Original Article

Influence of Moderate and Severe Exercise on Memory Formation and Anxiety-like Behaviors in Male Wistar Rat

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Abstract

Introduction: Recent researchers have showed that regular exercise induces positive effects on cognitive functions. Exercise intensity and timing of cognitive assessment may have an interactive effect on cognitive changes. Previous researches suggest that moderate intensity treadmill running has the most consistent benefit to cognitive function. In contrast, studies find positive, negative, or null effects to cognitive function after high intensity treadmill running. The primary objective of the present study was to compare the cognitive effects of intensity treadmill running protocol 1 (Low intensity), protocol 2 (Moderate intensity) and protocol 1 plus 2 (High intensity).
Materials and Methods: Male Wistar rats were divided into four groups (n=7 in each group) including: sedentary (Non-exercise), protocol 1, protocol 2, and combination of protocol 1 and 2. Step-through passive avoidance and elevated-plus maze apparatus have been used to test parameters of passive avoidance learning and anxiety-like behaviors.
Results: These findings showed that combination of both protocols (Protocol 1 with protocol 2) decreased step-through latency in the passive avoidance apparatus indicating memory impairment [$P<0.05$]. Moreover, the data revealed that different protocols for exercises did not alter %OAT [$P<0.001$], %OAE %OAT [$P>0.05$] and locomotor activity %OAT [$P<0.05$] compared to control group but not others.
Conclusion: High intensity exercise not only induced beneficial effect but also impaired memory formation.

Keywords: Moderated and severe exercise, Memory, Anxiety

1. Introduction

Physical activity leads to many health benefits [1] such as improving cognition and non-cognition behaviors. There is a growing body of knowledge about the beneficial effects of exercise on cognitive functions in experimental animals. Exercise enhances the expression of brain derived neurotropic factor (BDNF) in the hippocampus, which is essential for activity-dependent learning and memory [2]. Previous studies have shown that treadmill exercise alleviates the deficits in cognitive functions and anxiety behaviors among male rats [3]. In a few other studies, it was confirmed that treadmill running can improve learning and memory performance in 5- weeks-old rats [4], but the influence of exercise intensity on neurogenesis and the

transcription of neurogenesis-implicated molecular factors is not well understood in postnatal and juveniles.
Previous studies have shown that different intensity exercise has a various effects. Moderate impact forced exercise improves learning and memory, and yet, the forced exercise particularly at high intensity increases the secretion of glucocorticoids such as cortisol or corticosterone in the blood [5]. Elevated levels of glucocorticoids are known to inhibit neurogenesis in the hippocampus and disrupt hippocampal synaptic plasticity, resulting in impairment of learning [6]. Furthermore, some studies showed that physical exercise can improve anxiety behaviors [7, 8]. The purpose of the present study was a comparative investigation into the effect of two methods of moderate and high treadmill running and their combination on long-term memory and anxiety-like behaviors in Wistar male rat.

2. Materials and Methods

2.1 Animals

Male Wistar rats used in the study were inbred into animal house Institute of cognitive sciences studies (ICSS). The rats had free access to food and water and were housed in room with an ambient temperature of 22±2°C and 12:12 hr light/dark cycle (lights on at 07.00 h). The animals were only handled for weighing, and cage cleaning. All experiments were performed between 09.00 h and 14.00 h, and each rat was tested only once. The animals were allocated to four groups (n=7 in each group) including: sedentary (non-exercise), Exe_1 (protocol 1), Exe_2 (protocol$_2$) and $EX_{1,\ 2}$ (combination of protocol 1 and 2).

2.2 Exercise Protocols Postnatal Treatment (Exercise Protocol 1)

The rat pups (21 days after PND) in the exercise group were initially habituated to a motor-driven treadmill at a speed of 2 m/min for 10 min/day during first and second days of exercise (21 and 22 days after PND). Then they were made to run on treadmill for 30 min once a day for 18 consecutive days (until 50 days after PND) including 2m/min in the first 5 min, 5m/min for the next 5 min and 8 m/min for the last 20 min. Control rats were placed on sedentary treadmills for the same duration [9].
A summary of the experimental design in displayed in Fig1.

Exercise Protocol 2

In this protocol, 51-80-day old male Wistar rats at the beginning of all experiments were used. Rats ran as forced exercise on treadmill at 0° inclination, Sunday through Thursday during the light cycle between 9:00 am and 2:30 pm for a total of 4 weeks using the protocol 2 outlined in Fig 2 [10].

Exercise Protocol 1 and 2

The rats received protocol 1 on PND 21-50 and running exercise with protocol2 on PND 51-80, outlined in fig3.

2.3 Behavioral Tests

Behavioral assessments began after the termination of exercise procedure. All behavioral tests were conducted in a noiseless room during the light period (between 9:00 and 14:00) under bright and modest illumination. The rats were transferred in the testing room at least 1 hr before the behavioral assessment. At the end of each test session, the equipment was carefully cleaned using 70% ethanol, and the cage was transferred to the colony room.

2.4 Passive Avoidance Task

In the passive avoidance task, in order to avoid a mild foot shock, the rat must learn to remain in a brightly lit compartment and not enter the preferred dark compartment. The passive avoidance task was carried out using a step-through apparatus. The apparatus had two chambers: a dark one $(30 \times 30 \times 30 \text{ cm})$ with metal grid floor and a brightly illuminated (100 w) one $(8 \times 7 \times 30 \text{ cm})$.

The two chambers were separated by a guillotine- type door. One training trial method has been used for passive avoidance task [11]. The training trial started by placing the rat in the brightly lit compartment. Once the rat had entered the dark compartment, the guillotine door was closed and an electric shock (0.30 – 0.35 mA for 3 sec) was delivered to the animal through the grid floor. Retention test (no shocks) was performed 24h after the acquisition trial. At that time, the animal was returned to the illuminated compartment, and the step-through latency was estimated by measuring the time (latency time) for the rat to move to the dark compartment. A latency of at least 300 sec was used as a criterion for learning. Before each test, the apparatus was wiped clean and dried [11].

2.5 Elevated Plus Maze Task

The elevated-plus maze (EPM) consisted of four arms, two of which had no side or end walls (open arms; $50 \times 10 \text{ cm}$). The other two had side walls and end walls, but were open on the top (closed arms;$50 \times 10 \times 40 \text{ cm})$. Where the four arms intersected, there was a square platform of $10 \times 10 \text{ cm}$. Rats were individually placed in the center of the maze facing a closed arm and were allowed 5 min of free exploration. The number of entries into open or closed arms and the total time spent in the open or closed arms were recorded by means of a digital camera. The test room was illuminated by one 60w bulbs located 1.5 m above the apparatus. Raw data were used to manually calculate indices corresponding to the anxiety – like behaviors. Entries were defined when all four paws were in the arms and measured by a hand counter. The open arm entries percentage as well as the open arm time percentage (as standard anxiety – like behavior indices) was calculated as follows:
(a) % OAT (the ratio of time spent in open arms to total time spent in any arm $\times 100)$; (b) % OAE (the ratio of entries into open arms to total entries $\times 100)$. (c) Total closed open arm were considered as a relatively pure index of locomotor activity [12].

2.6 Statistical Analysis

Given the data normality of distribution and homogeneity of variance, the results were statistically evaluated by one-way ANOVA to compare the effects of exercise with the corresponding control group. Following a significant F-value, post-hoc analysis (Tukey test) was performed to assess the specific group comparisons. In all comparisons, $P<0.05$ indicated a statistical significance.

3. Results

3.1 The Effect of Different Protocols of Exercises on Memory Formation in the Sep-through Apparatus

The one-way ANOVA and post hoc Tukey's test analysis revealed that each protocol of exercise did not alter memory formation per se, while combination of them (protocol 1 with protocol 2) decreased memory formation [$F (3, 24) = 3.87$, $P<0.05$, Fig.4].

3.2 The Effect of Different Protocols of Exercises on Exploratory-like Behaviors in the EPM Apparatus

The one-way ANOVA and post hoc Tukey's test analysis indicated that all protocols of exercises did not alter %OAT [$F (3, 24) =7.17$, $P < 0.001$, Fig.5 panel A], %OAE [$F (3, 24) =1.9$, $P>0.05$, Fig.5 panel B] and locomotor activity [$F (3, 24) =3.0$, $P <0.05$, Fig.5 panel C] as compared to control group

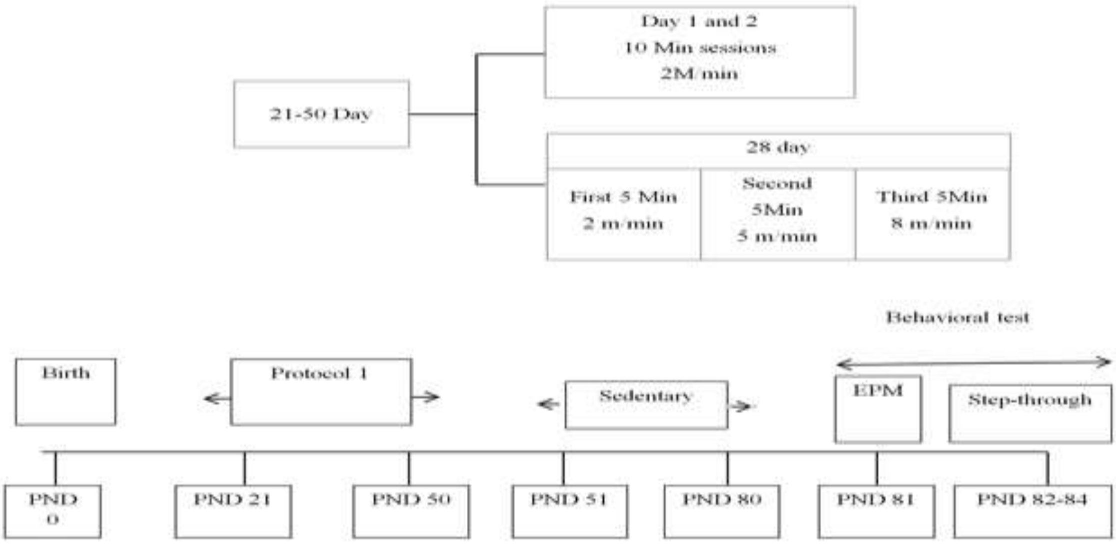

Figure 1. Timeline of treadmill exercise (Protocol1) on PND21-50and behavioral test

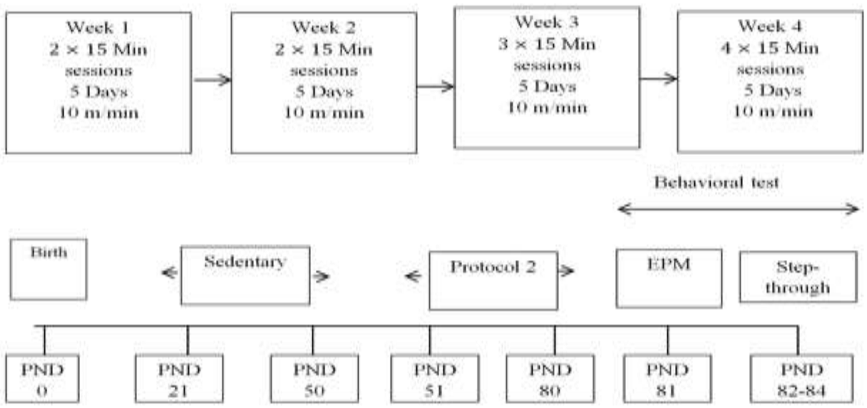

Figure 2. The exercise protocol utilized in this study (PND51-80) is moderate in that it gradually increased in speed and duration across the 4-week period

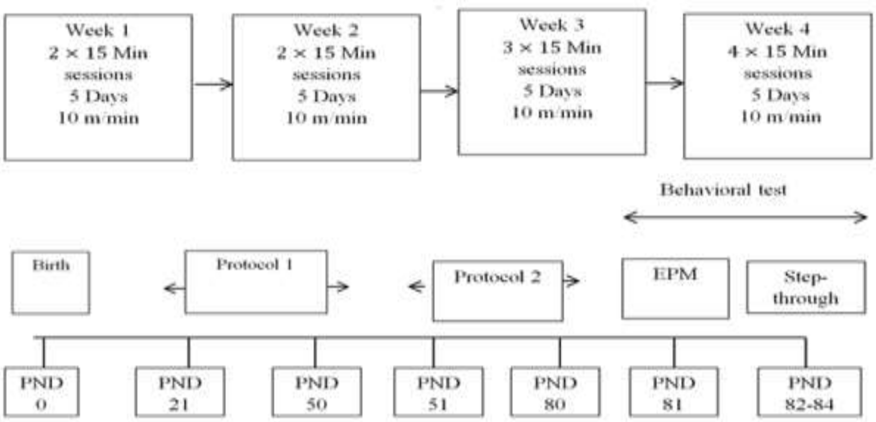

Figure 3. Timeline of treadmill exercise, combination of protocol1 plus Protocol 2 on PND21-80 as high intensity of exercise duration across the 6-week period

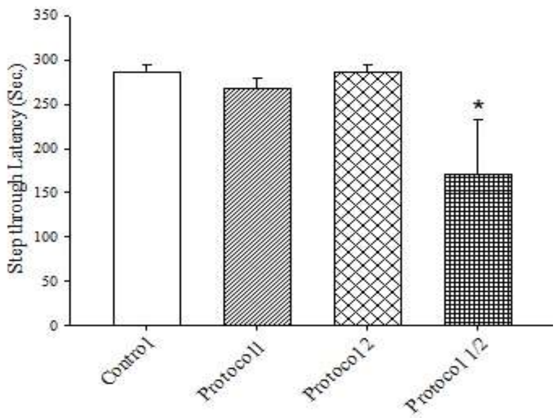

Figure 4. The effect of different protocols of exercises on memory formation in the step-through apparatus. The animals were treated by different protocols of exercises (control, protocol 1, protocol 2 and Protocol 1 with Protocol 2) as described in the method section. The data are shown as mean ± S.E.M. *P<0.05 as compared to sedentary group as control group.

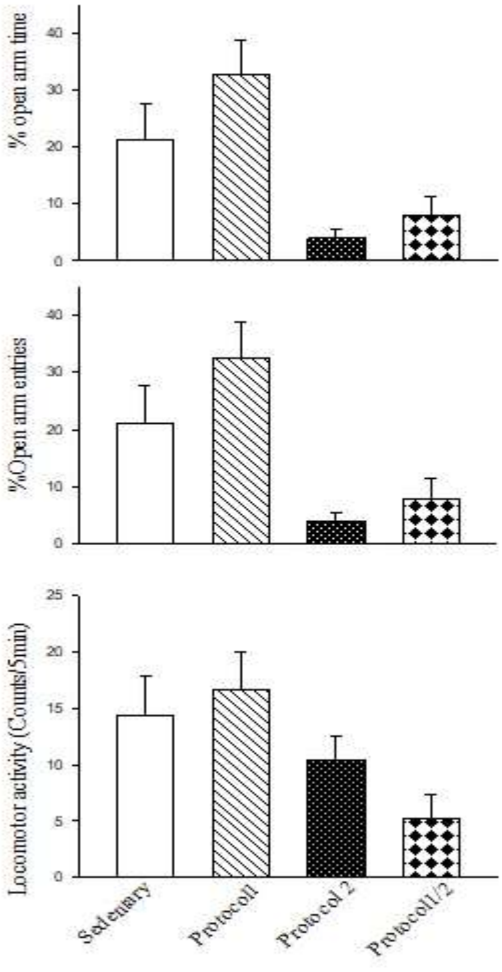

Figure 5. The effect of different exercises protocols on exploratory-like behaviors in the EPM apparatus. The animals were treated by different protocols of exercises (control, protocol 1, protocol 2 and protocol 1 with protocol 2) as described in the method section. Panels A, B and C show %OAT, %OAE and locomotor activity, respectively. The data are shown as mean ± S.E.M.

4. Discussion

The aim of the present study was to characterize the impact of different protocol treadmill running exercise on the behavior of rat regarding to general aspects as well as with particular emphasis on exploratory-related behavior. EPM and step-through latency test was used to gain a general overview of the behavior characteristics of the animals since this test allows the assessment of a variety of behavioral parameters such as memory formation, anxiety-related behavior, locomotion and exploration. The results of this study showed that protocol 1 plus protocol 2 (high intensity) induced impaired memory, while each of them did not caused memory impairment per se. This is in agreement with other evidence that high to maximal intensity exercise had a negative effect on cognitive function [13, 14]. The inverted-U hypothesis suggests that high intensity exercise would elicit maximal cognitive benefits [15]. High-intensity exercise has been reported to decrease global brain glucose uptake and increase lactate production in humans [16]. The high-intensity exercise condition may also alter energy metabolism in the brain in a manner that reduces BDNF (brain derived neurotropic factor) mRNA level expression [2].

The hypothalamic–pituitary–adrenal axis is affected by exercise, as evidenced by elevated glucocorticoid hormone levels [17]. Exercise effects on glucocorticoid hormone levels may influence learning and neurogenesis [18]. This is important given that cognition is known to be sensitive to stress, especially as related to glucocorticoid. For example, chronic stress is accompanied by deficits in hippocampal function and impaired neurogenesis [19, 20], while acute stress or glucocorticoid agonist can enhance memory consolidation [21]. In the present study, forced treadmill running task may influence the neurogenesis state, especially in the high-intensity exercise group. However, while the actual mechanisms of exercise-induced cognitive changes are still unclear, in some of the latest studies the potential role of cortisol, catecholamine's, BDNF, insulin-like growth factor 1 (IGF-1) and N-methyl-D-aspartate (NMDA) . It is plausible that two or more of these mechanisms work together to facilitate exercise-induced changes in cognitive function. Each of the potential mechanisms shares a common effect increased glutamate expression [22, 23].

Although there have been a number of neurobiological mechanisms proposed to explain the apparent loss of neural drive, referred to as "central fatigue "the neurotransmitter [24, 25]. The central fatigue hypothesis is based on that during prolonged exercise the synthesis and metabolism of central neurotransmitters such as monoaminergic systems might be changed. The serotonergic system has been suggested as an important modulator of memory formation and thus has been implicated in the control of numerous behavioral and physiological function [26]. Moreover, there is evidence that low-intensity exercise may have cognitive benefits in juvenile rats [27]. Therefore, neurogenesis induced by early life exercise may have a significant impact on brain structure and function development whereas moderate-intensity treadmill running enhances NMDAR1 mRNA levels.

5. Conclusion

High intensity exercise not only induced beneficial effect but also impaired memory formation. Future research should examine the influence of the anticipation of high exercise on cognitive function to better understand whether it is the psychological or physical stress imposed by exercise that decreased cognitive function.

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Conflict of Interest

The authors declare no conflict of interest.

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