Original Article

Effect of Continuous Environmental Enrichment and Aerobic Exercise on Rat Plasma and Hippocampal Brain-Derived Neurotrophic Factor

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Abstract

Objective: Environmental enrichment (EE) or exercise can positively affect memory function through increased long-term potentiation and neurogenesis, which is facilitated by brain-derived neurotrophic factor (BDNF). BDNF promotes blood vessel growth, angiogenesis linked to adult neurogenesis, and neuronal survival. Here, we investigated the effects of EE, aerobic exercise, and their combination on plasma and hippocampal BDNF levels. **Materials and Methods:** Twenty-four 7-month-old adult male Wistar rats weighing 300–400 g were randomly assigned to the following four groups: control (C), aerobic exercise (A), EE, and combined EE and aerobic exercise (EEA). Interventions were given for 8 weeks, and plasma and hippocampal BDNF levels were measured using enzyme-linked immunosorbent assay. **Results:** A combination of aerobic exercise and continuous EE produced the largest increase in hippocampal and plasma BDNF levels in adult rats. A positive correlation ($r = 0.686$, $P = 0.002$, $n = 24$) was observed between plasma and hippocampal BDNF levels in adult rats. **Conclusion:** We conclude that a combination of aerobic exercise and continuous EE increases plasma and hippocampal BDNF in adult rats.

Keywords: Aerobic exercise, brain-derived neurotrophic factor, environmental enrichment, hippocampus

Introduction

The ability to store and recall information is one of the many hallmarks of higher cognitive function. The underlying mechanism of memory and learning is synaptic plasticity.[1] Brain-derived neurotrophic factor (BDNF) is shown to modulate synaptic plasticity and facilitate learning and memory.[1,2] Among neurotrophic factors in the nervous system, BDNF (along with its main receptor, tyrosine receptor kinase B [TrkB]) is the most abundantly expressed and has widespread distribution. Its expression is fundamental for neuronal survival during development, as well as for neuronal integration in the adult mammalian brain.[2] BDNF is also implicated in the modulation of synaptic function and plasticity through increased long-term potentiation (LTP) and neurogenesis.[1] The action of BDNF in the adult central nervous system (CNS) is currently one of the most extensively studied subjects in neuroscience, likely because of its essential role in LTP.[2] Outside of the nervous system, BDNF is also expressed in the liver and skeletal muscles and is stored in thrombocytes and plasma. Suppressing the expression of BDNF and its receptor (TrkB) in mice was shown to influence memory and learning processes.[3] Hence, many attempts have been made to increase BDNF expression levels to improve memory function. Among these efforts to improve cognitive and memory function are aerobic exercise and environmental enrichment (EE). These treatments have been shown to increase BDNF protein levels both in the brain and peripheral organs.[4] Aerobic exercise can improve learning and memory function by inducing neurogenesis and neuroplasticity. This type of exercise increases nerve cell signaling activity in the form of LTP, which is known to occur through increased expression of neuropeptides, especially BDNF.[5] Similarly, EE can also affect learning and memory. EE can have long-lasting effects on the individual phenotype through developmental plasticity, and serum and plasma BDNF concentrations' increase is reported to elevate with EE.[6] Hence,

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in this study, we investigated the combined effect of aerobic exercise and continuous EE on plasma and hippocampal BDNF levels in adult rats.

Materials and Methods

The design and method of this study was reviewed and approved by the Medical Research Ethics Committee, Faculty of Medicine, Universitas Indonesia, with number 1018/UN2. F1/ETIK/2017, November 6, 2017.

Animals

Twenty-four adult male Wistar rats, aged 7 months, weighing from 300 to 400 g, were included in this study. Rats were randomly assigned to the following four groups: control (C), aerobic exercise (A), EE, and combined EE and aerobic exercise (EEA). The animals were maintained under a 12-h light and dark cycle, and food and water were available *ad libitum*. The rats were acclimatized for 2 weeks before the start of the experiment. At the end of an 8-week experimental period, the rats were sacrificed, intra-orbital blood was collected, and the hippocampus was isolated. Plasma and hippocampal BDNF levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (QY-A10582, Qayeebio, China).

Aerobic exercise

Aerobic exercise treatment was implemented in Groups A and EEA using a four-lane treadmill for rats. Treatment was given for 8 weeks, 5 days/week, with each session lasting for 30 min with a treadmill speed of 20 m/min. Before each session, there was a 3-min warm-up session with a treadmill speed of 8 m/min.

Environmental enrichment model (Marlau™ cage)

The EE model that was used is a standardized apparatus, the Marlau™ cage. The goal of continuous EE exposure is to improve the animals' quality of life by providing a combination of physical activity, enhanced social interaction, and natural exploration.[7] The EE model was used for Groups EE and EEA, and treatment was administered for 8 weeks.[8] The Marlau™ cage measured 800 mm × 600 mm × 510 mm [Figure 1], consisted of two floors, and had various enrichment objects such as running wheels, ladders, labyrinths, toys, tunnels, and nesting materials. The enrichment objects used were brightly colored to provide visual sensory stimulation. The ground floor height was 300 mm, with the G1 area measuring 296 mm × 600 mm [Figure 1] and the G2 area measuring 496 mm × 600 mm [Figure 1]. There was also a labyrinth on the upper floor, connected from the G2 area by stairs, and a sled tunnel that went down to the G1 area [Figure 1].[9]

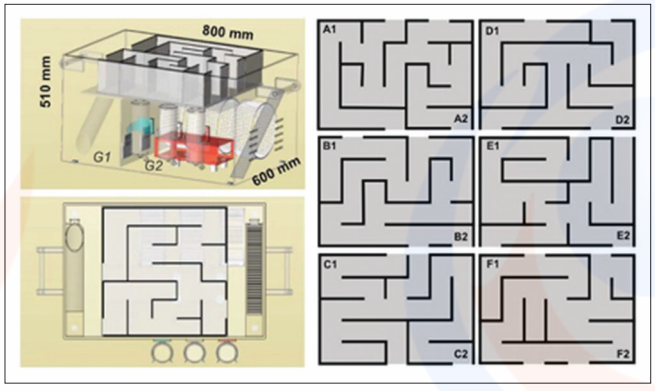

Figure 1: Marlau™ cage

The protocol that was used involved changing the labyrinth configuration three times per week, on Mondays, Wednesdays, and Fridays. Six labyrinths (A–F) were used, each consisting of two different possible configurations (1 and 2). The maze change began with the A1 model, followed by B1, C1, and so on, and ended with D2, E2, and F2. When all possible maze models had been used, ending with the F2 model, the cycle started again from A1 [Table 1].[10]

Brain-derived neurotrophic factor levels

Hippocampal tissue was homogenized with phosphate-buffered saline (pH 7.4) and centrifuged for 10 min at 3000 rpm before the supernatant was collected and stored at −80°C. Blood samples were collected and mixed in ethylenediaminetetraacetic acid for 20 min, centrifuged for 30 min at 3000 rpm, and the supernatant was collected and stored at −80°C. Whole protein concentrations were assessed using the Bradford protein assay and then compared with BDNF concentrations that had been measured with an ELISA kit (QY-A10582, Qayeebio, China).[11]

Statistical analysis

Plasma and hippocampal BDNF levels were analyzed using

Table 1: Configuration changes of the Marlau™ Cage maze

Marlau™	Day	Maze series
Week 1	Monday	A1
	Wednesday	B1
	Friday	C1
Week 2	Monday	D1
	Wednesday	E1
	Friday	F1
Week 3	Monday	A2
	Wednesday	B2
	Friday	C2
Week 4	Monday	D2
	Wednesday	E2
	Friday	F2
Week 5	Monday	A1
	Wednesday	B1
	Friday	C1
Week 6	Monday	D1
	Wednesday	E1
	Friday	F1
Week 7	Monday	A2
	Wednesday	B2
	Friday	C2
Week 8	Monday	D2
	Wednesday	E2
	Friday	F2

one-way ANOVA, whereas the correlation between plasma and hippocampal BDNF levels was analyzed with the Pearson's correlation coefficient. Data were considered statistically significant if $P < 0.05$.

Results

The combination of continuous EE and aerobic exercise induced the largest increase in both plasma and hippocampal BDNF concentrations [Figure 2]. The plasma BDNF level was lowest in Group C (2.87 pg/mg protein ± 0.31), followed by Group A (3.09 pg/mg protein ± 0.23), Group EE (3.34 pg/mg protein ± 0.50), and finally Group EEA (3.95 pg/mg protein ± 0.45). There were statistically significant differences in BDNF plasma levels between Groups EEA and C ($P = 0.001$) and Groups EEA and A ($P = 0.005$). This indicates that a combination of continuous EE and aerobic exercise is more effective in increasing plasma BDNF compared to exercise alone.

The hippocampal BDNF level was the lowest in Group C (34.15 pg/mg protein ± 5.06), followed by Groups A (35.23 pg/mg protein ± 5.38), EE (39.22 pg/mg protein ± 6.07), and EEA (45.82 pg/mg protein ± 6.01). There were statistically significant differences in hippocampal BDNF levels between Groups EEA and C ($P = 0.011$) and Groups EEA and A ($P = 0.024$). This indicates that a combination of continuous EE and aerobic exercise is more effective in increasing hippocampal BDNF compared to exercise alone.

Correlation of plasma and hippocampal brain-derived neurotrophic factor

There was a moderate positive correlation between plasma BDNF levels and hippocampal BDNF levels ($r = 0.686$; $P = 0.002$, $n = 24$) [Figure 3 and Table 2].

Discussion

BDNF is a member of the neurotrophic family, which plays important roles in neurogenesis, LTP, protection against neural cell death, and improving learning and memory by increasing neuronal plasticity.[10] BDNF is also a pro-survival signaling molecule that modulates synaptic plasticity and neurogenesis. BDNF is particularly abundant in the hippocampus and cerebral cortex, but it is also present in the blood stream.[12] Circulating BDNF can originate from both the CNS and the peripheral nervous system, and also from nonneuronal tissues such as vascular endothelial and immune system cells.[5]

Table 2: Data points of plasma BDNF and hippocampal BDNF levels

Subject	Plasma BDNF (pg/mg protein)	Hippocampal BDNF (pg/mg protein)
C1	2.96	28.05
C2	2.80	38.42
C3	2.37	39.30
C4	3.33	37.46
C5	2.88	28.26
C6	2.89	33.40
A1	2.94	31.58
A2	3.20	37.04
A3	2.74	27.79
A4	3.14	42.06
A5	3.42	33.06
A6	3.12	39.85
EE1	2.76	44.93
EE2	2.71	37.21
EE3	3.40	36.68
EE4	3.54	35.59
EE5	3.92	48.35
EE6	3.72	32.56
EEA1	3.08	39.49
EEA2	4.12	39.09
EEA3	4.01	45.02
EEA4	3.94	46.07
EEA5	4.37	51.56
EEA6	4.18	53.69

BDNF: Brain-derived neurotrophic factor, EE: Environmental enrichment, EEA: Combined EE and aerobic exercise

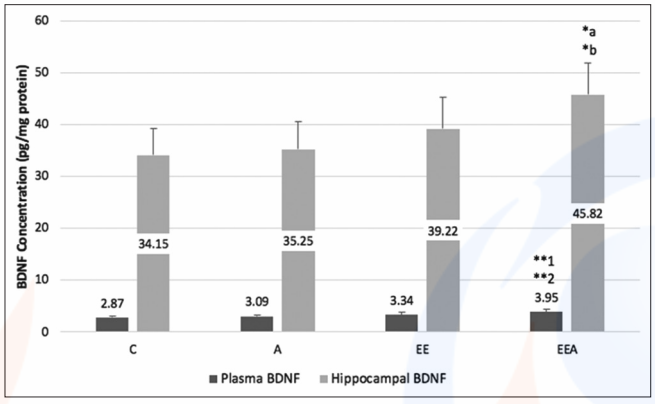

Figure 2: Mean concentrations of plasma and hippocampal brain-derived neurotrophic factor (BDNF) concentrations. C: Control, A: Aerobic exercise, EE: Environmental enrichment, EEA: Combined environmental enrichment and aerobic exercise. $^{**1}P = 0.001$ vs. C; $^{**2}P = 0.005$ vs. A; $^{*a}P = 0.011$ vs. C; $^{*b}P = 0.024$ vs. A

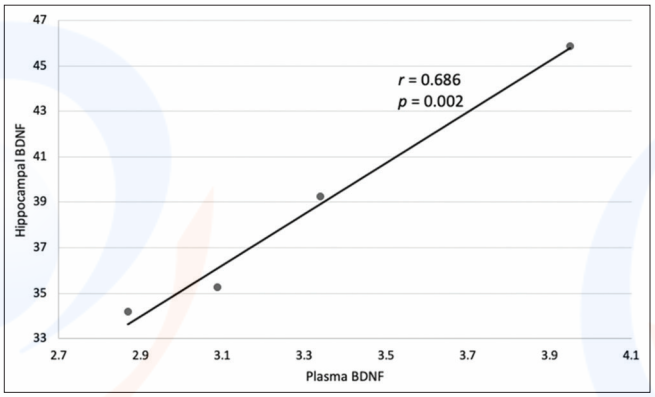

Figure 3: Correlation of plasma and hippocampal brain-derived neurotrophic factor levels

Several studies have investigated the effect of exercise on BDNF levels. Recent studies have shown that aerobic exercise has a positive impact on plasma BDNF.[5] The increase in plasma and hippocampal BDNF levels observed in our study following exercise was statistically insignificant. This may be caused by our study limitation in which the treadmill room was different from the cage room, with different lighting settings, which may cause a disruption in circadian rhythm and BDNF expression.[13] Another recent study reported that fibronectin type III domain-containing protein 5, a previously identified muscle protein that is induced by exercise and cleaved and secreted as irisin, can induce the hippocampal expression of BDNF and other neuroprotective proteins.[14]

EE involves complex sensorimotor stimulation and provides animals with increased opportunities for physical activity, various learning experiences, and social interactions.[15] These conditions can improve both the development and the function of the brain.[12,16] EE is also shown to increase plasma BDNF levels in animal studies.[6] BDNF that is secreted by skeletal muscles during physical activity can circulate in the blood and cross the blood–brain barrier. BDNF in the brain can then bind to its receptor, TrKB, as well as to p75 neurotrophic receptors.[1] This leads to the activation of a biochemical cascade, which results in the proliferation and survival of neuronal cells and their plasticity. This may in part explain the effect of EE and aerobic exercise on plasma and hippocampal BDNF levels.[12] In our study, EE increased both plasma and hippocampal BDNF almost as high as the combination of aerobic exercise and EE treatment, and the difference was statistically insignificant. This may be explained due to the fact that our EE protocol included conditions encouraging voluntary exercise, which supports our hypothesis that a combination of aerobic exercise and EE stimulates higher expression of BDNF.

There was a positive correlation between plasma and hippocampal BDNF in our study, which suggests that an increase in plasma BDNF is accompanied by an increase in hippocampal BDNF. However, the hippocampal BDNF levels were much higher than the plasma BDNF levels. From these data, we may thus assume that BDNF in the hippocampus does not come solely from plasma BDNF that crosses the blood–brain barrier, but that the majority comes from hippocampal tissue production. However, further investigation into BDNF expression levels in the hippocampus is needed to support this hypothesis.

Conclusion

This study supports the hypothesis that a combination of aerobic exercise and continuous EE increases plasma and hippocampal BDNF in adult rats.

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Conflicts of interest

There are no conflicts of interest.

References

1. Yamada K, Nabeshima T. Brain-derived neurotrophic factor/TrkB signaling in memory processes. J Pharmacol Sci 2003;91:267-70.
2. Cunha C, Brambilla R, Thomas KL. A simple role for BDNF in learning and memory? Front Mol Neurosci 2010;3:1.
3. Reda M, El-Nady HG, Rabie MA, Fawzy R, Adel S, Allah EA, *et al*. Comparing brain-derived neurotrophic factor levels, intelligence, and memory in clinical subtypes of attention-deficit hyperactivity disorder. Middle East Curr Psychiatry 2016;23:56-62.
4. Whiteman AS, Young DE, He X, Chen TC, Wagenaar RC, Stern CE, *et al.* Interaction between serum BDNF and aerobic fitness predicts recognition memory in healthy young adults. Behav Brain Res 2014;259:302-12.
5. Pereira DS, de Queiroz BZ, Miranda AS, Rocha NP, Felício DC, Mateo EC, *et al.* Effects of physical exercise on plasma levels of brain-derived neurotrophic factor and depressive symptoms in elderly women – A randomized clinical trial. Arch Phys Med Rehabil 2013;94:1443-50.
6. Mosaferi B, Babri S, Mohaddes G, Khamnei S, Mesgari M. Post-weaning environmental enrichment improves BDNF response of adult male rats. Int J Dev Neurosci 2015;46:108-14.
7. Mora F, Segovia G, del Arco A. Aging, plasticity and environmental enrichment: Structural changes and neurotransmitter dynamics in several areas of the brain. Brain Res Rev 2007;55:78-88.
8. Abdullah F. The Role of Aerobic Physical Exercise, Environmental Enrichment Model (EE) and Its Combination on the Spatial Memory of Male Wistar Rats: A Study of Perceptions of Neuroligin-1 and PSD-95 Proteins). Thesis; 2017.
9. Fares RP. Erythropoietin and Enriched Housing in Marlau™ Cages Protect Neurons and Cognitive Function in Epileptic Rats. Human Health and Pathology. Université Claude Bernard – Lyon I; 2009.
10. Sheikhzadeh F, Etemad A, Khoshghadam S, Asl NA, Zare P. Hippocampal BDNF content in response to short- and long-term exercise. Neurol Sci 2015;36:1163-6.
11. Qayeebio. Rat Brain Derived Neurotrophic Factor (BDNF) ELISA Kit Instruction Sample Collection and Storage. p. 1-6.
12. Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, *et al.* Blood BDNF concentrations reflect brain-tissue BDNF levels across species. Int J Neuropsychopharmacol 2011;14:347-53.
13. Coria-Lucero CD, Golini RS, Ponce IT, Deyurka N, Anzulovich AC, Delgado SM, *et al.* Rhythmic BDNF and TrkB expression patterns in the prefrontal cortex are lost in aged rats. Brain Res 2016;1653:51-8.
14. Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, *et al.* Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. Cell Metab 2013;18:649-59.
15. Will B, Galani R, Kelche C, Rosenzweig MR. Recovery from brain injury in animals: Relative efficacy of environmental enrichment, physical exercise or formal training (1990-2002). Prog Neurobiol 2004;72:167-82.
16. Maass A, Düzel S, Brigadski T, Goerke M, Becke A, Sobieray U, *et al.* Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. Neuroimage 2016;131:142-54.