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Moderate treadmill exercise rescues anxiety and depression-like behavior as well as memory impairment in a rat model of posttraumatic stress disorder

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Abstract

Post-traumatic stress disorder (PTSD) is a condition which can develop from exposure to a severe traumatic event such as those occurring during wars or natural disasters. Benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) are considered the gold standard for PTSD treatment, but their side effects pose a serious problem. While regular physical exercise is regarded as a mood elevator and known to enhance cognitive function, its direct role in rescuing core symptoms of PTSD including anxiety and depression-like behaviors and cognitive impairment is unclear. In the present study using the single-prolonged stress (SPS) rat model of PTSD (2h restrain, 20 min forced swimming, 15 min rest, and 1–2 min diethyl ether exposure), we examined the beneficial effect of moderate treadmill exercise on SPS-induced behavioral deficits including anxiety and depression-like behaviors and memory impairment. Male Wistar rats were randomly assigned into four groups: control (sedentary), exercised, SPS (no exercise), or SPS-exercised. Rats were exercised on a rodent treadmill for 14 consecutive days. Rats in all groups were tested for anxiety-like behaviors using open field (OF), light-dark and elevated-plus maze tests. All rats were tested for short-term and long-term memory in the radial arm water maze test. Rats were then sacrificed, blood was collected (for corticosterone levels), and individual organs (spleen, adrenals, and thymus) harvested. Results suggest that moderate physical exercise ameliorates SPS-induced behavioral deficits in rats.

Keywords

PTSD; stress; depression; anxiety; cognition; treadmill exercise

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1. Introduction

Post-traumatic stress disorder (PTSD) is a syndrome that can result from exposure to a severe traumatic event such as those occurring during wars or natural disasters [1]. The number of soldiers who return from combat zones and develop PTSD has been increasing in recent years. Although many individuals with PTSD recover in the first couple of years after experiencing the traumatic event, around 30-40% remain chronically symptomatic [2]. This serious disorder is believed to be, at least partly, due to dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis [3, 4]. Evidence indicates that, although similar in some aspects to chronic stress, PTSD is unique in its pathology [5]. Currently, the preferred treatment for PTSD is the use of antidepressants. Selective serotonin reuptake inhibitors (SSRIs) have proven to be effective toward anxiety and panic attacks commonly associated with PTSD and are the pharmacological treatment of choice for the disorder [6]. Other pharmacological options include repeated treatment with antipsychotics, anticonvulsants, adrenergic-inhibiting agents, opioid antagonists and benzodiazepines [7]. However, their use continues to be controversial due to their frequently reported negative side effects. Alternative treatments including the use of herbal medicines is being explored [8]. Other non-pharmacological interventions including physical exercise seem to be an excellent side-effect free approach. While regular physical exercise is known to enhance cognitive function and regarded as a mood elevator, its direct role in rescuing some of the core features of PTSD behaviors are unclear.

In the present study, we have used the single-prolonged stress (SPS) model of PTSD developed by Liberzon et al. [9], which replicates the specific neuroendocrinological abnormalities seen in PTSD patients. The SPS model comprises of three different types of stresses: 2 h restraint (psychological), 20 min forced swim (physical) and ether anesthesia (endocrinological), respectively. Application of these stresses increases serum corticosterone levels, and by combining three different kinds of stresses, the SPS model could accomplish severity of symptoms analogous to that of PTSD [10, 11]. Studies have shown that rats that undergo the SPS procedure suffer from increased anxiety, impairment of social and object recognition memory [12] and present with changes in glucocorticoid and mineralocorticoid receptors involved in the HPA system [13, 14]. Extensive evidence shows that regular exercise is beneficial in ameliorating memory impairment, enhancing cognitive function and preventing memory decline [15, 16]. Therefore, exercise may be beneficial in reversing PTSD-induced symptoms including anxiety and depressive behaviors and learning and memory function impairment [17]. Statistics show that individuals who suffer from PTSD tend, on an average, to exercise less (including activities such as shopping, walking, and sports) compared to before the onset of the disorder [18, 19]. Patients also reported poor compliance to medication [19].

In the present study we used the SPS rat model of PTSD to study the effect of regular exercise as a potential non-pharmacological therapeutic approach for attenuating SPS-induced deficits including anxiety-like (anxiety), depression-like (depression) behaviors, and learning and memory function impairment.

2. Materials and Methods

2.1. Animals

Male Wistar rats (175–200 g; Charles River, Wilmington, MA) were housed with a 12-h light, 12-h dark cycle (lights on at 0600 h) in a climate-controlled room with *ad libitum* food and water. After arrival at the research facility, all rats were allowed 1 week to acclimate before manipulations began (Scheme 1). All experiments were conducted in accordance with the NIH guidelines using approved protocols from the University of Houston Animal Care Committee.

2.2. Single-Prolonged stress model

Male Wistar rats were assigned into 4 groups; group 1: control, group 2: SPS, group 3: exercised, group 4: SPS-exercised.

Single Prolonged Stress (SPS)—The two SPS groups: SPS and exercise SPS rats (10 rats/group) were subjected to a one time combined stress paradigm applied consecutively in a day [9, 20]: immobilization (compression with double layered plastic Ziploc bag with edges covered in duct tape to prevent the rats from escaping and also an opening for the rats nose was provided in the Ziploc bag) for two hours followed immediately by 20 min of forced swimming (in a tall cylindrical tank of water 50X20 cm) then allowed to rest for 15 minutes, and finally exposure to ether anesthesia (with diethyl ether until loss of consciousness, 2–3 min).

2.3. Exercise Protocol

At the end of the SPS exposure, the exercise and exercise SPS groups of rats were treadmill exercised for 2 weeks (1st week – 10m/min for 30 min/day; 2nd week – 15m/min for 30 min/day; **scheme 1**) using our published treadmill exercise procedure [21, 22]. To encourage the rats to continue running, the metal bar grid at the beginning of each lane delivered a mild foot shock (0.5mA) that caused a tingling sensation. The rats eventually learned to continue running to avoid this shock. The other two groups control and SPS, were not exercised but were transported to the exercise room daily during the exercise protocol. All rats were observed while exercising to ensure they ran throughout the exercise sessions and to monitor for any signs of potential confounders such as pain or exhaustion [23].

2.4. Anxiety and depression-like behavior tests

First, open-field test was conducted followed by light-dark (LD) and elevated-plus maze (EPM) tests as previously published by us [21, 22].

2.4.1. Open Field (OF) activity—Rats were placed in the center of the OF (60×40 cm) and left free to explore the arena for 15 min and movement quantified using Opto-Varimex Micro Activity Meter v2.00 system (Optomax, Columbus Instruments; OH) as previously published by us [21, 22]. The light intensity was adjusted at 300 lux. Percent time spent in the center of the arena, total activity, ambulatory activity and distance covered were recorded.

2.4.2. Light-Dark (LD) exploration—Time spent in light is considered as a measure of anxiety behavior. The light-dark box consisted of a light and a dark compartment separated by a single opening for passage from one compartment to the other and total time spent in the lit area was recorded [21, 22].

2.4.3. Elevated plus-maze—A standard rat elevated plus-maze with two walled arms and two open arms extending 43 cm from a 10 cm central area (Med Associates Inc., St. Albans, VT). The arms of the maze were approximately 90 cm above the floor. The rat's movements were tracked visually. The observer was blinded to the group classification to avoid bias. Each session was started by placing the rat in the central area facing the open arms of the maze and lasted 5 min. In between each test animal, the maze was wiped down with alcohol. The amount of time the rat spent in the open arms was noted [24].

2.4.4. Sucrose Consumption—The sucrose consumption test has been used extensively to assess motivational state in rodents, including stress-induced anhedonia [25-27], which is regarded as a sign of depressive behavior [28]. The rats were given the choice between consuming water and a 1% solution of sucrose. The preference for sucrose over water is considered as a measure for rodents' sensitivity to a natural reward. Thus, anhedonia and depressive behavior is revealed by a reduction in sucrose preference [29-31]. All groups of rats were subjected to the 2-bottle paradigm from day 8 to day 28.

2.5. Memory Function Test

2.5.1. Radial Arm Water Maze (RAWM)—The RAWM procedures were done as previously published by us [32]. The RAWM consisted of a black circular pool filled with water at 25°C containing six swim paths in a dimly lit room. Each rat was randomly assigned a goal arm which contains a hidden black platform near the end of the arm. The rats were randomly released at an arm different from the goal arm, allowed to swim and locate the platform which is submerged 1 cm under water. The rats were allowed 1 minute for each learning trial or memory test. An error was counted when the rat entered more than halfway into an arm other than the goal arm or if the rat entered more than half of the goal arm but failed to approach the platform. Number of errors ranged from 1-7, as the rat can only swim into a total of 7 arms within 1 minute. If the rat failed to locate the platform within 1 minute, it was manually guided to the platform and scored with 7 errors. Upon reaching the platform, the rat was allowed 15 seconds rest before the next trial began.

Each rat was subjected to a set of six learning trials (trials # 1-6) followed by a five min rest period and then another set of six learning trials (trials # 7-12). The short-term memory test was conducted 30 min after the end of 12th trial. This was followed by the long-term memory test which was given 24 h later.

2.6. Corticosterone measurement

The blood was collected by cardiac puncture at least 48 hrs after the last behavioral test to avoid the behavioral test-induced increase in the corticosterone levels. Serum corticosterone levels, released in response to stress and anxiety were measured using an EIA based kit (cat#500651, Cayman Chem. Co., Ann Arbor, MI) per manufacturer's instructions [31].

3. Results

3.1. General body parameters and tissues weights

Daily food intake during the SPS procedure and 21-days after protocol was not significantly different among controls, exercise alone, SPS and SPS-exercised rats [$F(3,36) = 0.95$; ns] (**Fig. 1A**). However, daily water intake was markedly increased in SPS rats compared to controls, exercise alone and SPS-exercise rats [$F(3,36) = 4.16$; $p < 0.05$] (**Fig. 1B**). Furthermore, the SPS and control rats gained weight according to the normal weight gain profile, while exercise alone and SPS-exercise groups gained less weight than the controls and the SPS groups [$F(3,36) = 11.35$; $p < 0.05$] (**Fig. 1C**).

The absolute weights of the thymuses obtained from the SPS rats were significantly decreased compared to that of the control rats. Exercise, however, significantly improved the thymus weight in the SPS-exercise rats [$F(3,20) = 4.87$; $p < 0.05$] (**Fig. 2A**). Furthermore, there were no differences observed in the adrenal and spleen weights among the controls, exercise alone, SPS and SPS-exercise groups (**Fig 2 B, C**).

3.2. Plasma Corticosterone

Corticosterone level in plasma was measured using an EIA based kit [33]. SPS significantly increased serum corticosterone levels by 225% when compared to control and 220% when compared to exercised control rats (**Fig. 3**). The corticosterone levels were significantly decreased in SPS-exercise group compared to SPS rats. Although the corticosterone levels were higher than the control and exercise alone groups, they were significantly lower than the SPS alone group suggesting that exercise only partially alleviates the increased corticosterone levels. Serum corticosterone levels were 47 ng/ml in control rats; 49 ng/ml in exercise rats; 125 ng/ml in SPS rats and 95 ng/ml in SPS-exercised rats.

3.3. Anxiety and depression-like behaviors

Open-field activity and light–dark exploration tests were conducted to test for anxiety behavior. Rodents will typically spend more time exploring the periphery of the arena (thigmotaxis), than the unprotected center area. Rats that spend significantly more time exploring the unprotected center area demonstrate anxiolytic baseline behavior. The open-field test demonstrated that SPS rats spent only 25% of their time in the center of the open-field arena while control, exercise alone and SPS-exercise spent 44, 44 and 36% of their time in the center, respectively (**Fig. 4A**). Furthermore, SPS rats had lower total (**Fig. 4B**) and ambulatory (**Fig. 4C**) activity counts and covered lesser distance (**Fig. 4D**) than the control, exercise and SPS-exercise groups.

In light–dark test, a rat is exposed to a novel environment with protected (dark compartment) and unprotected (light compartment) areas. Indisposition to explore the lit, unprotected area and inclination to spend more time in the dark compartment during a 5-min test session is indicative of high anxiety behaviors. The light–dark exploration test results suggest that SPS rats spent 55 s in the light compartment while control, exercise alone and SPS-exercise rats spent 96, 106 and 83 s, respectively, in the light compartment [$F(3,36) = 3.58$; $p < 0.05$] (**Fig. 5A**).

Elevated-plus maze model is based on rat's aversion for open spaces. This aversion leads to the behavior termed as thigmotaxis, which means avoidance of open areas by restricting movements to enclosed spaces or to the edges of a confined space. Increased amount of time spent in the closed arms during a 5-min session is indicative of high anxiety behavior. The EPM results suggest that SPS rats spent 90 s in the open arms while control, exercise alone and SPS-exercise rats spent 127, 134 and 137 s, respectively, in the open arms [$F(3,36) = 3.48$; $p < 0.05$] (**Fig. 5B**).

The sucrose consumption assay was used to assess motivational state in rodents, including stress-induced anhedonia. SPS exposure influenced sucrose intake, a measure of natural reward, 24 h after the last stress session. As expected, the SPS rats exhibited anhedonia indicated by a marked decrease in preference for sucrose when compared to control, exercise and SPS-exercise groups [$F(3,36) = 12.79$; $p < 0.05$] (**Fig. 6**). However, exercise did not seem to completely reverse the anhedonic effect of SPS.

3.4. Memory function test

SPS rats made significantly higher number of errors both in the short-term (**Fig. 7A**) and long-term memory tests in the RAWM than all other groups (**Fig. 7B**). Thus, exercise normalized both short-term and long-term memory in the SPS-exercise group.

4. Discussion

Among the several proposed animal models of PTSD, the SPS model reproduces most of the neuroendocrinological deficits seen in PTSD patients [9]. In this study, we have demonstrated the beneficial effect of moderate physical exercise in a SPS rat model of PTSD. The SPS rats exhibited increased anxiety behavior, as demonstrated by reduced time spent in the light compartment of the light-dark box, decreased total and ambulatory activities and distance travelled in the open field, as well as reduced time spent in the open arms of the EPM test. Thus, moderate treadmill exercise reversed these effects of SPS suggesting a rescue effect of physical training. Furthermore, SPS rats displayed decreased sucrose preference, indicating anhedonia and depressive behavior. It is well known that rodents exhibit preference for sweetened solutions. In a separate study, we have reported reduced preference for sweet solution (1% sucrose) in a rat model of social stress [33], this reduction in sucrose preference can be reversed by treatment with antidepressants [27]. This is possibly an effect of stress on the brain's reward circuitry [34, 35]. Furthermore, 2 weeks of treadmill exercise improved sucrose preference in these rats suggesting a decrease in depressive behavior and anhedonia. It is known that depression is associated with low levels of physical activity [36] whereas physical exercise is known to be mood elevating and providing a diversion from negative thoughts [37, 38]. The mood elevating effects are perhaps due to the release of beta-endorphins, which create a “feel-good” effect that occurs following physical activity [39, 40].

The impaired short term memory and long term memory in SPS rats is in agreement with the findings of Bremner et al. [41] demonstrating that short-term memory deficits in a patient group consisting of 26 Vietnam veterans with combat related PTSD cases. In another study scientists have revealed impairment in memory consolidation in PTSD individuals [42]. In

the present study, moderate physical exercise improved both long-term and short-term memory impairments in SPS rats. The improved memory may be due to exercise-induced increase in brain derived neurotrophic factor (BDNF) protein expression in the brain (Zagaar et al. 2013). Long-term down regulation of BDNF mRNA in rat hippocampus has been observed in an animal model of PTSD [43]. BDNF is a key molecule [44-46]. Zhang et. al have shown that a single-nucleotide polymorphism in the *BDNF* gene (Val66Met) influences hippocampal volume, memory and appears related to susceptibility to a variety of neuropsychiatric disorders, including posttraumatic stress disorder (PTSD) [47].

As expected, our data showed a decrease in the normal body weight profile in the two rat groups (exercised and exercised-SPS) after 2 weeks of exercise training. The weight gain in unexercised SPS rats was similar to that of the control rats over the same time period. Interestingly, SPS rats consumed more water possibly due to changes in vasopressin or angiotensin systems, which could contribute to alteration in drinking behavior [3].

The principal physiological responses to stressful stimuli are mediated by the sympathoadrenal system and the HPA. Activation of the HPA is associated with marked elevation of plasma corticosterone levels [10, 11]. Furthermore, SPS can lead to enhanced negative feedback of the HPA axis 7 days after SPS and also in PTSD patients, through time-dependent sensitization, and this characteristic has been reliably replicated in the SPS model, is one of the advantages of SPS over other animal models of PTSD [10, 20, 48]. The corticosterone levels were markedly increased in the SPS rats. Interestingly, exercise caused significant reduction in the corticosterone levels, but did not completely normalize it. This may be due to the unusually high (more than 100%) stress-induced elevation of corticosterone, which may require longer periods of exercise and also suggesting that the neurochemical changes may be not as responsive as behavioral measures.

Additionally, our study showed significant decrease in the thymus weight in SPS rats. However, adrenal glands and spleen tissue weights were not affected. Previous studies have reported differential effects on these organs depending on the stressor applied [49-51]. Many stressors have been associated with involution of the thymus [52]. However, exercise normalized the thymus weight in the SPS rats signifying a protective role of physical activity. This could be due to exercise induced increase in blood circulation throughout the body including the thymus gland, which ensures that the waste products are removed promptly and thus help in thymus recovery from stress [53].

In summary, this study shows that SPS induced PTSD-like behavioral deficits in rats characterized by increased anxiety behavior, anhedonia, increased depressive behavior and memory impairment. Even though the biological, psychological, and social implications of PTSD have been under scrutiny for many years, and the treatment options have improved vastly, much remains to be understood. Although SSRIs are recommended to treat PTSD associated depression and anxiety, they cause adverse side effects such as sexual dysfunction, weight gain and sleep disturbances [54]. Participation in physical activity has been associated with decrease in a number of diseases [45]. In spite of the growing evidence for the importance of physical activity, 74% of adults do not meet the United States recommended guideline of at least 30 min of moderate-intensity physical activity for 5 days

a week [55]. The rat SPS induced PTSD model used in the present study, replicated most of the PTSD symptoms and the use of a nonpharmacological intervention, namely, moderate treadmill exercise, which alleviated the associated anxiety and depression-like behaviors, stress and cognitive deficits in rats [56-61].

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Research Highlights

- Single-Prolonged Stress (SPS)-induced behavioral and cognitive impairments in rats.
- Moderate treadmill exercise rescued SPS-induced behavioral and cognitive impairments in rats.

Scheme 1.

A schematic representation of the experimental regimen. Male Wistar rats were acclimatized for one week and assigned into 4 groups; group 1: control, group 2: SPS, group 3: exercised, group 4: SPS-exercised. The two SPS groups: SPS and exercise SPS (10 rats/group) were subjected to a one time combined stress paradigm [9, 20] for one day. One SPS group underwent 2 weeks of moderate treadmill exercise while the other group was not exercised. The control group (sedentary) was subjected to exercise without the SPS procedure. Behavior tests were conducted at the end of the treadmill exercise protocol. Animals were sacrificed at least 48 hrs after the last behavioral test.

Fig 1. Effect of SPS on body weight, food and water intake

Daily food intake (A), water consumption (B) and weight gain (C). (*) significantly different from control and SPS groups, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 10$ rats/group.

Group designations: control, exercise (Ex), single-prolonged stress (SPS), single-prolonged stress and exercised (SPS/Ex).

Fig 2. Effect of SPS on absolute tissue weights

Thymus (A), adrenals (B) and spleen (C). (*) significantly different from all other rat groups, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 10$ rats/group. Group designations: control, exercise (Ex), single-prolonged stress (SPS), single-prolonged stress and exercised (SPS/Ex).

Fig 3. Effect of SPS on corticosterone levels

SPS significantly increased the levels of corticosterone compared to control rats. However, exercise SPS rats had a rescue effect. (#) significantly different from exercise and control and (*) significantly different from all other rat groups, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 10$ rats/group. Group designations: control, exercise (Ex), single-prolonged stress (SPS), single-prolonged stress and exercised (SPS/Ex).

Fig 4. Anxiety-like behavior in the open field test

Percent time spent in center (A), Total activity (B), ambulatory activity (C) and total distance travelled (D). (*) significantly different from all other rat groups, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 10$ rats/group. Group designations: control, exercise (Ex), single-prolonged stress (SPS), single-prolonged stress and exercised (SPS/Ex).

Fig 5. Anxiety-like behavior in the light-dark test

Light dark (A) and elevated plus maze (B) tests were conducted in all rats. (*) significantly different from all other rat groups, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 10$ rats/group. Group designations: control, exercise (Ex), single-prolonged stress (SPS), single-prolonged stress and exercised (SPS/Ex).

Fig 6. Examination of depression-like behavior using sucrose consumption test

SPS significantly decreased sucrose consumption, while exercise improved the amount of sucrose consumed. (#) significantly different from SPS alone and (*) significantly different from all other rat groups, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 10$ rats/group. Group designations: control, exercise (Ex), single-prolonged stress (SPS), single-prolonged stress and exercised (SPS/Ex).

Fig 7. Effect of exercise on memory function

Short-term memory (A) and long-term memory (B) tests were conducted in all rats. (*) significantly different from all other rat groups, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 10$ rats/group. Group designations: control, exercise (Ex), single-prolonged stress (SPS), single-prolonged stress and exercised (SPS/Ex).