; Exercise

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder identified by social communication decrease and social interaction deficit, as well as respective behaviors [1] or stereotypic movements [2]. Evidence has shown that patients with ASD experience many mental disorders, indicating that 79% of ASD patients have at least one psychiatric disorder at some point in their

life [3]. The neurodevelopmental hypothesis described the etiology of ASD as impaired prenatal development and many pathological processes that begin before the brain fully matures [4]. Thimerosal (THIM), known as sodium ethylmercurithiosalicylate, is an ethylmercury-containing pharmaceutical compound containing 49.6% mercury by weight and metabolizes into ethylmercury and thiosalicylate [5]. Since 1999, the issue of THIM as a reason for ASD and other

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Email:info@gmj.ir



✉ Correspondence to:

Ramin Shabani, Faculty of Humanities, Department of Physical Education and sport science, Rasht Branch, Islamic Azad University, Rasht, Iran
Telephone Number: +989112324796
Email Address: shabani_msn@yahoo.com

important neurodevelopmental disorders has turned into a hot topic [6-8].

Unfortunately, there has been no certain medical treatment for ASD so far. Lack of effective treatments or medications showed a need to decrease stereotypical behaviors without critical side effects from medications. Evidence has shown the positive effects of exercise on ASD symptoms [9, 10]. It has been shown that customized physical education effectively decreases the stereotypical behaviors of a child with ASD and dramatically decreases anxiety in patients with ASD [11].

Iardanis and Apteslis performed physical education and exercise for children with ASD to determine the effects on socialization, communication, and behavior [12]. They also demonstrated positive behavior associated with increased physical activity [12]. Pan *et al.* [11] evaluated the impact of the swimming programs on behavioral and social skills in children with ASD. The results demonstrated that it had been associated with a reduction in antisocial behavior problems [11]. Previous studies have also evaluated the impact of physical activity and exercise interventions on individuals with ASD [13, 14], resulting in positive outcomes. Thus, we examined the effects of different models of forced exercise on stereotypical behaviors in a rat model of ASD-induced by THIM.

Materials and Methods

1. Animals

Male Wistar rats (weighing 200-250g) were obtained from the animal house Institute of cognitive sciences studies (ICSS; Tehran, Iran). All rats had ad libitum access to food and water and were housed in a room with a standard temperature ($22\pm 2^{\circ}\text{C}$) and a 12/12h light/dark cycle (lights on at 7). All tests were carried out between 9 h and 14 h, and each rat was tested only once.

2. Drugs

THIM was purchased from Merck (Germany) and Acros (Acros organic, Thermo fisher

scientific, USA) companies. THIM administration was done on postnatal days (PND) 7, 9, 11, and 15 at the dose of ($300\ \mu\text{g Hg/kg}$) ((per kg) in a volume of $50\ \mu\text{l}$, intramuscular into the glutei maximi.

3. Study Groups

The 56 newborn rats were randomly divided into eight groups, and each group consisted of some subgroups. The schedule was as follows [15, 16]:

Group 1. Saline (1 ml/kg) was injected without exercise.

Group 2. Saline (1 ml/kg) was injected plus protocol 1 exercise (PND 21-50).

Group 3. Saline (1 ml/kg) was injected plus protocol 2 exercise (PND 51-80).

Group 4. Saline (1 ml/kg) was injected plus protocol 1 exercise (PND 21-50) and protocol 2 exercise (PND 51-80).

Group 5. THIM ($300\ \mu\text{g Hg/kg}$) was injected without exercise

Group 6. THIM $300\ \mu\text{g Hg/kg}$ was injected per injection plus protocol 1 exercise (PND 21-50).

Group 7. THIM ($300\ \mu\text{g Hg/kg}$) was injected per injection plus protocol 2 exercise (PND 51-80).

Group 8- THIM ($300\ \mu\text{g Hg/kg}$) was injected per injection plus protocol 1 exercise (PND 21-50) and protocol 2 exercise (PND 51-80).

4. Exercise Protocols

4.1. Postnatal Treatment (Exercise Protocol 1)

The day of delivery was considered as PND. In PND groups, animals were put on the stationary treadmill (sedentary), pups from dams prenatally ran from PND 21 to PDN 50 (protocol 1).

At first, the rat pups of the exercise group were familiarized with the treadmill with a speed of 2m/min for 10 min/day on days 1 and 2 of exercise. Next, the animals were forced to run on the treadmill for 30 min once a day for 18 days.

The intensity of exercise applied for the exercise group was running at the speed of 2m/min for the first 5 min, 5m/min for the next 5 min, and the speed of 8 m/min for the last 20 min at an eight inclination. Control animals were put on stationary treadmills for the same period [17].

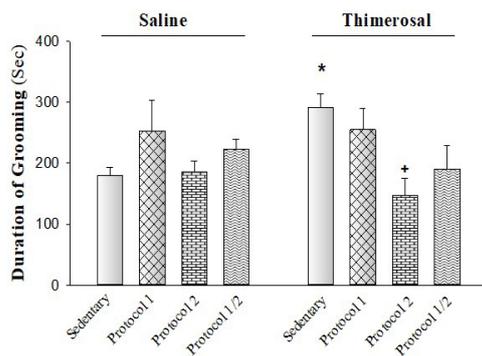


Figure 1. The effect of exercise protocols treatment on stereotypical behaviors (grooming) in the THIM-exposed rat. The rats received several kinds of exercise protocols in the presence or absence of THIM. The duration of grooming is an index of ASD-like behaviors. Seven rats were used in each group. The data are shown as mean±S.E.M. *P<0.05 as compared to the sedentary/saline group as the control group. +P<0.05 as compared to the sedentary/THIM group as a control group.

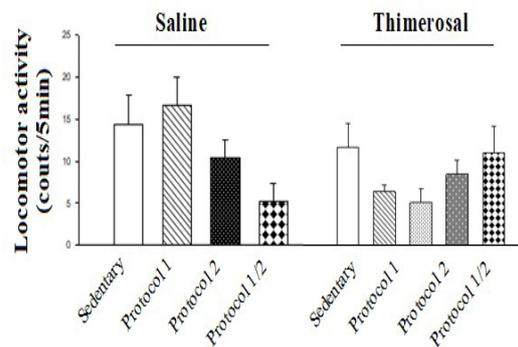


Figure 2. The effect of exercise protocols treatment on locomotor activity in the THIM-exposed rat. The animals received some kinds of exercise protocols in the presence or absence of THIM. Seven animals were used in each group. The data are exhibited as mean±S.E.M.

4.2 Exercise Protocol 2

For this protocol, 51-80 days male Wistar rats were used at the beginning of all experiments. Rats ran as forced exercise on the treadmill at 0° inclination Sunday to Thursday during the light phase (9:00 am to 2:30 pm) for up to four weeks.

The treadmills were equipped with grids that induce a slight electric shock (average intensity of 0-0.05 mA with an inter-pulse interval of 1-2 seconds) to force the animals to run constantly [18].

4.3. Exercise Protocol 1 + 2

Protocol 1 was achieved on PND 21-50, and the juvenile rat did running exercise with protocol 2 on PND 51-80 (Figure-1).

5. Grooming Behavior in Three-Chambered Social Interaction Room

The duration of this stage was 10 minutes. The time of direct interactions between the subject rat and the containment lodging (stranger 1) or not lodging (stranger 2) rat individually and the duration of other behaviors such as self-grooming was recorded and monitored.

6. Open Field Test (OFT)

The size of the open field cage in this study was 40 cm × 60 cm × 50 cm, which was prepared of white plastic. The cage floor was divided into 12 equal squares that were

observable by the experimenter. A video camera was located on top of the apparatus to record the tests. Each rat was located in the center of the cage. The number of locomotor activities in the apparatus was counted for 5 min. The apparatus was cleaned with ethanol 70% after each test [19].

7. Ethical Issues

Experiments were performed on Male Wistar rats (weighting 290-300 gr). The rats had free access to food and water and housed in room with an ambient temperature of 22±2°C and 12:12 hr light/dark cycle (lights on at 7.00 h). The animals were only handled for weighing, drug administration and cage cleaning (Co number: IR.IAU.LIAU.REC.1401.001).

All procedures were approved by the Research and ethics committee of Rasht Branch, Islamic Azad University, Rasht, Iran (dissertation code: 117214423962001).

8. Statistical Analysis

At first, data normality of distribution and homogeneity of variance were evaluated. To assess stereotype behaviors in the three-chamber paradigm test and locomotor activity on the open field test, two-way ANOVA was applied. All statistical tests were directed using SPSS (SPSS Inc., Chicago, IL). P<0.05 was considered to show statistical significance.

Results

Effect of Exercise Protocols Treatment On Stereotyped Behaviors (Grooming) in the THIM-Exposed Rats

Two-way ANOVA followed by posthoc analysis revealed that administration of THIM increased the duration of grooming while only protocol 2 restored this response (THIM effect $F [1, 48]=0.1, P<0.05$; protocols effect $F [3, 48]=3.25, P<0.05$; and THIM-protocol interaction effect $F [3, 48]=3.69, P<0.05$; Figure-1).

Effect of Exercise Protocols Treatment On Locomotor Activity in the THIM-Exposed Rat

Two-way ANOVA followed by posthoc analysis displayed that injection of THIM had no significant effect on locomotor activity (THIM effect $F [1, 48]=2.85, P>0.05$, protocols effect $F [3, 48]=1.28, P>0.05$; and THIM-protocol interaction effect $F [3, 48]=1.14, P>0.05$; Figure-2).

Discussion

This research evaluated the effects of duration-intensity of exercise on stereotyped behaviors in autistic rats. According to studies, many physiological changes in THIM-treated animals are similar to the symptoms of ASD. ASD is identified according to the characteristic neurobehavioral patterns such as stereotyped behaviors and movement impairment [20, 21], an increase in anxiety [20]. Thus in the present research, we measured these similar behaviors in a rat model of ASD. One of these behaviors is grooming. As we know, grooming is the main activity during the wakefulness phase of the rats [21]. Grooming usually manifests itself in response to unexpected stimuli, conflict situations, or frustration. This stress and conflict-related grooming are described as a displacement activity. Note that both novel and stressful stimuli can significantly augment the pituitary-adrenal system. This relationship between the novel and/or stressful stimuli and grooming as displacement activity is so important due to

the potency of adrenocorticotrophic hormone (ACTH) and other pituitary peptides (ACTH, melanocyte-stimulating hormone [MSH], and β -MSH) to induce excessive grooming through intraventricular administration [22]. In this study, we showed that THIM administration could alleviate the ASD-like behavioral time (grooming) compared to the control group. Previous studies have also shown that THIM increased repetitive and stereotyped behaviors, the core symptoms of ASD [23, 24]. In these studies [23, 24], high-intensity aerobic activity can worsen stereotypic behaviors in children with ASD, while mild- and moderate-intensity aerobic activities induce great reductions in these behaviors. Previous studies have revealed that physical activity can improve stereotyped behaviors [9, 24]. It has been suggested that exercise-induced short-term decrease of stereotypic behaviors in individuals with ASD [14]. It has also been reported that a mere 3-min bout of running improves stereotypic behaviors in an autistic patient [25]. Furthermore, water-based exercise along with social skills training can dramatically improve the behaviors of patients with ASD [26]. Additionally, a previous study has reported that short- and low-moderate-intensity exercise in patients with ASD can significantly reduce self-stimulatory behaviors [26]. The therapeutic effect of exercise in ASD has been shown in many studies [27-29]. Unlike the present research, many previous studies have suggested that high-intensity exercise greatly decreased stereotyped behavior, and its therapeutic effect was much more than low-intensity exercise [26, 30-33]. On the other hand, some research did not reveal a therapeutic effect of intensity exercise on stereotyped behavior in individuals who do a running/jogging protocol for up to fifteen minutes. This result may be associated with the intensity of the exercise [31]. Moreover, researchers have declared that disruptions in neurotransmitter systems such as serotonin and dopamine have a main role in the induction of stereotypical behaviors in ASD [34]. Evidence links stereotypical behaviors to the function and level of dopamine and serotonin. It has been revealed

that hyperserotonemia is interconnected with declarative abilities and self-injuries behaviors in patients with ASD [35]. Research indicates that physical activities significantly change dopaminergic and serotonergic systems and decrease stereotypical behaviors in ASD [36]. Several other factors play a strong role in decreasing grooming behavior in autistic rats. It has been shown that N-methyl-D-aspartate (NMDA) gene can induce long-lasting changes in cognition and behaviors [37]. NMDA can improve ASD-like behaviors via balancing excitatory-inhibitory circuits [38]. Exercise can be mediated through Ca²⁺ influx via NMDA receptor, whose expression is enhanced following exercise. This process activates the mitogen-activated protein kinase (MAPK) cascade through Ca²⁺/

calmodulin-dependent protein kinase[39, 40]. The activated MAPK affects many important factors, such as the cAMP response element-binding protein that its expression is enhanced following exercise [41].

Conclusion

Treatment with THIM increased the stereotypical behaviors. Also, stereotypical behaviors were reversed by moderate exercise. Hence, moderate exercise may be a beneficial method for the treatment of ASD.

Conflict of Interest

The authors declare that they have no conflict of interest.

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