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The enhancing effect of peripheral physical activity on spatial memory by elevating central neurotrophic factor and synaptic plasticity-related proteins in the hippocampus of adolescent rats

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ABSTRACT: Investigation of spatial memory entails studying the process for encoding and retrieving spatial information in working memory that is required for animal and human survival. Synaptic plasticity in the hippocampus is necessary for working memory. Physical activity stimulates the N-methyl-D-aspartate receptor (NMDAR) and subsequently increases NR2A or NR2B subunits of NMDAR-enabling synaptic plasticity in the hippocampus. Reduction of NMDAR and the postsynaptic density (PSD)-95 protein can be found in learning and memory impairment. An increasing amount of evidence has shown that peripheral physical activity has an enhancing effect on learning and memory and elevates the expression of brain-derived neurotrophic factor (BDNF). However, the mechanisms of physical activity that improve working memory have not been fully understood. In the current study, we study the potential effects of peripheral physical activity on spatial memory and synaptic plasticity-related proteins. Physical activity was performed by having the rats do voluntary wheel running throughout P25–P40. The Morris water maze task was performed on P40 for 6 days to study spatial memory. The hippocampal protein expression was ascertained by Western blot analysis. We found that physical activity enhanced spatial memory and BDNF, PSD-95, NR2A, and NR2B levels in the hippocampus. Physical activity can be effective in improving the central synaptic plasticity-related proteins that are essential for learning and memory. Peripheral physical activity is a promising candidate for further investigation as a potential treatment for learning and memory impairment.

KEYWORDS: physical activity, learning and memory, hippocampus, N-methyl-D-aspartate receptor, brain-derived neurotrophic factor

INTRODUCTION

Cognition is crucial for functional dependence. Increasingly severe levels of cognitive decline induce more restrictions on activity dependence and lead to a subsequently poorer quality of life [1]. Cognitive ability contributes to several skills, including attention, visuospatial skills, learning, memory, and language. The learning and memory domain of a cognitive function is necessary for individuals operating in daily living. Spatial memory involves a form of short-term memory or working memory that stores and recalls information. This cognitive domain is required for the survival of animals. According to Hebbian theory, learning and memory retention is an activity-dependent process to refine synaptic efficacy [2].

The hippocampus is a critical brain region that has high synaptic plasticity and responds to learning and memory processes [3]. The refinement of structure and function of glutamate receptors is required for two types of the synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD). The animal models of learning and memory impairment exhibited decreases in the protein levels of NMDAR and PSD-95 [4, 5]. The peripubertal stage is susceptible to emotional and social disturbances. Adolescent stressed rats exhibited slower spatial learning and memory later in life [6]. An increasing amount of evidence postulates that peripheral physical exercise alleviated hippocampal neuron apoptosis [7] and improved the impairment of spatial learning ability, which was related to enhancing hippocampal neurogenesis in the prenatal stress in rats [8]. The exercise was applied as an intervention to improve object recognition memory in rodents, which elucidated an increase in cell proliferation and an elevation of BDNF mRNA expression in the dentate gyrus [9]. However, the mechanisms of how peripheral physical activity can enhance spatial learning and memory have not been fully understood.

The aim of this study is to investigate the potential effects of peripheral physical activity on spatial memory and synaptic plasticity-related proteins.

MATERIALS AND METHODS

Animal experiments

Eight 21-day Sprague Dawley rats, weighing 40–45 g, were obtained from the National Experimental Animals Center of Mahidol University, Salaya, Thailand.

This experimental procedure was approved by the Institute of Molecular Biosciences Animal Care and Use Committee (MB-ACUC), Mahidol University, Thailand (COA.NO.MB-ACUC 2010/003.1). The rats were housed in a single housing condition in temperature and humidity-controlled environment and maintained on a 12-h light/dark cycle with free access to food and water. The rats were distributed evenly as control and exercise groups ($n = 5$).

Voluntary wheel running exercise

The rat pups were trained by housing them in a cage with plastic running wheels after weaning on P21–P24. The running wheels, 100 cm in diameter, were equipped with a recorder for the timing and rotation of the wheel. Wheel running activity was recorded daily at 10 a.m., and the pups who ran less than 100 m were excluded. The running performances were conducted on P25–P40.

Behavioral study

In addition, spatial learning and memory performances were measured on P40 with the Morris water maze test (MWM). The MWM experiments were performed by using a polypropylene circular pool (painted black intentionally, 150 cm in diameter) that was filled with clear water. The water temperature was maintained at approximately 26±1 °C. The pool was divided into 4 equal quadrants on 2 axes (North-South and East-West). Each quadrant was given a geometric shape as a hint. A circular platform (15 cm in diameter, 23 cm in height) was submerged under the water by approximately 2 cm and located in the middle of the southwest quadrant. To avoid any other external spatial cues apart from the maze, a black curtain was used to surround the pool. A digital camera was mounted on the ceiling above the pool, which was connected to a computer equipped with tracking software (S-MART: PanLab Co., Barcelona, Spain). After 30 min of acclimation to the room, the rat was then put in the water maze. On the first day of the test, the visible platform trial, the escape platform was placed in the target quadrant with 2 cm above the water surface. Animals were allowed to swim for 60 s per training time. If the animals could not find the visible platform within 60 s, they were guided and left on the platform for 20 s. From the second to the fifth day, the platform was submerged 2 cm under the water surface for the acquisition test. The experimental animals were put in the Morris water maze for four trials per day. The rats were allowed to search for the escape platform for 60 s. If the rats failed to reach the platform within a given time, it was placed on the platform for 20 s. The water was stirred from one trial to the next in order to erase the olfactory traces of the previous rat swimming patterns. The rats were placed in the pool facing the wall in a random quadrant for four trials and were towel dried each time a trial was done. The time spent to find the platform (or escape latency) was recorded and analyzed. An hour after the last session of the fifth day, the platform was removed from the pool for the retention test of the probe trial. The rats were given 60 s to search in the water maze. The time and distance that the rats spent searching in the target quadrant versus the other three quadrants were recorded and measured for each rat.

Tissue preparation

After performing the last session of MWM task, the whole hippocampal tissues were collected. Brain tissues then were suspended in lysis buffer composed of 50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% Na Deoxycholate, 1% SDS, 1 mM PMSF, and 1% Triton-X-100 and supplemented with complete protease and phosphatase inhibitor cocktail set (Calbiochem, Germany), then homogenized for 10 s and centrifuged at 14,000 rpm at 4 °C for 15 min. The supernatant was collected for protein determination. The protein concentration of each sample was determined by Bradford protein assay.

Western blot analysis

Protein samples (20 µg) were resolved in a 7–10% SDS–PAGE and underwent electrophoresis at 150 V for 1 h. The protein bands were then transferred to a nitrocellulose membrane (Amersham Bioscience, Piscataway, NJ, USA). The transfer efficiency was checked by Ponceau-S red staining. Membranes were washed with Tris-buffered saline (TBS) for 5 min and then incubated in a blocking buffer for 1 h at room temperature. After that, the membranes were incubated overnight at 4 °C with the following primary antibodies: a mouse monoclonal anti-NR2A (sc-515148), a mouse monoclonal anti-NR2B (sc-365597), and a mouse monoclonal anti-PSD-95 (sc32290, 1:2000) from Santa Cruz Biotechnology, USA, a rabbit monoclonal anti-BDNF (ab108319) from Abcam, UK, and a mouse polyclonal anti-β-actin (AB3563, 1:5000) from Chemicon International, USA. The membranes were then washed 3 times with 0.1% Tween TBS for 5 min each and incubated with the appropriate HRP-conjugated secondary antibodies for 1 h at room temperature, and then washed 3 times with 0.1% Tween TBS for 5 min each. Finally, the signals were visualized using an ECL reagent (Amersham Biosciences) and the immunoreactive bands were exposed using the Azure c300 Chemiluminescent Western Blot Imaging System™ (Azure Biosystems, Inc., Dublin, USA). The immunoblot band densities were quantified using a densitometer with the ImageJ software. The density of each band was neutralized by the density of β-actin as the internal control.

Data and statistical analysis

Quantitative results were expressed as Mean ± SEM calculated from the duplicate experiments. The statistical significance of differences between the means was evaluated using Student's *t*-test (unpaired, unless otherwise stated). The probability level of $p < 0.05$ was considered a statistically significant difference between the two sets of data. The data were statistically analyzed using GraphPad Prism software.

RESULTS

Peripheral physical activity enhances spatial learning and memory

The spatial learning and memory performance was measured by the MWM task. The average time reaching the target quadrant of each rat was recorded as escape latency (Fig. 1a). The control rats significantly decreased the escape latency on Day 5 as compared to Day 1 ($p < 0.05$). Correspondingly, marked decrease in escape latency of the exercise group on Day 5 ($p < 0.001$), and significant decrease in escape latency on Day 4 ($p < 0.01$) have been elucidated. Interestingly, the exercise group showed significantly higher time in the target quadrant than the control group ($p < 0.01$) (Fig. 1b). The schematic diagram represents the tracking pattern of control and exercise group on the retention test of the probe trial (Fig. 1c).

Peripheral physical activity enhances brain neurotrophic factor

BDNF is a member of the neurotrophin family. It is widespread in the developing and adult mammalian brain. The data have revealed that BDNF is expressed in the hippocampus of adolescent rats. The peripheral physical activity significantly enhanced the levels of BDNF in the exercise group, compared to the control group (Fig. 2) ($p < 0.05$).

Peripheral physical activity enhances the synaptic plasticity-related proteins

Synaptic plasticity in the hippocampus is associated with the existence of NMDAR on postsynaptic membrane. NR2A and NR2B subunits are mentioned in NMDAR in the rat hippocampus. The results showed that peripheral physical exercise could significantly elevate the expression of NR2A and NR2B levels (Fig. 3c,d) ($p < 0.05$), compared to the control group. NMDAR is anchored on the surface membrane by scaffolding protein. Our data showed that peripheral physical exercise could significantly elevate the expression of the major scaffolding protein PSD-95 (Fig. 3b) ($p < 0.001$), when compared with the control group.

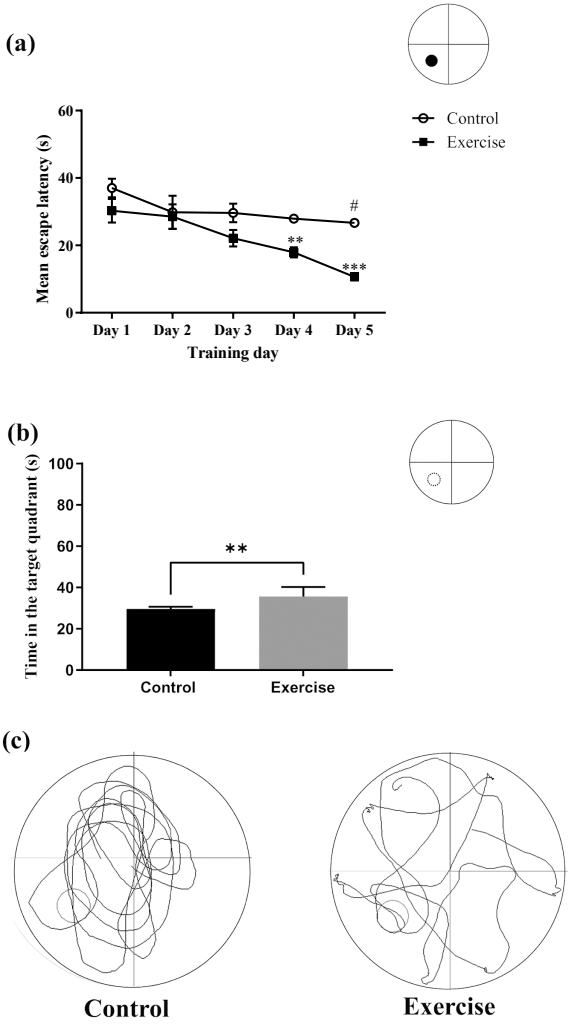

Fig. 1 The effects of peripheral physical activity on spatial learning and memory performance. (a) The escape latency during five consecutive days of the acquisition test. Each data point is expressed as mean ± SEM. The control group (the empty circles) showed a significant difference on Day 5 when compared with Day 1 at # $p < 0.05$; the exercise group (the black squares) showed significant differences on Day 4 at ** $p < 0.01$ and Day 5 at *** $p < 0.001$ when compared with Day 1. (b) The time spent in the target quadrant during the probe trial. Values are expressed as mean ± SEM of 5 animals. There was a significant difference when compared with the control group at ** $p < 0.01$. (c) The schematic pictures of the control and exercise groups' swim tracking on the probe trial. The behavioral test was performed on P40.

DISCUSSION

For over a decade now, physical activity has revealed benefits when it comes to improving learning and memory. But the mechanisms of learning and memory improvement have not been fully demonstrated. The hippocampus is crucial for spatial working memory, which is reflected by the MWM test. The task was assigned and repeated to demonstrate memory for-

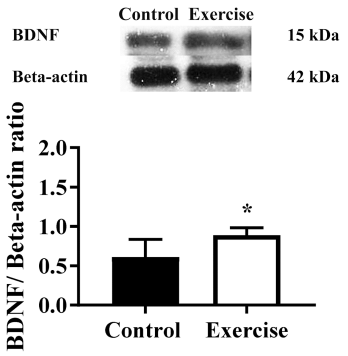

Fig. 2 The effects of peripheral physical activity on BDNF protein expression. The upper panel represents Western blot analysis performed for BDNF in the hippocampal tissue, in a comparison between the control group and exercise group at P40. The lower bar graph displays the results from the Western blot analysis. The data are expressed as protein band densities/ β-actin ratio; values are represented as mean ± SEM of 5 animals. There was a significant difference when compared with the control group at * $p < 0.05$.

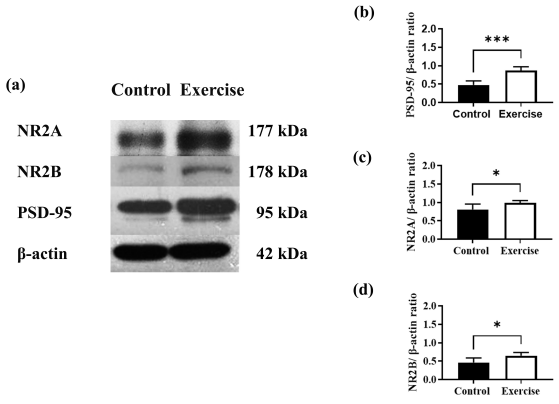

Fig. 3 The effects of peripheral physical activity on synaptic protein expressions in the hippocampal tissue. The bar graphs displaying the results from the Western blotting relative levels of (b) PSD-95 protein, (c) NR2A protein, and (d) NR2B protein, when compared each to β-actin. Values are expressed as mean ± SEM of 5 animals. There was a significant difference when compared with the control group at * $p < 0.05$ and *** $p < 0.001$.

mation. All rats showed their improved learning and memory by reducing the mean escape latency. In addition, it has been shown that physical activity shortens the duration of short-term memory formation. The time spent in the target quadrant during the probe trial was then determined for memory retention. Physical activity showed an enhancement of acquired memory consolidation [10].

The improvement in learning and memory is due to an increase in the number of newborn neurons in the hippocampus. The process of neurogenesis requires BDNF during the critical period of brain development throughout adulthood [11]. Our current study reveals that physical activity enhanced the level of BDNF in the hippocampus of adolescent rats. Correspondingly, a recent study demonstrated that physical activity induced an increase in BDNF and was able to improve working memory in young adults [12].

Learning and memory are regulated by the modification of synaptic strength through NMDAR-dependent mechanisms. An activity-dependent form of LTP requires Ca^{2+} influx through NMDAR, which is involved in consolidation of learning and memory. In addition, animal models with hypofunction or down-regulation of NMDAR showed reduced hippocampal LTP [13], which in turn revealed schizophrenic behavior [14, 15]. This implies that the molecular mechanism of neuroplasticity responds to cognitive impairment and psychiatric problems. Enhancement of NMDAR function could improve cognitive symptoms in rat models of schizophrenia [16]. Here, we can show the efficacy of peripheral physical activity on enhancing NR2A and NR2B subunits in the hippocampus of adolescent rats. Furthermore, this study can exhibit an increase in PSD-95 in the exercise group, as well. PSD-95 is the principal scaffolding protein, which has an important role in regulating synaptic maturation and anchoring NMDAR onto the surface membrane. Perturbation of the NMDAR-PSD-95 complex induced a decrease in LTP [17] attributed to schizophrenia and developmental disorder [18]. It is well known that physical activity can be a curative intervention to improve spatial working memory impairment and enhance BDNF in NMDAR antagonist-induced schizophrenic mouse models [19]. In addition, there is a report that peripheral physical activity prevented forgetting of new vocabulary and increased blood BDNF in healthy young adults [20].

Taken together, the results of this and other studies show the efficacy of peripheral physical activity to promote learning and memory behavior, corresponding to the elevations of BDNF, NR2A, NR2B, and PSD-95, which play an essential role in the learning and memory domains of cognitive function. Our work suggests that, since physical activity has the potential effect of enhancing learning and memory, it should be encouraged more in healthy adolescents.

CONCLUSION

Our data demonstrate that peripheral physical activity enhances the level of BDNF, which is necessary for learning and memory. In addition, we succeeded in demonstrating that the molecular mechanism of peripheral physical activity induces learning and memory by enhancing the expression of synaptic plasticity-related proteins, NMDAR and PSD-95, in the hip-

pocampus of adolescent rats.

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