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RESEARCH ARTICLE

The Effects of Physical Exercise on Spatial Learning and Serotonin Levels in the Brain of Adult Rats

Annisa Rahmah Furqaani,[1] Sri Redjeki,[2] Dwirini Retno Gunarti[3]

[1]Department of Histology and Biomedical Sciences, Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia, [2]Department of Physiology, [3]Department of Biochemistry & Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta Pusat, Indonesia

Abstract

Physical exercise can enhance tryptophan transport into the brain so that it will also increase serotonin levels in the brain. Therefore, it may influence many brain functions, such as learning and memory. This study aimed to determine the effect of physical exercise on spatial learning and serotonin levels in the brain of adult male Wistar rats. Biochemistry Laboratory of Department of Biochemistry & Molecular Biology, Faculty of Medicine, Universitas Indonesia was the study place which conducted in January–April 2013. Sixteen adult male rats randomly divided into two groups, the control group, and the treatment group. Physical exercise for the treatment group for four weeks using the animal treadmill at 15 m/min in speed for 15 minutes in the 1st week and 25 minutes for the next three weeks. Learning and memory test using water-E maze apparatus once a week. At the end of the exercised period, animals were sacrificed, and the brains were isolated. The measurement of serotonin and tryptophan levels was done using high-performance liquid chromatography (HPLC). The results showed that physical exercise improved animals performance in learning and memory test, exercised group made fewer errors at third and fourth week ($p<0.05$). Serotonin levels in the brain of exercised group was significantly higher than that in control group ($p<0.05$). These results indicated that the enhancement of serotonin levels in the brain induced by physical exercise is involved in improving spatial learning and memory.

Keywords: Brain, learning and memory, physical exercise, serotonin

Pengaruh Latihan Fisik terhadap Kemampuan Belajar Spasial dan Kadar Serotonin pada Otak Tikus Dewasa

Abstrak

Latihan fisik diketahui dapat meningkatkan transpor triptofan melewati sawar otak sehingga dapat meningkatkan kadar serotonin di otak. Oleh karena itu, latihan fisik berperan memengaruhi berbagai fungsi otak termasuk proses belajar dan memori. Penelitian ini bertujuan mengetahui pengaruh latihan fisik aerobik dengan intensitas yang ringan terhadap kemampuan belajar spasial serta kadar serotonin pada otak tikus Wistar dewasa. Penelitian ini dilakukan di Laboratorium Biokimia, Departemen Biokimia & Biologi Molekuler, Fakultas Kedokteran, Universitas Indonesia, Jakarta periode Januari–April 2013. Enam belas ekor tikus jantan dewasa dibagi secara acak menjadi dua kelompok, yaitu kelompok kontrol dan kelompok perlakuan. Latihan fisik diberikan kepada kelompok perlakuan selama 4 minggu menggunakan *animal treadmill* dengan kecepatan 15 m/menit selama 15 menit pada minggu pertama dan 25 menit pada 3 minggu berikutnya. Uji belajar dan memori dengan perangkat *water-E maze* dilakukan satu kali/minggu. Setelah masa latihan fisik selesai, hewan coba dikorbankan dan jaringan otak diisolasi. Pengukuran kadar serotonin dan triptofan pada otak dilakukan menggunakan kromatografi cair kinerja tinggi (KCKT). Hasil penelitian menunjukkan bahwa jumlah kesalahan yang dilakukan oleh kelompok perlakuan lebih sedikit secara signifikan pada uji belajar dan memori ke-3 dan ke-4 ($p<0{,}05$). Kadar serotonin lebih tinggi secara signifikan pada otak kelompok perlakuan ($p<0{,}05$). Hasil penelitian ini mengindikasikan bahwa peningkatan kadar serotonin pada otak yang diinduksi oleh latihan fisik aerobik intensitas ringan terlibat dalam meningkatkan kemampuan belajar dan memori spasial.

Kata kunci: Belajar dan memori, latihan fisik, otak, serotonin

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Correspondence: Annisa Rahmah Furqaani. Department of Histology and Biomedical Sciences, Faculty of Medicine, Universitas Islam Bandung. Jln. Tamansari No. 22, Bandung 40116, West Java, Indonesia. Phone: (6222) 4203368. Fax: (6222) 4231213. Mobile: 6281221688757. E-mail: annisarahmahf@gmail.com

Introduction

Physical exercise defined as any structured physical activity undertaken in a certain period to maintain or improve body health and physical fitness. Regular physical exercise contributes to maintaining muscle tone, ideal body weight, and cardiovascular fitness.[1] It also plays a vital role in improving mental health, maintaining positive self-esteem, preventing stress and depression. Moreover, physical exercise has a beneficial effect on improving cognitive function, including learning and memory.[2,3]

The physical exercise involved in improving cognitive function through several mechanisms, such as increased nutrients and oxygen distribution into the brain, enhancing neurogenesis, increasing neurotrophic and neuroprotective levels that induce nerve cells growth, maintaining synapses plasticity, activating antioxidant systems, and formatting long-term potentiation (LTP).[4–11] Moreover, physical exercise also improves cognitive function by affecting the neurotransmitter systems, such as serotonin (5-HT),[12–14] dopamine (DA),[14] epinephrine (E), two norepinephrine (NE),[4,14] glutamate (Glu),[6] and acetylcholine (ACh).[14]

Physical exercise can enhance serotonin levels in the brain by increasing the transport of plasma tryptophan (Trp) into the brain as a consequence of the increased fat catabolism during the physical exercise. Physical exercise also enhances the uptake of branched-chain amino acids (BCAA) by the skeletal muscles so that BCAA levels in the plasma decreased. Since BCAA and tryptophan using the same protein carrier (sizeable neutral amino acid, LNAA, protein carrier), the increased of free tryptophan levels along with the decreased of BCAA levels in plasma will enhance the utilization of LNAA protein carrier by tryptophan. Therefore, more tryptophan can pass through the blood-brain barrier. Increased tryptophan availability in the brain provides more abundant substrates for serotonin synthesis and release by serotonergic neurons.[15–18]

Increased serotonin levels in the brain will be able to affect various brain functions, including learning and memory. Serotonin is known as a neurotransmitter that involved in the learning and memory processes due to serotonergic neurons innervate different regions of the central nervous system, such as the cerebellum, neocortex, thalamus, limbic system, medulla oblongata, and medulla spinalis. In addition, various serotonin receptors can be found in almost all parts of the brain that enables serotonin affects learning and memory processes through several mechanisms depend on the receptor-activated, among others, depolarizing neurons directly via activation of 5-HT_3 receptor, increasing certain neurotransmitters release through cyclic adenosine monophosphate (cAMP) cascade, involving in neurogenesis, and formatting LTP.[19–25] It mentioned earlier that serotonergic neurons innervate different regions of the central nervous system, one of the densest parts innervated by serotonergic neurons is hippocampus, which is part of the limbic system. The hippocampus plays a vital role in learning and memory, emotion, and spatial navigation.[26] Therefore, increased levels of serotonin in the brain induced by physical exercise implicated in increasing this neurotransmitter levels in the hippocampus. Furthermore, it will also improve hippocampal-dependent learning and spatial memory. Therefore, this research aimed to determine the effect of physical exercise on spatial learning and serotonin levels in the brain of adult male Wistar rats.

Methods

This study was experimental in vivo research. Sixteen adult male Wistar rats (*Rattus norvegicus*), age 8–10 weeks, initial weight 150–200 g, were used. This study was done at the Biochemistry Laboratory of Biochemistry & Molecular Biology, Faculty of Medicine, Universitas Indonesia in January–April 2013. Rats obtained from the Department of Biochemistry & Molecular Biology, Universitas Indonesia. Animals maintained under the controlled condition of light (12 hours light:12 hours dark), temperature 24±1°C, and humidity 60–85%. They fed on standard food produced by PT Charoen Pokphand Indonesia and allowed to have free access to food and tap water ad libitum. This study had been reviewed and approved by the Health Research Ethics Committe, Faculty of Medicine, Universitas Indonesia-Dr. Cipto Mangunkusumo General Hospital Jakarta, with ethical approval number: 759/H2.F1/ETIK/2012.

Animals randomly divided into two groups of eight animals each. The control group and the exercised group that performed a low intensity of aerobic exercise. The exercise was done for four weeks (5 days/week). The animals of the exercised group ran on a motorized treadmill with a slope of 0° at 15 m/min in speed for 15 minutes in the

1st week and 25 minutes for the next three weeks. In the first two minutes of every exercise session, the speed of motorized treadmill set at 10 m/min in order to warm up the animals.

Learning memory test in this study was done once a week. Water-E maze apparatus was used to assess the animal's learning and memory capability. The apparatus was made up of E shaped glass, with the main ditch, center ditch, and two side ditches (left and right). Stairs used as the goal (finish point) and placed in one side of the ditch. The animals placed at the end part of the center ditch as the start point of a water-E maze. The number of errors and the duration to finish the test recorded. The test was repeated three times for each animal without resting.

By the end of the treatment period, animals sacrificed, and the brains isolated in order to measure tryptophan and serotonin levels using HPLC. The brain of each individual rat was homogenized at 10,000 rpm in $HClO_4$ 0.1 M, 0.025% Na_2EDTA, and 0.025% L-cystine. Homogenates centrifuged at 12,500 g, 4˚C, for 15 minutes. The supernatants from the centrifugation were ready for analysis using HPLC. Chromatographic separation performed with octadecyl (C18) column, 5 µm, 4.6 × 250 mm. Flow rate set at one mL/min and UV detection at 210–400 nm. The mobile phase was KH_2PO_4 10 mM : acetonitrile (9:1). 50 µL sample injected into the column. The external standard of tryptophan and serotonin produced by SIGMA, USA.

The results of the study presented as mean ± standard deviation (SD). T test independent with $\alpha=0.05$ was performed to analyze all parameters in the study, except the number of errors at the fourth week was analyzed using Mann-Whitney with $\alpha=0.05$.

Results

The results of the learning and memory test presented in Table 1. The number of errors and duration needed by the animals to finish the test assessed. The average travel time required to reach the target (stairs) and the average number of errors made by the animals in completed learning and memory test decreased in both groups (control and exercised groups). However, the exercised group was able to show better progress than the control group in resolving water-E maze test. The results showed that physical exercise could improve animals performance in learning and memory tests, exercised group made fewer errors at third ($p<0.05$) and fourth week ($p<0.05$). Although there was no significant difference in duration needed by both groups to complete learning and memory tests ($p>0.05$).

The results of the measurement of tryptophan and serotonin levels in the brain presented in Table 2. Serotonin levels in the brain of exercised group significantly higher than that in control group ($p<0.05$). Meanwhile, tryptophan levels were not significantly different in both group ($p>0.05$). By observing the levels of serotonin in the brain, it concluded that tryptophan levels were not significantly different might be caused by more tryptophan in the exercised group has been converted into serotonin.

Discussion

The results showed that exercised group was able to show better performance than the control

Table 1 Duration and Number of Errors in Learning and Memory Test

Parameters	Week	Control Group	Exercised Group	p Value
Duration (second)	0	24.63±16.07	27.43±12.50	0.704
	1st	15.36±5.62	15.83±5.06	0.847
	2nd	14.50±7.54	12.48±3.90	0.604
	3rd	13.13±6.11	9.50±3.57	0.900
	4th	8.62±4.02	5.78±2.75	0.118
Number of errors (times)	0	1.42±1.15	1.46±0.53	0.928
	1st	1.08±0.83	0.96±0.55	0.728
	2nd	1.13±1.08	0.71±0.42	0.642
	3rd	1.04±0.74	0.37±0.45*	**0.039**
	4th	0.62±0.55	0.04±0.12*	**0.008**

*Showed significantly different from the control group (t test independent for all paramater, Mann-Whitney test for number of errors at 4th week, $p<0.05$)

Table 2 Tryptophan and Serotonin Levels in the Brain

Parameter (ppm/g brain tissue)	Control Group	Exercised Group	p Value
Tryptophan	2.54±0.97	3.41±1.26	0.115
Serotonin	9.90±2.46	15.88±3.93*	**0.003**

*Showed significantly different from the control group (t test independent, $p<0.05$)

group in finishing learning and memory test, especially after three weeks of exercising. The exercised group was able to resolve learning and memory test with fewer errors than the control group at the third and fourth week. It concluded that low intensity of aerobic exercise in this study improved learning and spatial memory assessed with the water-E maze. The results of this study were consistent with other studies which showed that physical exercise has a positive contribution to cognitive functions, including learning and memory ability. Van Praag et al.[6] showed that running enhances learning and spatial memory with the Morris water maze, increase neurogenesis in the hippocampus and improve LTP at medial perforant path synapses toward the dentate gyrus (DG). The increased in neurogenesis and synaptic plasticity in the runner mice thought to contribute to improving spatial navigation ability. Another study showed that low intensity of aerobic exercise could improve spatial learning and memory ability of elderly rats, but the exercise procedure had not been able to increase brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the forebrain.[27]

The study by Borght et al.[8] showed that running could increase neurogenesis in rat's hippocampus which allegedly associated with the improvement of animal performance in completing learning and memory test with Y-maze. Liu et al.[28] concluded that both, voluntary exercise and forced exercise on a treadmill, can improve spatial navigation of mice on the Morris water maze. Those exercise procedures also increase BDNF and synaptotagmin one levels in the hippocampus. The increased BDNF levels positively correlated with improvement of learning and memory ability, mainly through neurogenesis, synaptogenesis, and activation of another neurotrophic factor. While synaptotagmin is a molecule that required in synaptic vesicles aggregation when new synapses formation take place. In their study, Kobilo et al.[29] showed that running could increase BDNF levels in the hippocampus. BDNF plays a role in increasing neurogenesis in that brain region. Their study also showed that running could improve animals (mice) performance in the open-field test which reflects the improvement in processing information and decreasing anxiety levels in new environments. Another research by Ke et al.[30] concluded that voluntary exercise was the most effective exercise in increasing BDNF levels in the hippocampus and improving motor function in rats with ischemic stroke.

Exercise also affects brain functions by modulating neurotransmitter systems. Chaouloff et al.[17] showed that light intensity of running increased tryptophan levels in mice's brain, without affect the total tryptophan levels in the body. According to Chaouloff et al.[17] results, it was clearly indicated that fat catabolism which occurred during the physical exercise was the factor that responsible for the enhancement of tryptophan transport into the brain so that this amino acid levels increased in the brain. More free fatty acids (FFA) was available in the plasma when fat catabolism occurred. FFA and tryptophan had the same binding sites on albumin so that the enhancement FFA levels in plasma disrupted chemical bond between tryptophan and albumin. As a result, there would be more free tryptophan found in plasma. Along with the increased free tryptophan levels and the increased of FFA concentrations, there was also an increased BCAA uptake by the skeletal muscles. Therefore BCAA levels in the plasma decreased, and the number of competitors for tryptophan to pass through the brain barrier decreased because LNAA (including BCAA) used the same protein carrier. Increased free tryptophan levels along with decreased BCAA levels in plasma resulted in enhancing tryptophan transport into the brain so that more tryptophan available in the brain. Tryptophan is an essential amino acid which is a precursor of serotonin. Increased levels of tryptophan providing more substrate for serotonin synthesis by the serotonergic neurons in the brain, including the hippocampus. Increased serotonin levels in the brain may affect various brain functions, including learning and memory.[15–18]

In line with Chaouloff et al.,[17] this study showed that tryptophan levels in the brain of the exercised group were higher than that in the control group although not significantly different. The average tryptophan levels were not significantly different can be explained by observing serotonin levels in the brain. The results showed that serotonin levels in the brain of exercised group significantly higher than that in the brain of the control group. Tryptophan levels were not significantly different might be caused by more tryptophan in the exercised group had been converted into serotonin. These results indicated that low intensity of aerobic exercise in this study not only enhanced serotonin levels in the brain but also improved learning and spatial memory. The enhancement of serotonin levels in the brain implicated in the enhancement of this neurotransmitter in the hippocampus. Hippocampus is a small region of the brain that forms part of the limbic system and associated with learning processes, memory, and spatial navigation.[20]

The increased levels of serotonin in the hippocampus induced by physical exercise played essential roles in improving hippocampus structures and functions through several mechanisms, such as increase neurogenesis, increase other neurotransmitters production and their release, synaptogenesis, and synapses facilitation. Improvement of hippocampus structure and function enhanced hippocampal-dependent learning and memory.[23–25] Activation of the 5-HT_3 receptor involved in increasing some neurotransmitters release by activated cAMP cascade, depolarize neurons directly so that the transmitting of information will move quicker in the nervous system. Activation of this receptor may also increase the release of other neurotransmitters, especially the excitatory neurotransmitter, encephalin, and gamma-aminobutyric acid (GABA).[31,32] Physical exercise can also increase neurotrophic levels, such as NGF, BDNF, synaptotagmin I, insulin-like growth factor I (IGF-I), vascular endothelial growth factor (VEGF) that involve in increasing neurogenesis, synaptogenesis, and angiogenesis in the brain and also hippocampus.[28–30,33–36]

Activation of 5-HT_{2A} receptor on cholinergic and glutamatergic axon terminals can increase the release of acetylcholine and glutamate. Increased levels of both neurotransmitters are significant in memory formation, the process of LTP, and the learning process.[22] 5-HT_{2A} is also involved in neurogenesis, increased levels of 5-HT_{2A} are increasing proliferation rate of precursor cells in the DG of the hippocampus. Activation of 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors were involved in the regulation of neurogenesis in the DG.[22,25,37] Activated 5-HT_4, 5-HT_6, and 5-HT_7 receptors are also involved in enhancing cell proliferation in the DG indirectly through cAMP cascade, activation of cAMP can increase the expression of cAMP response element-binding protein (CREB), cAMP-CREB complex is capable of increasing the expression of BDNF.[24,25,38] BDNF involved in enhancing serotonin release in the hippocampus, as a result, it is also enhancing neurogenesis in that region.[7–9] The DG of the hippocampus also contributes to anxiety. Modulating the activity of the ventral dentate gyrus by stimulating neurogenesis in that area is suggested can lower anxiety.[20]

The enhancement of serotonin levels has essential roles in improving hippocampus structure so that it will also improve hippocampus function, including hippocampal-dependent learning and spatial memory. This study showed that low intensity of aerobic exercise involved in modulating serotonin levels in the brain. Moreover, it was also improved learning ability and spatial memory of rats with the water-E maze.

Conclusion

The enhancement of serotonin levels in the brain induced by low intensity of aerobic exercise is involved in improving learning ability and spatial memory.

Conflict of Interest

The authors declare no conflict of interests.

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