Heliyon 9 (2023) e20641

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

The effect of low- and moderate-intensity interval training on cognitive behaviors of male and female rats with VPA-induced autism

Nazanin Sabet [a], Banafshe Abadi [b,c], Amirhossein Moslemizadeh [c], Mohammad Amin Rajizadeh [d], Fatemeh Arabzadeh [e], Seyyed Sajjad Vakili Shahrbabaki [f], Zahra Soltani [g], Forouzan Rafie [h,i], Hamideh Bashiri [f,j,k,*]

[a] Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[b] Herbal and Traditional Medicines Research Center, Kerman University of Medical Sciences, Kerman, Iran
[c] Brain Cancer Research Core (BCRC), Universal Scientific Education and Research Network (USERN), Kerman, Iran
[d] Department of Immunology, Tehran University of Medical Sciences, Tehran, Iran
[e] Cardiovascular Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran
[f] Student Research Committee, Kerman University of Medical Sciences, Kerman, Iran
[g] Department of Biology, University of Turku, Turku 20014, Finland
[h] Endocrinology and Metabolism Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran
[i] Department of Physiology and Pharmacology, Afzalipour Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[j] Visiting fellow Department of Medicine Division of Geriatrics and Gerontology Emory University School of Medicine
[k] Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

ARTICLE INFO

Keywords:
Valproic acid
Autism
Exercise training
Anxiety-like behaviors
Rat
Social impairments

ABSTRACT

Introduction: This study was performed to evaluate the effects of low and moderate treadmill exercise for one month on social interaction, anxiety-like behaviors, and spatial learning and memory in male and female autistic rats.

Methods: Pregnant rats received valproic acid (VPA) (600 mg/kg/i.p) once on gestational day 12.5 to induce autism-like symptoms in the offspring. After delivery, the offspring were divided into six main groups, each with male and female subgroups: Control (CTL, prenatal normal saline), autism (prenatal VPA), low-intensity training (LIT,normal saline + low treadmill exercise), moderate -intensity training (MIT, normal saline + moderate treadmill exercise), VPA + LIT, and VPA + MIT. On the 60th day, the offspring were tested by the elevated plus maze (EPM), open field test (OFT), social interaction test (SIT), and Morris water maze (MWM).

Abbreviations: ASD, Autism spectrum disorder; ANOVA, Analysis of variance; BDNF, Brain-derived neurotrophic factor; EPM, Elevated plus maze; HPA, Hypothalamic–pituitary–adrenal; LIT, Low-intensity training; MIT, Moderate-intensity training; MWM, Morris water maze; SI, Sociability index; SIT, Social interaction test; SNI, Social novelty index; OAE, Open arm entries; OAT, Open arm times; OFT, Open field test; SEM, Standard error of mean; VPA, Valproic acid.

* Corresponding author. P. O. Box 76169 13555, Kerman, Iran.

E-mail addresses: Nazanin_5157@yahoo.com (N. Sabet), banafshe.abadi@yahoo.com (B. Abadi), ahmz1374@gmail.com (A. Moslemizadeh), aminrajizadeh@yahoo.com (M.A. Rajizadeh), ftmarabzade@gmail.com (F. Arabzadeh), farshad.v.fard@gmail.com (S.S. Vakili Shahrbabaki), soltaniy@yahoo.com (Z. Soltani), frafie@emory.edu (F. Rafie), h.bashiri@kmu.ac.ir, bashiry_h@yahoo.com (H. Bashiri).

https://doi.org/10.1016/j.heliyon.2023.e20641
Received 8 February 2023; Received in revised form 23 August 2023; Accepted 3 October 2023
Available online 5 October 2023

Results: The results showed that both LIT and MIT could partly alleviate anxiety-like behaviors induced by prenatal VPA exposure in two sexes. Social impairment was observed in the autistic rats and was improved by LIT in both sexes and MIT in females. No significant change was seen in the spatial learning and memory of autistic rats by exercise.
Conclusion: The findings suggest that treadmill exercise can be helpful for improving some autism-like behaviors. Further studies are needed to investigate the involved mechanisms.

1. Introduction

Autism spectrum disorder (ASD) refers to a complex behavioral disorder resulting from defects in neurobiological development [1,2]. The prevalence of autism is significantly increasing. It is estimated that about 1 in 100 children is diagnosed with ASD [3]. Studies have shown that ASD is 4.5 times more common in males than females [4], but the behavioral disorders associated with the disease are more severe in females than in males [5,6]. This disorder is congenital and occurs in the first 3 years of life. It is usually detected between the ages of 2 and 4 years with clinical observations and a questionnaire for parents [7]. The main symptoms are impaired social communication and interaction, difficulty in speaking, a tendency for repetitive behaviors, and monotonous activities and interests [8]. The complexity of symptoms and comorbidities associated with this disorder make the diagnosis difficult [9]. According to available evidence, genetic and environmental factors may lead to autism [10]. Studies have displayed many genetic deficiencies associated with this disease [11]. Exposure to teratogenic substances such as valproic acid (VPA) as an antiepileptic drug in the early days of pregnancy can contribute to the induction of autism [12]. Animal investigations have shown that VPA administration during specific stage of prenatal growth representing the first trimester of human pregnancy induces autistic-like behaviors, including social behavior disorders and cognitive deficits, similar to that of people with ASD.

Due to the high prevalence of ASD, costs of treatment, and it's devastating effect on families, this syndrome has received a great deal of attention [13]. The therapeutic role of exercise has been indicated in many neurological diseases, including autism. Exercise is a major treatment program for autism. Exercise causes the release of certain neurotransmitters in the brain. Findings show that it has positive effects on brain activity through various mechanisms, such as increased neurogenesis, mood enhancement, etc. [14]. Exercise can offer benefits to autistic individuals through rhythmic movements that are similar to stereotyped behaviors. Researchers have found that exercise can reduce repetitive behaviors, but this knowledge is often marginalized [15,16]. Intense exercise stimulates oxidative stress, but regular and MIT lessens oxidative stress by increasing antioxidant defense, ultimately reducing the symptoms of autism. Additionally, it can suppress Purkinje cell deficiency by down-regulating reactive astrocytes and microglia and improve motor function in mice with autism [17,18].

Low intensity activities require the least amount of effort, compared to moderate activities. The definition for low intensity activity is an activity that is classified as < 3 metabolic equivalents (METS). One MET, or metabolic equivalent, is the amount of oxygen consumed while sitting at rest. Moderate intensity activities are defined as activities ranging between 3 and < 6 METS. These activities require more oxygen consumption that light activities. Also, moderate-intensity activity is usually made up of exercises that get your heart rate up to 50 %–60 % higher than its rate when you are at rest. Several studies have revealed that treadmill exercise at low speeds (up to 8 m/min) could positively affect motor and cognitive disorders in autistic rats [19,20]. Also, in another study, treadmill exercise at moderate speeds (up to 25 m/min) could improve neurological deficits such as autism [16]. The effects of exercise in low and moderate intensities have been examined on autism, but no comparison has been made between these two types of exercise intensity in the two sexes. This study aimed to investigate the effects of low and moderate endurance exercise on anxiety-like behaviors, social interaction, and learning and memory in male and female rats.

2. Materials and methods

2.1. Animals

This experimental study was approved by the Ethics Committee of Kerman University of Medical Sciences. The Ethic Approval Code is IR.KMU.REC.1399.448. Wistar rats with controlled fertility were used for this study. One male and two female rats with a weight range of 220–250 g were kept in one cage to mate for three days.

The evaluation of the estrous cycle was randomly conducted based on the proportion of cell types observed in the vaginal secretion. The estrous cycle was daily monitored by taking and staining the vaginal smears with the Papanicolaou method between 7.00 and 8:00 a.m [21].

Briefly, the specimen was treated with alcohol in graded concentrations, stained with solutions including eosin-azure (EA50), orange G (OG6), and Harris haematoxylin, re-treated with alcohol in graded concentrations, and placed in xylene. The estrous cycle stage was specified by a light microscope with a 40 $\times$ objective lens. The estrous phase was confirmed by the presence of enucleated cornified cells [21,22]. The morning in which spermatozoa were observed in vaginal smear or vaginal plugs was set as day 0 of pregnancy [21]. After ensuring the female rats' pregnancy, they were kept in separate cages until parturition. All rats were kept under 12 h of light/dark cycle and controlled temperature (23 ± 1) and had free access to food and water. VPA was purchased as sodium salt from Sigma Company. VPA solution was prepared by dissolving the sodium salt in 0.9 % saline to achieve a concentration of 250 mg/ml, which was injected into pregnant rats at a dose of 600 mg/kg on day 12.5 of pregnancy [4]. After parturition, the offspring

were lactated until day 21. Thereafter, based on their sexes, they were randomly divided into 12 groups. All tests were performed between 9:00 to 15:00 on days 60–70. Offspring started the treadmill exercise with two intensities, low and moderate, on day 30 onward for one month. The effects of treadmill exercise on anxiety-like behaviors, spatial learning and memory, and social interaction were evaluated in this study.

The study groups were as follows.

1) Control (CTL) group: Female and male rats received prenatal normal saline (1 ml/kg).
2) Autistic group (VPA): Female and male rats received prenatal VPA [23].
3) Low-intensity training (LIT): Female and male rats received prenatal normal saline (1 ml/kg) and did low-intensity treadmill exercise for one month [24].
4) Moderate-intensity training (MIT): Female and male rats received prenatal normal saline (1 ml/kg) and did moderate-intensity treadmill exercise for one month [25].
5) Autistic rats + low-intensity training (VPA + LIT): Female and male rats received prenatal VPA (600 mg/kg/i.p. through the mother) and did low-intensity treadmill exercise one month after birth for one month.
6) Autistic rats + moderate-intensity training (VPA + MIT): Female and male rats received prenatal VPA (600 mg/kg/i.p. to mother) and did moderate-intensity treadmill exercise one month after birth for one month.

2.2. Exercise protocol

The 30-day-old male and female rats in the exercise groups started running on the treadmill five times a week, 30 min daily at a speed of 8–10 m/min [24](low intensity, for one month, and the moderate-intensity exercise group began running on the treadmill for 30 min at a speed of 20 m/min daily, five times a week for one month [25].

2.3. Elevated plus maze

The elevated plus maze (EPM) is known as one of the methods which can be applied to measure the anxiety-like behavior of animals. The test was conducted in the same ways as a previous study [26]. The apparatus is a wood-based device with two enclosed arms (50 × 10 × 40 cm) and two open arms (50 × 10 cm) placed at 50 cm above the floor. A 1 cm plexiglass margin was set on the sides and at the end of the open arms to prevent rats from falling. In each experiment, the animal was placed in the 10 × 10 cm square in the center zone of the maze and allowed to move freely toward any arm for 5 min. The entrance frequency and time spent in each arm were measured by a video tracking system (Borj Sant Azma), and finally, %OAE (%open arm entries) and %OAT (%open arm times) were calculated as follows [27]:

$$\mathrm{OAE\%} = \frac{\textit{Open Arm Entries}}{\textit{Total enteries in both arms}} \times 100$$

$$\mathrm{OAT\%} = \frac{\textit{Open Arm Time spent}}{\textit{Total time in both arms}} \times 100$$

2.4. Open field test

The open field test (OFT) was performed to evaluate the motor activity and stereotyped behaviors in an unfamiliar area. The apparatus was a 90 × 90 × 30 cm Plexiglas box with its floor divided into 16 small equal squares. At the beginning of the test, each rat was placed individually in the center zone and permitted to explore the box freely. Mean speed, total distance moved, and the duration spent in the center and peripheral area were recorded for 5 min using the video tracking system (Borj Sant Azma). Also, the number of grooming and rearing behaviors were measured as stereotypic behaviors by a human observer who was blind to experiment groups. Moving toward the peripheral area of box, grooming, and rearing were considered anxiety-like behaviors [28].

2.5. Three-chamber sociability and social novelty test

In order to evaluate the social behavior of animals, the three-chamber sociability test was done as previously described [21]. This test is based on animals' natural inclination to discover a novel context. The social interaction test (SIT) includes three sessions: habituation period, sociability, and social novelty period. The apparatus in the size of 180 × 420 × 160 cm is divided into three chambers connected by doorways, with the right and left chambers, containing a wire cage each. In the habituation period, the rat was placed in the center of the middle chamber for 5 min. After the habituation phase, a rat without any previous contact with the subject rat, Stranger 1, was placed in a wire cage in the right chamber. Then, the doorways were opened to permit the subject rat to explore all the chambers for 10 min. The time spent in each chamber (S1, S2) and active interaction with Stranger1 or with the empty wire cage was recorded by the video tracking system (Borj Sant Azma) and a human observer who was blind to the experiment groups. In the third phase, a new unfamiliar rat, Stranger 2, was placed in the other, empty wire cage. The duration of active interaction with Stranger1 and 2 and the duration spent in each chamber were recorded separately for 10 min. During the second and third phase, sociability index (SI) and social novelty index (SNI) was calculated as follows [29]:

$$SI\ (Sniffing) = \frac{Time\ exploring\ (novel\ rat\ 1) - Time\ exploring\ (novel\ object)}{Time\ exploring\ (novel\ rat\ 1) + Time\ exploring\ (novel\ object)}\ \text{SNI (Sniffing)}$$

$$= \frac{Time\ exploring\ (novel\ rat\ 2) - Time\ exploring\ (known\ rat)}{Time\ exploring\ (novel\ rat\ 2) + Time\ exploring\ (known\ rat)}\ \text{SI (Chamber time)}$$

$$= \frac{Time\ spent\ in\ chamber\ (novel\ rat\ 1) - Time\ spent\ in\ chamber\ (novel\ object)}{Time\ spent\ in\ chamber\ (novel\ rat\ 1) + Time\ spent\ in\ chamber\ (novel\ object)}\ \text{SNI (Chamber time)}$$

$$= \frac{Time\ spent\ in\ chamber\ (novel\ rat\ 2) - Time\ spent\ in\ chamber\ (known\ object)}{Time\ spent\ in\ chamber\ (novel\ rat\ 2) + Time\ spent\ in\ chamber\ (known\ object)}$$

2.6. Morris water maze

The Morris water maze (MWM) was used to estimate spatial learning and memory in the rats. The MWM is a dark circular tank, 160 cm in diameter and 60 cm in depth, filled with water (35 cm in height, 23–25 °C). In this method, animals escape from water onto a hidden platform with a 10 cm diameter placed 1.5 cm *below* the surface of the *water* in the center of the tank. Various spatial cues are mounted on the walls around the tank, and their location is constant during the test. The spatial learning of animals and memory-related parameters such as the total time spent to detect the hidden platform (escape latency) and the distance moved to reach the hidden platform were measured by a video-tracking system software (Ethovision, Noldus Information Technology, Netherlands).

Firstly, spatial learning was evaluated in three blocks with an interval of 30 min, and each block contained four trials. The animal was placed into the water from one of the four quadruple circles that the machine randomly selected for each trial. The animal was allowed to swim for 60 s to use the surrounding spatial cues to find the hidden platform under the surface of the water and rest on it for 30–35 s. The animal was then placed in its cage for 30–35 s before the next trial. Two hours after the training trial, the probe trial was

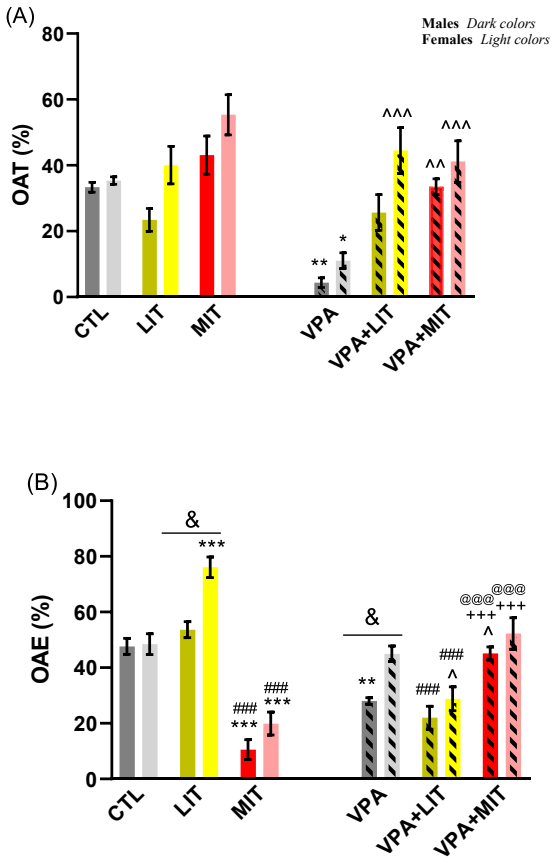

Fig. 1. The effects of LIT and MIT on open arm time (%OAT) (A) and open arm entries (%OAE) (B) in the EPM in autistic rats. Each bar represents mean ± SEM. Significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with CTL group in similar sex; $^{\hat{}}P < 0.05$, $^{\hat{}\hat{}}P < 0.01$, $^{\hat{}\hat{}\hat{}}P < 0.001$ compared with VPA group in the similar sex; $^{\#\#\#}P < 0.001$ compared with LIT group in the similar sex; $^{+++}P < 0.001$ compared with MIT group in the similar sex; $^{@@@}P < 0.001$ compared with VPA + LIT group in the similar sex; $^{\&}P < 0.05$, male groups in comparison with the female groups ($n = 7$ in each group).

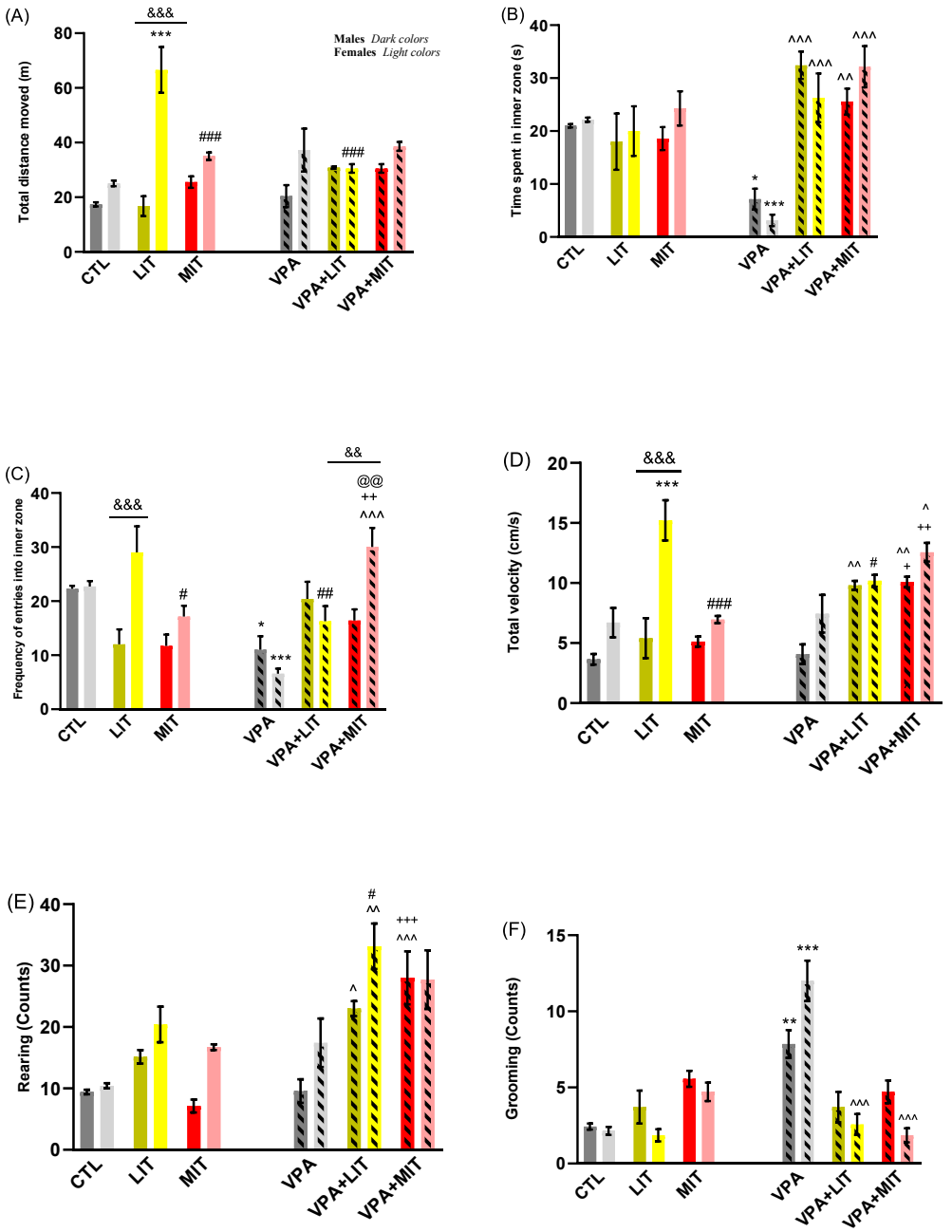

Fig. 2. The effects of LIT and MIT on total distance moved (A), time spent in inner zone (B), frequency of entries into inner zone (C), total velocity (D), rearing (E) and grooming (F) in the OFT in autistic rats. Each bar represents mean ± SEM. Significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with CTL group in the similar sex; $^{\hat{}}P < 0.05$, $^{\hat{}\hat{}}P < 0.01$, $^{\hat{}\hat{}\hat{}}P < 0.001$ compared with VPA group in the similar sex; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with LIT group in the similar sex; $^{++}P < 0.01$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with MIT group in the similar sex; $^{@@}P < 0.01$ compared with VPA + LIT group in the similar sex; $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$ male groups in comparison with female groups ($n = 7$ in each group).

performed for 60 s to evaluate the spatial memory via removing the platform. The total time spent and distance traveled in the target quadrant, which previously contained the platform, were considered as spatial memory retention [23].

2.7. Statistical analysis

Results were reported as mean (SEM). The analysis of the obtained data was performed using two-way analysis of variance (ANOVA). In order to compare the significance of the groups, Tukey post hoc test was used. For analysis of spatial learning in the water maze, we used repeated-measures ANOVA. In all statistical comparisons, *P*-values less than 0.05 were considered statistically significant. Data analyses were done using GraphPad Prism version 8.00, (GraphPad Software, San Diego, USA).

3. Result

3.1. The effect of LIT and MIT on %OAT and %OAE in the EPM in autistic rats

Fig. 1 shows the effects of LIT and MIT on EPM indices as anxiety-like behaviors. Two-way ANOVA revealed a significant interaction among treatments [F (5, 30) = 17.10, $P < 0.001$] and sexes [F (1, 6) = 71.28, $P < 0.001$], but not for treatment & sex [F (5, 30) = 1.04, $P > 0.05$] in the %OAT. Tukey post hoc analysis revealed a significant decrease in VPA groups compared with ($P < 0.01$ for males, and $P < 0.05$ for females), indicating anxiety-like behaviors in the autistic rats. VPA + LIT in females ($P < 0.001$), and VPA + MIT in males and females ($P < 0.01$ and $P < 0.001$, respectively) significantly increased %OAT compare with VPA groups, suggesting a reversal effect of these two kinds of training (Fig. 1A).

As seen in Fig. 1B for %OAE, two-way ANOVA indicated a significant relationship among treatments [F (5, 30) = 51.66, $P < 0.001$], between sexes [F (1, 6) = 43.42, $P < 0.001$,] and treatment & sex, [F (5, 30) = 3.137, $P < 0.05$]. Tukey post hoc analysis revealed that % OAE in the LIT female group was more than the CTL group ($P < 0.001$). There was a remarkable difference between the male and female LIT groups ($P < 0.05$). On the other hand, this index in MIT groups was lower than the CTL ($P < 0.001$) and LIT ($P < 0.001$) groups in both sexes. VPA administration decreased %OAE in male rats compared with the CTL group ($P < 0.01$), indicating anxiety-like behavior in autistic males, and MIT could reverse it ($P < 0.05$) and exerted anxiolytic effects. However, VPA + LIT in female rats had a considerable decrease compared to VPA group ($P < 0.05$). There was a remarkable difference between the male and female VPA groups ($P < 0.05$). sexes in addition, %OAE in the VPA + LIT groups were less than the LIT groups ($P < 0.001$). Furthermore, this index in the VPA exercise + MIT groups was significantly higher than the MIT ($P < 0.001$) and VPA + LIT ($P < 0.001$) groups in both sexes (Fig. 1B).

3.2. The effect of LIT and MIT on OFT indices in autistic rats

Fig. 2 shows the effects of low and moderate endurance exercise training on OFT indices as anxiety-like and stereotypic behaviors and locomotor activity. Two-way ANOVA revealed significant interaction among treatments [F (5, 30) = 6.78, $P < 0.001$], between sexes [F (1, 6) = 25.36, $P < 0.01$] and treatment & sex [F (5, 30) = 11.91, $P < 0.001$] in the total distance moved. Tukey post hoc analysis revealed that this parameter in the LIT female group was significantly higher than the CTL group ($P < 0.001$). Also, there was a significant difference between both sexes about LIT ($P < 0.001$). On the other hand, total distance in the MIT female and VPA + LIT female groups were lower than the LIT female group ($P < 0.001$) (Fig. 2A).

For time spent in the inner zone, as shown in Fig. 2B, two-way ANOVA revealed a significant interaction among treatments [F (5, 30) = 11.95, $P < 0.001$], but not for sexes [F (1, 6) = 0.31, $P > 0.05$] and treatment & sex [F (5, 30) = 1.88, $P > 0.05$]. Tukey post hoc analysis revealed that rats that received VPA in both male ($P < 0.05$) and female ($P < 0.001$) groups spent less time in the inner zone, indicating anxiety-like behaviors in autistic rats. Our results showed that VPA + LIT and VPA + MIT in both sexes significantly increased this parameter compared to the VPA groups, suggesting the reversal effect of exercise on anxiety behaviors ($P < 0.001$ and $P < 0.01$) (Fig. 2B).

Two-way ANOVA revealed a significant interaction among treatments [F (5, 30) = 6.598, $P < 0.001$], between sexes [F (1, 6) = 8.481, $P < 0.05$] and treatment & sex [F (5, 30) = 8.96, $P < 0.001$] in the frequency of entries into the inner zone (IFREQ). Further analysis showed that the LIT females had more IFREQ than males ($P < 0.001$). VPA injection decreased the IFREQ) in the autistic male ($P < 0.05$) and female ($P < 0.001$) groups compared to the CTL groups, proposing anxiety-like behaviors in the VPA groups. The in females, MIT ($P < 0.05$) and VPA + LIT ($P < 0.01$) groups had less IFREQ than the LIT group. Furthermore, in females, the VPA + MIT group had more IFREQ than the VPA ($P < 0.001$), MIT ($P < 0.01$), and VPA + LIT ($P < 0.01$) groups. There was a considerable difference between male and female rats in the VPA + MIT group ($P < 0.01$) (Fig. 2C).

In Fig. 2D, two-way ANOVA revealed a significant interaction among treatments [F (5, 30) = 12.33, $P < 0.001$], between sexes [F (1, 6) = 25.46, $P < 0.01$] and treatment & sex [F (5, 30) = 9.5, $P < 0.001$] in total velocity. Further analysis showed that rats in the LIT female group had more velocity compared to the CTL group ($P < 0.001$). There was a significant difference between male and female in the LIT group ($P < 0.001$). On the other hand, in females, the MIT ($P < 0.001$) and VPA + LIT ($P < 0.05$). groups had less velocity than the LIT group. VPA + LIT and VPA + MITmale groups ($P < 0.01$), and VPA + MIT female group ($P < 0.05$) had more velocity than the VPA group ($P < 0.01$). It should be considered that the VPA MIT male ($P < 0.05$) and female ($P < 0.01$) groups had less velocity than the MIT groups (Fig. 2D).

About rearing, two-way ANOVA revealed a significant interaction among treatments [F (5, 30) = 19.10, $P < 0.001$] and between sexes [F (1, 6) = 8.88, $P < 0.05$], but not for treatment & sex [F (5, 30) = 1.86, $P > 0.05$]. Tukey multiple comparison revealed a

significant difference for number of rearing in the VPA + LIT male ($P < 0.05$) and female ($P < 0.01$) groups, and also the VPA + MIT male group ($P < 0.001$) compared to the VPA group. There was a significant increase in rearing of the VPA + LIT female group compared to LIT group ($P < 0.05$). The VPA + MIT male group increased rearing compared to the MIT rats ($P < 0.001$) (Fig. 2E).

Fig. 2F illustrates the grooming number in the experimental groups. Two-way ANOVA revealed a significant interaction among treatments [$F(5, 30) = 29.06$, $P < 0.001$] and treatment & sex [$F(5, 30) = 4.07$, $P < 0.01$], but not for sexes [$F(1, 6) = 3.77$, $P > 0.05$]. This index in the injected rats was dramatically higher than the CTL groups in male ($P < 0.01$) and female ($P < 0.001$) animals. Also, the grooming number in female VPA + LIT and VPA + MIT groups was lower than the VPA groups ($P < 0.001$) (Fig. 2F).

3.3. The effect of LIT and MIT on social interaction task indices in autistic rats

Fig. 3 shows the effects of LIT and MIT on SI and SNI indices as social interaction indices. Two-way ANOVA revealed a significant interaction among treatments [$F(5, 30) = 12.21$, $P < 0.001$] and treatment & sex [$F(5, 30) = 4.68$, $P < 0.01$], but not for sexes [$F(1, 6) = 1.44$, $P > 0.05$] in SI (sniffing). Tukey post hoc analysis showed that this parameter in the male ($P < 0.05$) and female ($P < 0.001$) VPA groups was significantly less than the CTL groups, indicating social interaction impairments in the autistic rats. This index in the VPA + LIT and VPA + MIT female groups ($P < 0.01$ and $P < 0.05$, respectively) was higher than the VPA group, suggesting the improving effect of exercise in social behaviors of female autistic rats. On the other hand, this index in the VPA + MIT male rats was significantly less than the MIT ($P < 0.001$) and VPA + LIT ($P < 0.01$) groups. Also, there was a remarkable difference between the male and female VPA + MIT groups ($P < 0.01$) (Fig. 3A).

For SNI (sniffing), as shown in Fig. 3B, two-way ANOVA revealed a significant interaction among treatments [$F(5, 30) = 22.25$, $P < 0.001$], between sexes [$F(1, 6) = 32.82$, $P < 0.01$] and treatment & sex [$F(5, 30) = 5.556$, $P < 0.001$]. Tukey post hoc analysis revealed that the MIT female group had more SNI than the LIT group ($P < 0.001$). A significant reduction was seen in the male VPA group compared to the CTL group ($P < 0.05$), proposing social disorders in male autistic rats. Also, SNI in the VPA + LIT male group

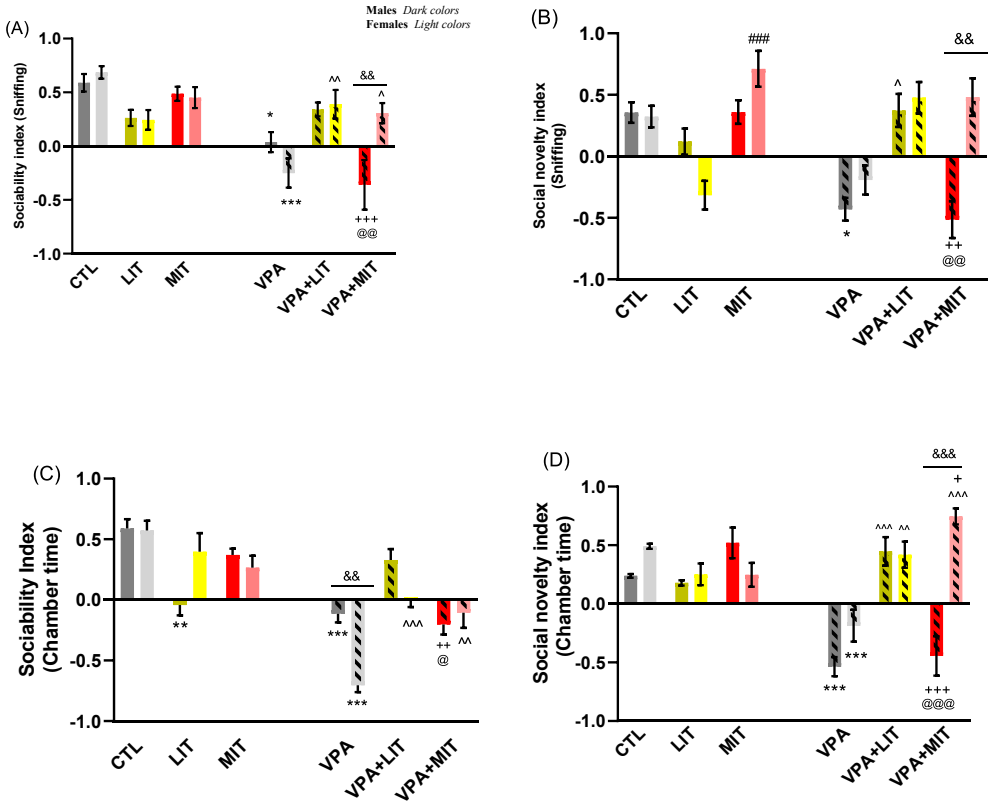

Fig. 3. The effects of LIT and MIT on sociability index (sniffing) (A), social novelty index (sniffing) (B), sociability index (chamber time) (C) and social novelty index (chamber time) (D) in the SIT in the autistic rats. Each bar represents mean ± SEM. Significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with CTL group in the similar sex; $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ compared with VPA group in the similar sex; $^{\#\#\#}P < 0.001$ compared with LIT group in the similar sex; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with MIT group in similar sex; $^{@}P < 0.05$, $^{@@}P < 0.01$, $^{@@@}P < 0.001$ compared with VPA + LIT group in the similar sex; $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$ male groups in comparison with female groups ($n = 7$ in each group).

was more than the VPA group ($P < 0.05$) which shows that LIT can improve social interactions in autistic male rats. This index in the VPA + MIT male group was significantly less than the MIT ($P < 0.01$) and VPA + LIT ($P < 0.01$) groups. In addition, there was a remarkable difference between the male and female VPA + MIT groups ($P < 0.01$) (Fig. 3B).

About SI (chamber time), two-way ANOVA revealed a significant interaction among treatments [$F(5, 30) = 30.64$, $P < 0.001$] and treatment & sex [$F(5, 30) = 7.76$, $P < 0.001$], but not between sexes [$F(1, 6) = 1.76$, $P > 0.05$]. Tukey analysis showed a reduction of this index was observed in the LIT male group compared to the CTL group ($P < 0.01$). SI (chamber time) in the VPA-injected groups and in both sexes significantly decreased compared to the CTL groups ($P < 0.001$), indicating social disorders in the autistic groups. Also, there was a remarkable difference between the male and female autistic groups ($P < 0.01$). There was a significant difference between VPA + LIT, and also VPA + MIT female groups ($P < 0.001$, $P < 0.01$, respectively) compared to the VPA groups, which indicates the beneficial effect of these two kinds of exercise on social behaviors in female rats. Furthermore, the results showed a diminished SI in the

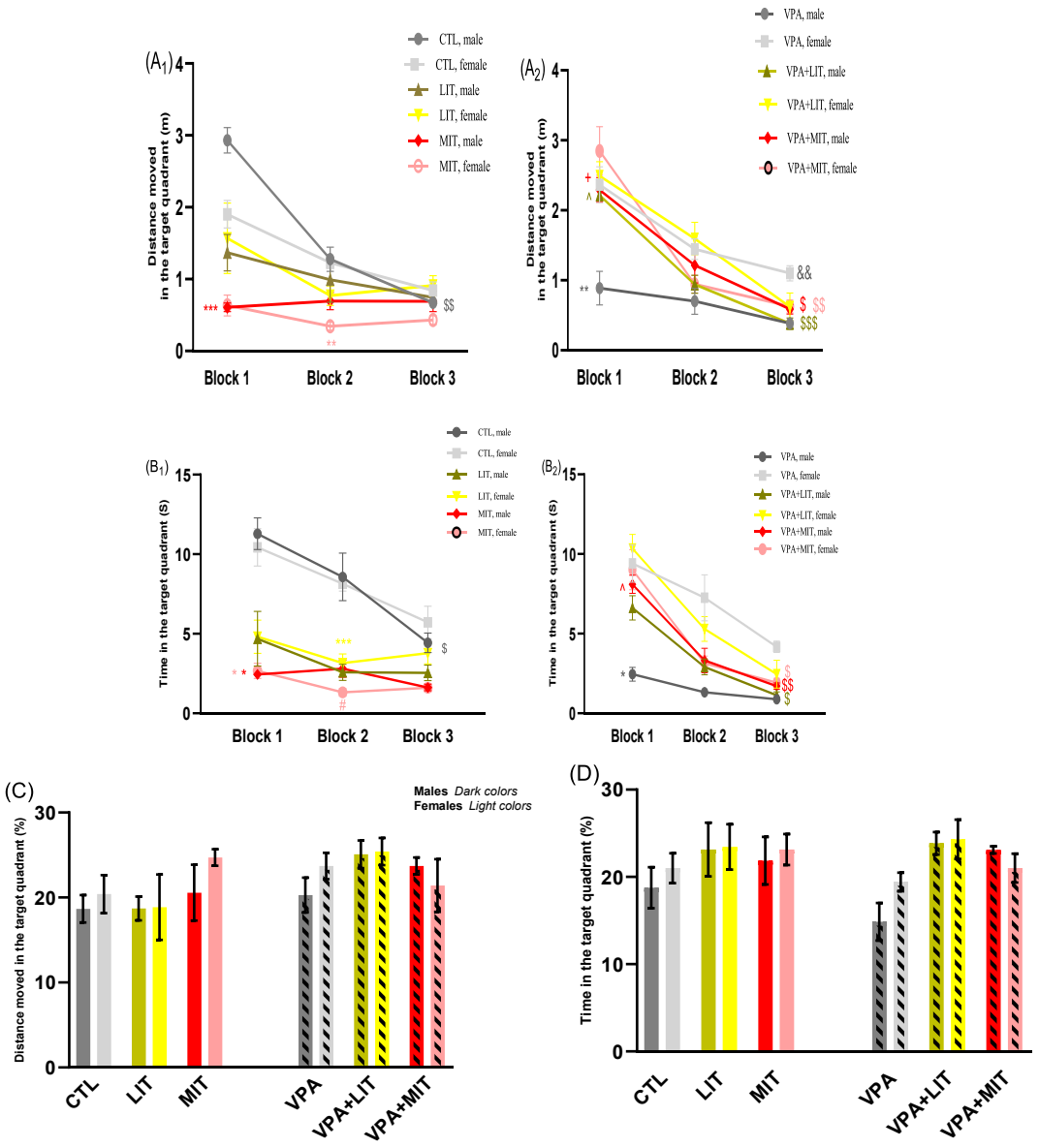

Fig. 4. The effects of LIT and MIT on distance moved in the target quadrant in each block (learning phase) (A), time in the target quadrant in each block (learning phase) (B), distance moved in the target quadrant (memory phase) (C) and time in the target quadrant (memory phase) (D) in the MWM in autistic rats. Each bar represents mean ± SEM. Significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with CTL group in similar sex; $\hat{}P < 0.05$ compared with VPA group in the similar sex; $^{+}P < 0.05$ compared with MIT group in the similar sex; $^{\&\&}P < 0.01$, male groups in comparison with female groups in the first block; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ compared with female groups in the second block. ($n = 7$ in each group).

VPA + MIT male group compared to the MIT ($P < 0.01$) and VPA + LIT ($P < 0.05$) groups (Fig. 3C).

In Fig. 3D, two-way ANOVA revealed a significant interaction among treatments [F (5, 30) = 12.46, $P < 0.001$], between sexes [F (1, 6) = 104.6, $P < 0.001$] and treatment & sex [F (5, 30) = 14.74, $P < 0.001$] in SNI (chamber time). Further analysis showed that this index decreased in the VPA-treated male and female rats, suggesting defects in social interaction in autistic rats ($P < 0.001$). VPA + LIT in the male ($P < 0.001$) and female ($P < 0.01$) and VPA + MIT in the female ($P < 0.001$) groups could increase SNI and ameliorate social behavior. In the VPA + MIT male group, SNI (chamber time) was significantly less than the MIT ($P < 0.001$) and VPA + LIT ($P < 0.001$) groups. In addition, SNI in the VPA + MIT female group was more than the MIT group ($P < 0.05$). Also, there was a remarkable difference between the male and female VPA + MIT groups ($P < 0.001$) (Fig. 3D).

3.4. The effect of LIT and MIT on spatial learning and memory in the MWM in autistic rats

The effects of LIT and MIT on spatial learning and memory in the MWM are presented in Fig. 4. As seen in Fig. 4A, statistical analysis indicated that the distance moved in the target quadrant of the autistic male ($P < 0.01$) and MIT male ($P < 0.001$) groups was significantly reduced in the 1st block compared to the CTL group. Furthermore, the distance moved in the VPA + LIT male group was more than the VPA male group in the first block ($P < 0.05$). Also, male rats in the VPA + MIT group moved a longer distance than the MIT group in the 1st block ($P < 0.05$). In the 2nd block, the distance moved in the MIT female group was significantly less than the CTL group ($P < 0.01$). In the 3rd block, the CTL male ($P < 0.01$), VPA + LIT male ($P < 0.001$), VPA + MIT male ($P < 0.05$) and female ($P < 0.01$) groups moved less distance to find the hidden platform compared to the same groups in the 1st block. In addition, the distance moved in the target quadrant by the autistic female rats was more than the male rats in the 3rd block ($P < 0.01$) (Fig. 4A).

Time spent in the target quadrant in the first block in the VPA male and MIT male and female groups ($P < 0.05$) was dramatically less than the CTL groups. This parameter was higher in the VPA + MIT male group than the VPA group ($P < 0.05$). In the 2nd block, time spent in the target quadrant in the LIT female group was higher than the MIT female group ($P < 0.05$) and was lower than the CTL groups ($P < 0.001$). In the 3rd block, this index was less in the CTL male ($P < 0.05$), VPA + LIT male ($P < 0.05$), VPA + MIT male ($P < 0.01$) and female ($P < 0.05$) groups compared to the same groups in the 1st block (Fig. 4B).

In the probe test, which has been illustrated in Fig. 4C and D, two-way ANOVA revealed that there was no significant interaction among treatments [F (5, 30) = 2.06, $P > 0.05$], between sexes [F (1, 6) = 0.97, $P > 0.05$] and treatment & sex [F (5, 30) = 0.54, $P > 0.05$] for distance moved in the target quadrant, and also among treatments [F (5, 30) = 2.48, $P > 0.05$], between sexes [F (1, 6) = 1.35, $P > 0.05$] and treatment & sex [F (5, 30) = 0.85, $P > 0.05$] for time in the target quadrant (Fig. 4C and D).

4. Discussion

In the present study, we aimed to investigate the effects of LIT and MIT for one month on the behaviors of male and female autistic rats, including alteration in social and anxiety-like behaviors and spatial learning and memory. Our results showed that offspring of mothers that had received VPA had more stereotyped repetitive activities, anxiety, and social behavior deficits [30].

Studies have reported that typically 500 mg/kg or 600 mg/kg VPA is a sufficient dose to induce autism-like state without the death of rodents [31]. In addition, it has been found that VPA administration in mice on gestational day 12.5 leads to the best match with behavioral and neuronal alternations associated with autism [31].

In order to evaluate the effects of treadmill exercise on the anxiety-like behaviors of rats, EPM and OFT were performed. EPM and OFT results indicated that LIT and MIT could alleviate the anxiety of autistic rats. Furthermore, exercise positively affected the social behaviors of rats owing to the social interaction test. Interestingly, the effect of exercise on social interactions was sex-dependent, and moderate treadmill exercise reversely influenced the social behaviors of male autistic rats in contrast to female ones. In some cases, the difference in results between females and males can be attributed to gender-related factors, including sex hormones, behavioral variables, the change of the hypothalamic-pituitary-adrenal (HPA) axis activity, and the involvement of different neurotransmitters and brain areas [21,32]. For instance, the high activity of the HPA axis and disturbance in corticosterone levels can lead to substantial male-biased social behavior impairment [33]. In regard to morphological studies on rats, the abnormalities in social interaction in males and females can result from VPA-induced anomalies in the somatosensory cortex [34]. Besides, it has been proved that any hormonal changes in the placenta during prenatal growth can lead to life-lasting effects [35]. The important role of sex hormones such as estrogen and progesterone in the developmental stages and neurotransmitter systems, along with their protective effects, should be taken into consideration by researchers for interpreting the sex-related differences [36]. It has been stated that estrogen and oxytocin levels in the brain may be responsible for reducing anxiety-like behaviors by treadmill exercise in female mice exposed to anxiety-inducing stress conditions [32]. A classic exploratory behavior recorded in the open field is rearing behavior, in which the animal temporarily stands on its hind legs to sample the environment. In the study of Zhongqing et al. (2023), the rearing increased by using an intervention and improved the autism symptoms [37].

According to the MWM results, exercise could not improve spatial learning and memory in rats. Additionally, in line with previous studies, prenatal VPA administration could not impair learning and memory [23,38,39]. One interesting finding is that males displayed enhanced spatial learning following prenatal VPA administration. This can be associated with the fact that some autistic children have a high ability in learning [40].

The present study results were in accordance with Seo et al.'s report, indicating that exercise ameliorated social behaviors and anxiety-like symptoms in autistic rats [24], but they also showed that treadmill exercise improved spatial memory in autistic rats [24]. The contrary results might result from the different intensity of physical activity, which was lower than the LIT in the present study, or the method used to evaluate the rats' spatial learning and memory. Although many studies have indicated that moderate treadmill

exercise can improve learning and memory, the frequency, duration, and intensity of the exercise determines the optimal learning and memory effects [41,42].

The relationship between physical exercise intensity and mood effects is U-shaped according to previous studies. This means that neither very low nor high intensity of exercise is helpful for cognitive function improvement, and only a specific range of physical activity intensity is effective [43–45]. Presently, the lower and upper limits of the efficient intensity of exercise are unknown. There is not enough evidence for the interaction between activity types, exercise intensity, and training frequency; thus, further studies are required [46].

It is well known that exercise improves cognitive function, learning ability, and memory function [47,48]. Moreover, it has been shown that physical exercise can enrich memory deficits and social behavior complications and decreases hyperactivity in autism [49–51]. It has been revealed that treadmill exercise can augment neurogenesis in autistic rats. Continuous neurogenesis can lead to the recovery of autism-like symptoms in rats [24,52]. Seo et al. showed that treadmill exercise increased the expression of reelin, an essential factor for neuronal survival and differentiation [24]. Regarding the important role of reelin in brain development, dysregulation of reelin may be responsible for some functional abnormalities in the brain observed in autism. Thus, enhancing the expression of reelin is one of the suggested mechanisms for the role of exercise in improving the cognitive and behavioral function of autistic rats. An increase in B-cell lymphoma-2 and brain-derived neurotrophic factor (BDNF) and a decrease in Bax expression have been proposed as the possible mechanisms of the effects of exercise on autism [53,54]. Altogether, physical exercise can improve autism-like symptoms by different mechanisms; however, the main influential factor is not clear. Hence, more studies are needed to investigate the pathways that explain these responses.

5. Conclusion

Overall, the summary of our findings provides some support for the beneficial effects of MIT on ameliorating anxiety-like behaviors in both sexes and also the usefulness of LIT in improving social disturbances in male and female offspring. Therefore, exercise may act as a potential clinical agent, which can amend some behavioral impairments in autism. The precise mechanism of the neuroprotective effect of exercise in the VPA model of autism remains to be elucidated.

Author contribution statement

Nazanin Sabet: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
Banafsheh Abadi: Performed the experiments; Wrote the paper.
Amirhossein Moslemizadeh, Mohammad Amin Rajizadeh, Fatemeh Arabzadehe: Performed the experiments.
Seyyed Sajjad Vakili Shahrbabaki, Zahra Soltani, Forouzan Rafie: Contributed reagents, materials, analysis tools or data.
Hamideh Bashiri: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

Funding

This research was funded by Student Research Committee, Kerman University of Medical Sciences (Code: 99000265).

Ethics approval

The study was approved by the Ethical Committee of Kerman University of Medical Sciences with Ethics code IR.KMU.REC.1399.448.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to acknowledge the Vice-Chancellor for Research & Technology of Kerman University of Medical Sciences for their support of the study.

References

[1] J. Baio, et al., Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2014, MMWR Surveillance Summaries 67 (6) (2018) 1.
[2] D. Dufour-Rainfray, et al., Behavior and serotonergic disorders in rats exposed prenatally to valproate: a model for autism, Neurosci. Lett. 470 (1) (2010) 55–59.
[3] J. Zeidan, et al., Global prevalence of autism: a systematic review update, Autism Res. 15 (5) (2022) 778–790.
[4] V. Bambini-Junior, et al., Resveratrol prevents social deficits in animal model of autism induced by valproic acid, Neurosci. Lett. 583 (2014) 176–181.
[5] C.S. Carter, Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders? Behav. Brain Res. 176 (1) (2007) 170–186.
[6] N. Sabet, Z. Soltani, M. Khaksari, Multipotential and systemic effects of traumatic brain injury, J. Neuroimmunol. 357 (2021), 577619.
[7] R.J. Landa, Diagnosis of autism spectrum disorders in the first 3 years of life, Nat. Clin. Pract. Neurol. 4 (3) (2008) 138–147.
[8] R.B. Silvestrin, et al., Animal model of autism induced by prenatal exposure to valproate: altered glutamate metabolism in the hippocampus, Brain Res. 1495 (2013) 52–60.
[9] S. Miot, et al., Comorbidity burden in adults with autism spectrum disorders and intellectual disabilities—a report from the EFAAR (Frailty Assessment in Ageing Adults with Autism Spectrum and Intellectual Disabilities) study, Front. Psychiatr. 10 (2019) 617.
[10] P. Chaste, M. Leboyer, Autism risk factors: genes, environment, and gene-environment interactions, Dialogues Clin. Neurosci. 14 (3) (2022) 281–292.
[11] R. Bromley, et al., Autism spectrum disorders following in utero exposure to antiepileptic drugs, Neurology 71 (23) (2008) 1923–1924.
[12] R.R. Dietert, J.M. Dietert, J.C. DeWitt, Environmental risk factors for autism, Emerg. Health Threats J. 4 (1) (2011) 7111.
[13] B. Barrett, Substantial lifelong cost of autism spectrum disorder, J. Pediatr. 165 (5) (2014) 1068–1071.
[14] M. Andoh, et al., Exercise reverses behavioral and synaptic abnormalities after maternal inflammation, Cell Rep. 27 (10) (2019) 2817–2825.
[15] N. Sabet, et al., The effect of two types of diet on apoptosis indexes, lipid profile and histopathological outcome in acute kidney injury during exercise, BMC Nephrol. 23 (1) (2022) 1–15.
[16] N. Sabet, et al., The effects of two different dietary regimens during exercise on outcome of experimental acute kidney injury, J. Inflamm. 19 (1) (2022) 1–14.
[17] N. Sabet, Z. Soltani, M. Khaksari, The effects of exercise on kidney injury: the role of SIRT1, Mol. Biol. Rep. 49 (5) (2022) 4025–4038.
[18] C.V. Toscano, H.M. Carvalho, J.P. Ferreira, Exercise effects for children with autism spectrum disorder: metabolic health, autistic traits, and quality of life, Perceptual and motor skills 125 (1) (2018) 126–146.
[19] F. Mirzavandi, et al., Effects of varied-intensity endurance exercise training on oxidative and antioxidant factors in the liver of rats with valproic acid-induced autism, Acta Neurobiol. Exp. 83 (1) (2023) 25–33.
[20] A. Al Awamleh, A. Wo ll, The influence of physical exercise on individuals with autism: is physical exercise Able to Help autistic? J. Soc. Sci. 10 (2) (2014) 46.
[21] K.B. Juybari, et al., Sex dependent alterations of resveratrol on social behaviors and nociceptive reactivity in VPA-induced autistic-like model in rats, Neurotoxicol. Teratol. 81 (2020), 106905.
[22] E. Imado, et al., Prenatal exposure to valproic acid causes allodynia associated with spinal microglial activation, Neurochem. Int. 160 (2022), 105415.
[23] S.S.V. Shahrbabaki, et al., Early postnatal handling alters social behavior, learning, and memory of pre-and postnatal VPA-induced rat models of autism in a context-based manner, Physiol. Behav. 249 (2022), 113739.
[24] T.-B. Seo, et al., Treadmill exercise improves behavioral outcomes and spatial learning memory through up-regulation of reelin signaling pathway in autistic rats, Journal of exercise rehabilitation 9 (2) (2013) 220.
[25] C. Bernardi, et al., Treadmill exercise induces hippocampal astroglial alterations in rats, Neural Plast. 2013 (2013) 1–10.
[26] H. Bashiri, et al., Modulatory effects of the basolateral amygdala α2-adrenoceptors on nicotine-induced anxiogenic-like behaviours of rats in the elevated plus maze, Neuropharmacology 105 (2016) 478–486.
[27] M. Shamsi Meymandi, et al., Prenatal pregabalin is associated with sex-dependent alterations in some behavioral parameters in valproic acid-induced autism in rat offspring, Int. J. Dev. Neurosci. 80 (6) (2020) 500–511.
[28] A. Moslemizadeh, et al., Combination therapy with interferon-gamma as a potential therapeutic medicine in rat's glioblastoma: a multi-mechanism evaluation, Life Sci. 305 (2022), 120744.
[29] T. Schneider, J. Turczak, R. Przewłocki, Environmental enrichment reverses behavioral alterations in rats prenatally exposed to valproic acid: issues for a therapeutic approach in autism, Neuropsychopharmacology 31 (1) (2006) 36–46.
[30] F.I. Roullet, J.K. Lai, J.A. Foster, In utero exposure to valproic acid and autism—a current review of clinical and animal studies, Neurotoxicol. Teratol. 36 (2013) 47–56.
[31] O. Larner, et al., A need for consistency in behavioral phenotyping for ASD: analysis of the valproic acid model, Autism Research and Treatment (2021) 2021.
[32] F.-Q. He, et al., Effects of treadmill exercise on anxiety-like behavior in association with changes in estrogen receptors ERα, ERβ and oxytocin of C57BL/6J female mice, IBRO Neuroscience Reports 11 (2021) 164–174.
[33] T. Schneider, et al., Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid, Psychoneuroendocrinology 33 (6) (2008) 728–740.
[34] Y. Hara, et al., Effect of prenatal valproic acid exposure on cortical morphology in female mice, Journal of pharmacological sciences 118 (4) (2012) 543–546.
[35] H.A. Wilson, et al., Endocrine insights into the pathophysiology of autism spectrum disorder, Neuroscientist 27 (6) (2021) 650–667.
[36] G.C. Wagner, et al., A new neurobehavioral model of autism in mice: pre-and postnatal exposure to sodium valproate, J. Autism Dev. Disord. 36 (2006) 779–793.
[37] Z. Qiu, et al., Lactiplantibacillus plantarum N-1 improves autism-like behavior and gut microbiota in mouse, Front. Microbiol. 14 (2023) 659.
[38] V. Bambini-Junior, et al., Animal model of autism induced by prenatal exposure to valproate: behavioral changes and liver parameters, Brain Res. 1408 (2011) 8–16.
[39] A. Shafaghi, et al., The effect of early handling on anxiety-like behaviors of rats exposed to valproic acid pre-and post-natally, Neurotoxicol. Teratol. 89 (2022), 107050.
[40] L. Kanner, Autistic disturbances of affective contact, Nervous child 2 (3) (1943) 217–250.
[41] A. Pietrelli, et al., Effects of moderate and chronic exercise on the nitrergic system and behavioral parameters in rats, Brain Res. 1389 (2011) 71–82.
[42] A. Pietrelli, et al., Aerobic exercise upregulates the BDNF-Serotonin systems and improves the cognitive function in rats, Neurobiol. Learn. Mem. 155 (2018) 528–542.
[43] J.R.A. Catellier, Z.J. Yang, The role of affect in the decision to exercise: does being happy lead to a more active lifestyle? Psychol. Sport Exerc. 14 (2) (2013) 275–282.
[44] M. Conradsson, et al., Effects of a high-intensity functional exercise programme on depressive symptoms and psychological well-being among older people living in residential care facilities: a cluster-randomized controlled trial, Aging Ment. Health 14 (5) (2010) 565–576.
[45] A.L. Dunn, , et al.O. Heather, Chambliss Exercise treatment for depression: efficacy and dose response, Am. J. Prev. Med. 28 (1) (2005) 1–8.
[46] A. Jiang, chin Tiffany, The effect of physical activity on affect, JIANG Changhao; CHEN Tingting 22 (12) (2014) 1889.
[47] S.-S. Baek, et al., Effects of postnatal treadmill exercise on apoptotic neuronal cell death and cell proliferation of maternal-separated rat pups, Brain and Development 34 (1) (2012) 45–56.

[48] H. Parsa, et al., Swimming training and Plantago psyllium ameliorate cognitive impairment and glucose tolerance in streptozotocin–nicotinamide-induced type 2 diabetic rats, J. Physiol. Sci. 71 (1) (2021) 1–12.
[49] D.B. Allison, V.C. Basile, R.B. MacDonald, Brief report: comparative effects of antecedent exercise and lorazepam on the aggressive behavior of an autistic man, J. Autism Dev. Disord. 21 (1) (1991) 89–94.
[50] F. Bahrami, et al., Kata techniques training consistently decreases stereotypy in children with autism spectrum disorder, Res. Dev. Disabil. 33 (4) (2012) 1183–1193.
[51] C. Petrus, et al., Effects of exercise interventions on stereotypic behaviours in children with autism spectrum disorder, Physiotherapy Canada 60 (2) (2008) 134–145.
[52] C.M. Teixeira, et al., Cell-autonomous inactivation of the reelin pathway impairs adult neurogenesis in the hippocampus, J. Neurosci. 32 (35) (2012) 12051–12065.
[53] J.-E. Kim, et al., Treadmill exercise ameliorates motor disturbance through inhibition of apoptosis in the cerebellum of valproic acid-induced autistic rat pups, Mol. Med. Rep. 8 (2) (2013) 327–334.
[54] L. Xu, et al., Moderate Exercise combined with enriched environment enhances learning and memory through BDNF/TrkB Signaling pathway in rats, Int. J. Environ. Res. Publ. Health 18 (16) (2021) 8283.