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Effects of exercise on depression and hippocampal neurogenesis in the post stroke depression rats

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Abstract: Stroke induces mood impairments, about 30% of which are antidepressants-resistant. To investigate the evidence and related molecular mechanisms of physical exercise on post stroke depression (PSD) rats. Wistar rats after middle cerebral artery occlusion/reperfusion and chronic unpredictable mild stress were randomly divided into Sham, PSD and physical exercise groups. The behavioral tests (sucrose preference test for depression, open field test for anxiety) were performed before and after intervention. We investigated regional changes in neurogenesis by Nissl staining, western blotting and immunohistochemical staining for BDNF, doublecortin (DCX) in the dentate gyrus (DG). The depression/anxiety-like behaviors and impaired hippocampal neurogenesis in the DG were found in PSD rats. Physical exercise intervention reversed both depression/anxiety-like behaviors, increase the expression of DCX, BDNF in the DG. Physical exercise could be a viable adjunctive strategy in combination with antidepressants as an effectively protective intervention for PSD rats, might via increasing neurogenesis in DG.

Keywords: Stroke; Exercise; Depression; Neurogenesis
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1. Introduction

Stroke remains the leading cause of death after cancer and cardiac diseases[1], with high rate of mood and cognitive impairments. Around 31% stroke patients have been suffering from depression, namely post-stroke depression (PSD), which is one of the highly prevalent psychiatric disorders after stroke and represents an important burden on the family and society[2]. Accumulating evidences demonstrate that aerobic exercise contributes to establishing recovery and prevent relapse of depression symptoms[3, 4]. The people with depression engage in low levels of physical activity and high levels of sedentary behavior[5]. However, the evidence of the curative effect of research into its related molecular mechanisms in animal experiments are still lacking. Adult hippocampal neurogenesis is sensitive to external factors: both antidepressants and physical exercise promote proliferation and survival of new hippocampal neurons[6]. Thus, the present study aimed to explore whether there are functional differences among emotional behaviors, including depression and anxiety, or hippocampal neurogenesis in PSD rats after physical exercise.

2.Methods

2.1 Animals

Healthy male wistar rats (weighing 240–260 g, 8-week old) were singly housed in standard plastic cages (cage size: 26 × 19 × 15cm) with food and water available ad libitum. They were adapted to the environment for one week, with a room temperature of 22 ± 2 °C and humidity of 50-60% under 12-h light-dark cycle (lights on at 8:00 a.m., lights off at 8:00 p.m.). All experiments and procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and approved by the local Ethical Committee of Animal Research for minimizing the suffering of animals.

2.2 Middle cerebral artery occlusion/reperfusion (MCAO/r) surgery

The MCAO/r was performed according to the previously reported method[7]. Briefly, the rats were deeply anesthetized with chloral hydrate (0.3ml/100g, i.p.) and then to expose the left common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA). The ECA was ligated, dissected and inserted gently with a round-tip nylon thread (Beijing Cinontech Co. Ltd, China) to block the blood flow at the middle cerebral artery (MCA). The blood flow of middle cerebral artery was monitored by laser speckle blood flow meter (Figure 1). After 90 min the nylon thread was removed. We evaluated the neurological deficit level with the Longa score 24 h after MCAO/r surgery. Rats with a score between 1 and 3 were enrolled for the further experiment. The sham group rats were subjected to the same procedure but without occluding the MCA.

2.3 Chronic unpredictable mild stress (CUMS)

The CUMS procedure was performed according to a method described previously with minor modifications[8]. In brief, the rats in PSD, Ex groups were expose to different discontinuous and irregular stress every day for 28 consecutive days, including: water or food deprivation for 24 h, tail pinching for 1 min, cage tilting for 24 h, cold swimming in 4 °C for 5 min, overnight illumination, behavior restriction for 2 h, moist bedding for 24 h. The rats in the sham group were

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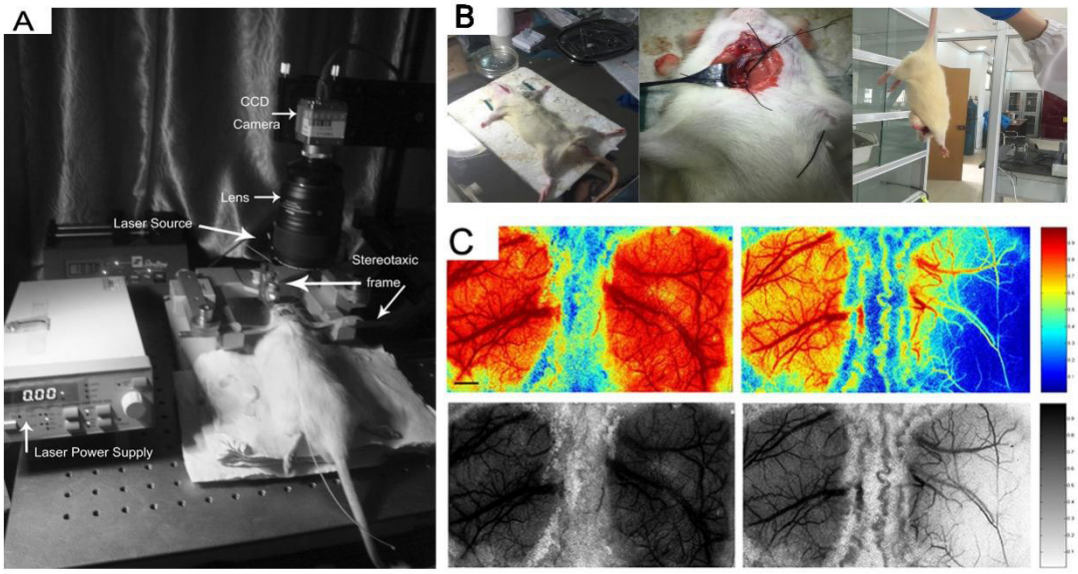

Figure 1. MCAO/r surgery and cerebral blood flow monitoring.

A. The blood flow of middle cerebral artery was monitored by laser speckle blood flow meter; B. MCAO/r surgery and evaluation of neurological deficit level; C. Representative laser speckle images showing baseline and intra-ischemic (left MCAO) CBF.

Abbreviations: MCAO/r, middle cerebral artery occlusion/reperfusion; CBF, cerebral blood flow.

housed with no contact with the stressed rats.

2.4 Exercise protocols

Exercise training was performed on the 8-lane motor-driven treadmill. Sessions were composed of 4x5min treadmill running at a speed of 12 m/min (40-60% VO_2max) interrupted by 30s rest. Each rat in exercise groups was trained twice times per day at an interval of 6-8h between two sessions for 28 consecutive days. The remaining rats in other groups were housed individually in a ventilated cage (cage size: 26 × 19 × 15cm) with no access to the treadmill.

2.5 Behavioral tests

Behavioral tests were performed one day after MCAO/r, CUMS, and exercise treatment.

2.5.1 Sucrose preference test (SPT)

On the first day, rats were free to drink two bottles of 1% sucrose solution. The next day, one of the sucrose solution was replaced by distilled water. On the third day, rats were deprived of water and food for 23 h. The rats were then given one bottle of 1% sucrose solution and one bottle of distilled water weighed in advanced. After 1 h, the volumes of consumed sucrose solution and distilled water were recorded to calculate the sucrose preference (%) by the following formula: sucrose consumption/(sucrose consumption + water consumption) × 100%. A decrease of sucrose preference indicated anhedonia and depression-like behavior[9].

2.5.2 Open field test (OFT)

The OFT was performed according to a method described previously with minor modifications[10]. Rat was placed in the open field apparatus which was made of a plastic box (45 cm × 45 cm × 50 cm) and allowed to explore freely for 10 min. We recorded the total distance to assess the exploratory behavior and the locomotion activity. The less total distance indicated low levels of exploration and anxiety-like behavior.

2.6 Western blotting

Whole protein extraction of the tissue samples were performed with RIPA lysis buffer (Beyotime, China), protease Inhibitor (Solarbio, Beijing, China) and phosphatase inhibitors (Calbiochem, Germany). The protein concentrations were determined by BCA method (Kangwei, China). The extracted protein was mixed with the SDS-loading buffer (Kangwei, China) and boiled for 5 min. Proteins sample were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then the proteins were electrotransferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Next, the membranes were blocked with 5% bovine serum albumin for 1 h and incubated with primary antibodies BDNF (1:2000, Abcam), DCX (1:1000, Santa Cruz) and GAPDH (1:1000, Bioss, China) at 4°C overnight in a refrigerator. Next day, after washing with TBST three times for 10 min, the PVDF membranes were incubated for 1h at room temperature with the secondary antibody (1:5000, Absin, China). Following, the membranes were washed by TBST three times for 10 min. Finally, imaging analysis was performed on a Quanta LAS 4010 imaging system (UVP, USA).

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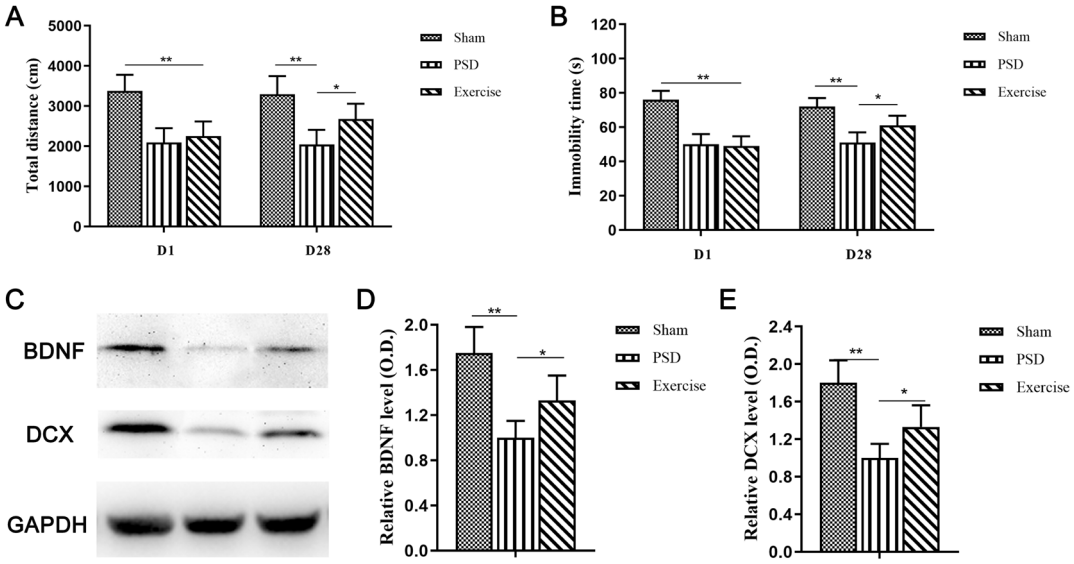

Figure 2. Effects of chronic treatment with exercise on depression/anxiety-like behaviors and adult hippocampal neurogenesis.

(A) Exercise intervention significantly increased total distance during OFT. (B) Exercise intervention significantly increased sucrose preference during SPT. (C&D&E) Western blot showed that exercise intervention significantly increased the BDNF and DCX expression level in the DG of the PSD rats. All values are presented as means±SD. *p < 0.05 and **p < 0.01.

Abbreviations: Abbreviations: SPT, sucrose preference test; OFT, open field test; BDNF, brain derived neurotrophic factor; DCX, doublecortin; DG, dentate gyrus; D1, first day of training treatment; D28, 28 days after training.

2.7 Nissl staining and immunohistochemistry

In brief, rats in each group were anesthetized and transcardially perfused using a 0.9% saline solution followed by 4.0% paraformaldehyde in PBS. Coronal sections (5μm, Leica, Germany) of rat brain at the hippocampus plane were performed, permeabilized, and blocked in PBS 1X with 0.3% Triton X-100 and 5% normal goat serum. For Nissl staining, sections were degreased through graded alcohol (70%, 95% and 100% alcohol) for 3min respectively and then hydrated through graded alcohol (95%, 70% and 50% alcohol) for 3min, respectively. Subsequently, the sections were stained in 0.1% toluidine blue solution for 20min, quickly rinsed in distilled water and differentiated in 95% ethyl alcohol for 15min. For immunohistochemistry, the sections were incubated in the same medium containing anti-BDNF antibody (1: 200, Abcam), DCX (1:100, Santa Cruz) at 37˚C for 2h. After rinsing three times in PBS, the sections were further incubated with horse radish peroxidase (HRP)-conjugated secondary antibody (1: 300, Bioss) diluted in PBS at 37˚C for 30min.Thereafter, the sections were developed with diaminobenzidine for 5min at room temperature and counterstained with hematoxylin. After dehydrated, cleared and mounted, the slides were observed with Olympus Fluorview-500 confocal microscope (40×; 1.0 NA) and quantified by Image J software.

2.8 Statistical analysis

All data were expressed as mean ± standard deviation (SD). The data were analyzed statistically by one-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey's post hoc test on GraphPad Prism (version 5). A value of $p < 0.05$ was considered statistically significant.

3.Results

3.1 Effects of chronic treatment with exercise on depression/anxiety-like behaviors

Chronic stressed rats after ischemic stroke manifested depression and anxiety-like behaviors, as demonstrated by SPT and OFT tests. In SPT study, rats after ischemic stroke exposed to CUMS for 4 weeks resulted in decreased sucrose preference ($F = 30.51$, $p < 0.01$). In OFT study, PSD rats showed limited exploratory behavior and the locomotor activity, with lower total distance ($F = 18.01$, $p < 0.01$) when compared with the sham group. Exercise successfully prevented depression/anxiety-like behaviors in PSD rats, as indicated by significant increase in sucrose preference ($q = 4.76$, $p < 0.05$) during SPT and total distance ($q = 4.40$, $p < 0.05$) during OFT as compared to vehicle-treated sedentary group (Figure 2A&2B).

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3.2 Effects of chronic treatment with exercise on hippocampal neurogenesis

Western blot showed that the BDNF and DCX relative levels increased in the DG in Ex group (BDNF: $q = 11.00$, $p < 0.05$; DCX: $q = 11.31$, $p < 0.05$). These results indicated that the antidepressants-resistant effect might be related to the impaired hippocampal neurogenesis in the DG in PSD rats (Figure 2).

Nissl staining showed that the neuronal cells in the DG were loosely arranged, lightly stained or missing in the PSD group. The neuronal cells and Nissl bodies were arranged more neatly and densely after chronic treatment with exercise (Figure 3A). In line with Nissl staining, rats after ischemic stroke exposed to 4-week of CUMS caused a significant decrease in DCX relative levels in the DG ($q = 10.32$, $p < 0.01$). Chronic treatment with

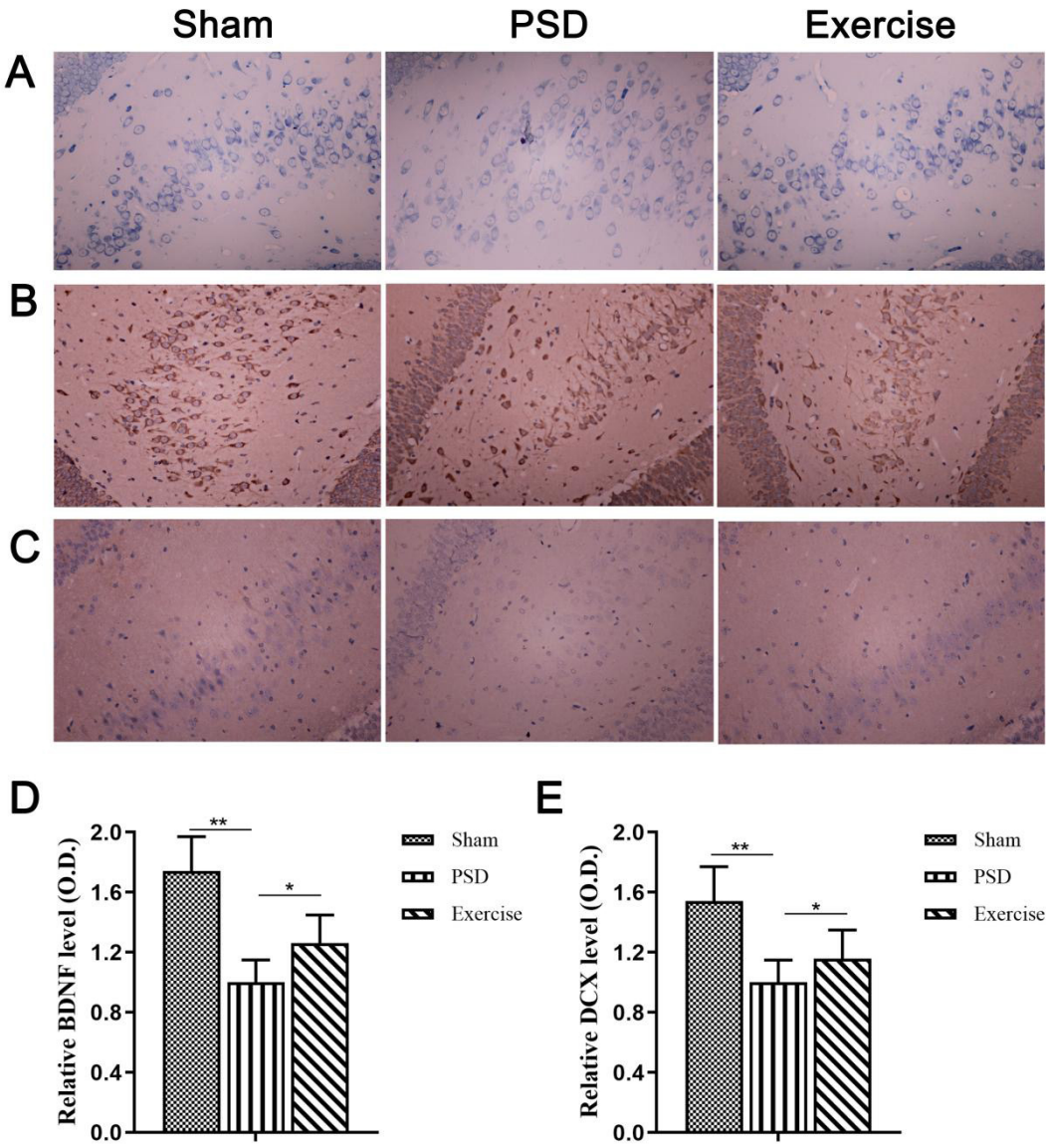

Figure 3. Effects of chronic treatment with exercise on adult hippocampal neurogenesis in the PSD rats. (A) Nissl staining in the DG.

(B) Illustration of BDNF immunostaining in the DG. (C) Illustration of DCX immunostaining in the DG and DG. (D) Exercise intervention significantly increased BDNF level in the DG. (E) Exercise intervention significantly increased DCX level in the DG.. All values are presented as means ± SD. *$p < 0.05$ and **$p < 0.01$.

Abbreviations: PSD, post stroke depression; DG, dentate gyrus; BDNF, brain derived neurotrophic factor; DCX, doublecortin.

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exercise (q = 4.38, p <0.05) significantly increased the DCX expression in the DG. Rats after ischemic stroke exposed to 4-week of CUMS caused significant decrease in BDNF proteins levels in the DG (q = 6.25, p < 0.01). Chronic treatment with exercise (q = 4.32, p < 0.05) in the DG significantly reversed these changes (Figure 3).

4. Discussion

In the present study, we investigated both the behavioral and cellular effects of physical exercise in a rodent model of post-stroke depression. We have demonstrated that chronic treatment with exercise to chronically stressed rats after ischemic stroke reversed both depression/anxiety-like behaviors and promoted hippocampal neurogenesis, survival of newborn neurons and neurotrophic factors in the DG.

In agreement with previous reports[11, 12], rats after ischemic stroke exposed to CUMS for 4 weeks profoundly worsened several aspects of the brain function, including depression-like behavior. These consequences were slightly ameliorated by physical exercise. Our result coincides with previously systematic reviews which reported a significant improvement in depression following exercise as an adjunctive treatment with low financial costs and side effects[13, 14]. Blumental and colleagues[15] observed that aerobic exercise achieved significant symptomatic relief in patients with mild to moderate depression and refractory patients. In addition, Pilu and colleagues[16] also found that long term use of exercise showed significant improvements in depressive (HAMD) and general psychopathological (CGI) in MDD patients with no responsiveness to antidepressant treatment.

In our study, we showed that chronic mild stress in the rat model of stroke caused significant decrease in hippocampal neurogenesis and cell death of newborn neurons in the DG. By contrast, in line with previous studies[17], physical exercise significantly enhanced the neurogenesis and prevented cell death throughout the DG. Combined with the behavioral outcomes, the differential activation and regulation in hippocampal neurogenesis after exercise might be linked to the functional segregation along the septo-temporal axis of the hippocampus. The ventral sub-region has reciprocal connections the prefrontal cortex (PFC), limbic system and amygdale involved in emotionally charged situations[18] and stress susceptibility and resilience[19]. The positive modulation of neurogenesis by running correlates in rodents with amelioration of symptoms of depression/anxiety induced by chronic stress and ischemic stroke. BDNF is known to be the regulator of the proliferation, differentiation and survival of neural progenitor cells (NPCs), and plays important roles in the development of depression and antidepressant treatments[20]. In addition, BDNF contributes to the survival and functions of GABAergic, dopaminergic, noradrenergic, and serotonergic neurons[21]. Recent evidence proved that increasing hippocampal BDNF level combined with AHN activation, not increasing AHN alone, is sufficient to improve cognition impairment in Alzheimer's mouse model. And increasing AHN genetically and pharmacologically alone combined with elevating BDNF levels in hippocampus could mimic the beneficial effects of exercise-induced improvements in cognition[22]. BDNF interacts preferentially with its specific high-affinity receptor TrkB to exert the positive influence. BDNF binds TrkB to activate multiple signaling cascades, including PI3K/Akt pathway and ERK1/2 pathway, and inhibit GSKβ, producing an antidepressant effect[23]. Our findings showed that the chronic administration of exercise significantly increased the BDNF level in the DG, which agreed with previous studies that muscarinic acetylcholine receptor (mAChR) agonist oxotremorine (Oxo) ameliorated the stress-induced anxiety-like behavior and up-regulated the BDNF expression in hippocampus[24].

5.Conclusion

Although the functional discrepancy between dorsal and ventral hippocampus might be far more complex, it is necessary to explore the region-specific effects of stress on neurogenesis and region-specific changes in emotional behaviors. Traditional antidepressants for treating depressive disorders have limited efficacy. Physical exercise as an effective intervention for depression could be a viable adjunctive strategy in combination with antidepressants.

Disclosure of interest
None

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