

PHYSIOLOGY

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Effect of Prolonged Physical Exercises on Brain-Derived Neurotrophic Factor Levels in the Hippocampus of Rat's Subjected to the Influence of Lead Acetate

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It has been suggested that physical exercise modulates cognitive functions through various signaling mechanisms that lead to brain-derived neurotrophic factor (BDNF) up-regulation, especially in the hippocampus, a major hub for learning and memory formation [1-3]. BDNF is needed for the healthy brain functioning. Studies on BDNF have shown differences in brain region size, memory functioning and anxiety-related behavior when BDNF expression is altered [4]. It is important for cell survival in neurogenesis studies and has been thought to play an important role in antidepressant action [5]. This has promoted the research on BDNF levels in health and disease in the hope of better understanding the etiology and treatment effects [6].

The effect of short-term exercise (15 min step-exercise) on serum BDNF levels was evaluated in healthy human subjects [6]. Results showed a short-term, significant increase in serum BDNF levels after exercise. Intra-individual differences in serum BDNF levels were remarkably small on the rest day and also when compared to rest values on the day of the exercise test. Inter-individual differences, on the other hand, were larger by comparison.

Studies found that the exercise-induced increase in BDNF is transient. This is similar to the other studies cited here. Thus, the prolonged reduction in serum BDNF in affective disorder patients is most likely associated with other factors, in addition to whatever transient effect one sees after physical exertion. It should be

noted that a recent study by A.B. Cunha et al [7] observed reduced serum BDNF levels in both manic and depressed bipolar disorder patients. Considering that depressed patients have been reported to be more inactive physically than non-depressed controls, while manic patients have the reverse activity profile [8, 9]. However, while most studies that correlated physical activity with BDNF expression used the "voluntary wheel running" model, a major critical issue is that the exercise parameters, i.e. intensity, duration, and frequency, are highly variable and dependent on the motor activity of the animal. To resolve this, in this study, we use a special treadmill running protocol in order to examine hippocampus activation and BDNF expression and present evidence that treadmill running at highly controlled mild intensities differentially affect the time-course of BDNF induction in the rat hippocampus.

Materials and methods. *Animals' Condition.* 50 days aged male Wistar rats (256-290 g, $n = 40$) were maintained under standard laboratory conditions (12-h light/12-h dark), with room temperature of $22 \pm 2^{\circ}\text{C}$ and food and water ad lib. Animals were acclimated for a week before treadmill exercise began.

Exercise training. Rats were randomly assigned to 1) basic group (Base-B, $n = 10$) 2) control group (Sham-S, $n = 10$) 3) group with lead injection (lead-L, $n = 10$) 4) treadmill exercise with lead injection group (train + lead - TL, $n = 10$). All rats were weighed on a daily basis during the exercise training phase. Animals were acclimatized to ambient rearing conditions for 4-5 days in group housing conditions (four rats per cage) and then habituated to run on a treadmill (KN-73, Natsume Ltd., Japan) for a total of seven sessions over 8 weeks. The running speed and distance was gradually increased from 15 to 22 m/min and from 25 to 64 m/min. Belt speed was 10 m/min, a walking rate for adult rats, and a speed that improves Morris maze performance in the adolescent rat [10]. At the end of the belt were stationary wire loops, which were electrified. A mild shock (0.75 mA, 500 ms duration, 0.5 Hz rate) was delivered through these loops to motivate the rats to continuously walk on the moving belt and thus avoid foot shock. The wire loops were activated during all exercise sessions, and an experimenter monitored all treadmill sessions. Rats quickly learned to stay on the belt and avoid shock, except of one rat, which would not stay on the moving belt, and thus was quickly removed from the exercise group. The L and TL groups' rats were run 5 days a week, with primary time of 25 up to 64 min at the end of the 8th week (Table 1).

Injections. The groups S received physiological solution and L and LT groups got % 2 lead acetate (20 mg/kg, i/p) 3 days in a week during all 8 training weeks. Until the sacrifice, all the rats were kept in their own cages. Rats were sacrificed by decapitation approximately 15 h after last exercises. After an intraperitoneal injection of 1% ketamine (30 mg/kg) and xylazine hydrochloride (4 mg/kg) the rats

were rapidly decapitated and the brains were quickly removed. The brain region of hippocampus were quickly dissected out. Transverse sections of hippocampus were prepared using a McIlwain tissue chopper. Then they were frozen with dry ice and cryopreserved at -80 C for inset hybridization and BDNF ELISA experiments. Blood samples were mixed with 100 mg/ml of EDTA to suppress coagulation and cooled with ice for analysis.

Table 1

The scheme of trainings for 8 weeks

| Training days | Speed & time of trainings | Number of week | | | | | | | |
|---------------|---------------------------|----------------|----|----|----|----|----|----|----|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | Treadmill speed (m/min) | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| | Time (minute) | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 |
| 2 | Treadmill speed (m/min) | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| | Time (minute) | 26 | 31 | 36 | 41 | 46 | 51 | 56 | 61 |
| 3 | Treadmill speed (m/min) | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| | Time (minute) | 27 | 32 | 37 | 42 | 47 | 52 | 57 | 62 |
| 4 | Treadmill speed (m/min) | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| | Time (minute) | 28 | 32 | 38 | 43 | 48 | 53 | 58 | 63 |
| 5 | Treadmill speed (m/min) | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| | Time (minute) | 29 | 34 | 39 | 44 | 49 | 54 | 59 | 64 |

Analysis of BDNF Concentration. BDNF protein was assessed using the ELISA kit (Demeditec 'ITALIA) according to manufacturer's recommendations. The hippocampus was individually homogenized in lysis buffer containing (in mM): 137 NaCl, 20 Tris-HCl (pH 8.0), Igepal (1%), glycerol (10%), 1 PMSF, 0.5 sodium vanadate, 0.1 EDTA and 0.1 EGTA. Then it was 10 min centrifuged at 14000 rpm under 4°C. Supernatant was diluted in sample buffer and incubated on 96-well flat-bottom plates, previously coated with anti - BDNF monoclonal antibody. After blocking, plates were incubated with polyclonal anti-human antibody for 2 h and horseradish peroxides - for 1 h. Then color reaction with tetraethyl Benzedrine was quantified in a plate reader at 450 nm. The standard BDNF curve ranged from 0 to 500 pg/ml.

Data analysis. Statistical analyses were conducted by two-tailed Student's t-test or two-way ANOVA followed by Tukey's test, when indicated. All data are presented as mean (±SEM).

Results and discussion. Experiments were performed on 40 Wistar male rats. Available results obtained by isolated studies suggest that exercise alters BDNF levels and oxidative status. It has been described that physical activity induces members of the neurotrophins family, especially BDNF, that modulate neuronal

survival and plasticity [11], maturation and outgrowth in the developing brain and that exert neuroprotective actions in the mature brain submitted to metabolic insults [12]. In addition exercise induces BDNF mRNA in the hippocampus [14, 1, 13]. There is evidence indicating that physical activity may reduce age-induced cognitive decline and it is recommended as a therapeutic strategy to prevent, or recover from, neurodegenerative disease [15]. Although the exact molecular mechanisms by which physical exercise affects brain function are unclear, it has been suggested that it might activate cellular and molecular pathways that contribute to neuroprotection.

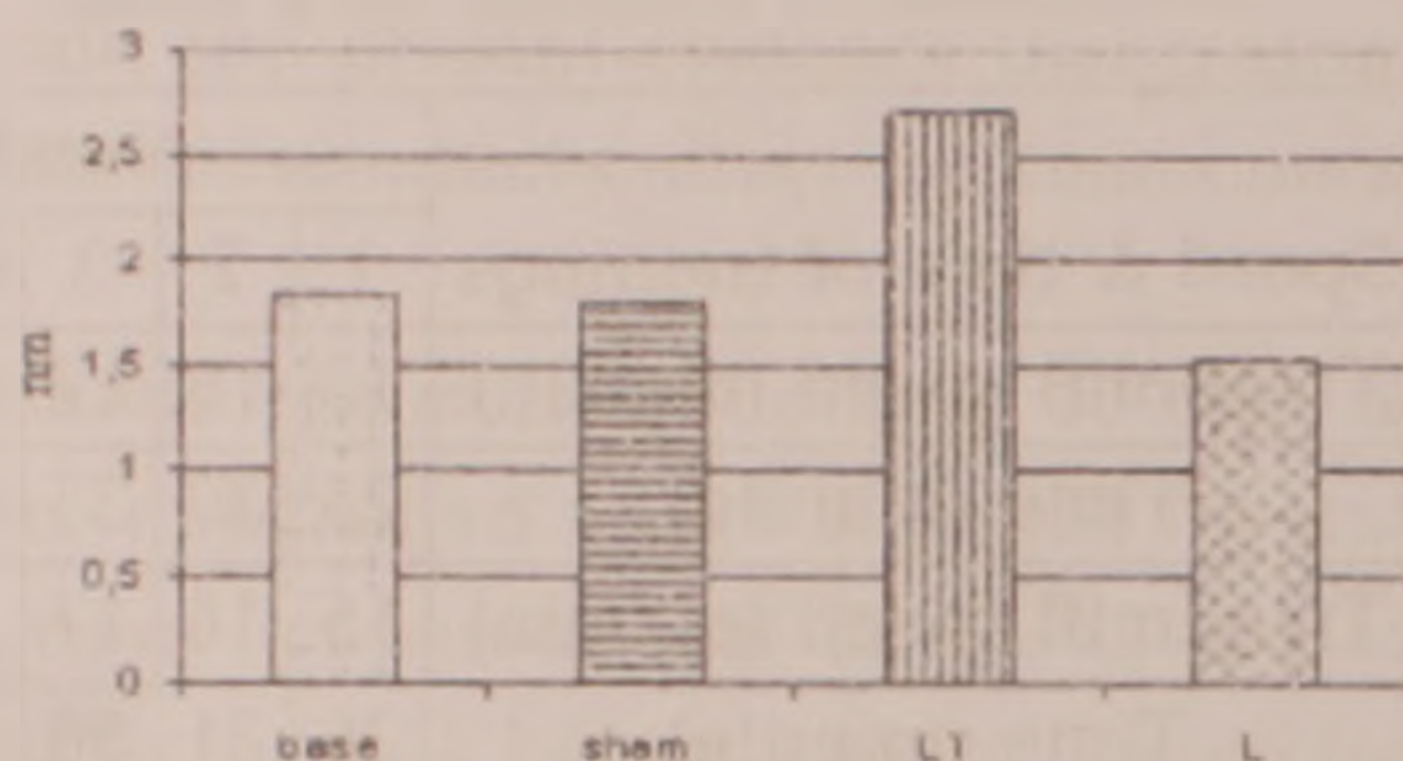


Fig. 1.

Table 2

Hippocampus BDNF values in different groups at 8th week

| Groups | Number of rats | Mean | Std. Deviation | Std. Error | Minimum | Maximum |
|--------------|----------------|--------|----------------|------------|---------|---------|
| Base | 8 | 1,8375 | 0,14772 | 0,05223 | 1,65 | 2,11 |
| Shame | 8 | 1,7988 | 0,65221 | ,23059 | 0,36 | 2,32 |
| Train + Lead | 7 | 2,7057 | 1,66047 | 0,62760 | 1,43 | 5,14 |
| Lead | 7 | 1,5400 | 0,21602 | 0,08165 | 1,24 | 1,87 |

In our experiments it was shown that at the end of tests (8th week) the value of BDNF in L group in comparison with those of base group (1.8375 ± 0.14772 SD, $n = 8$, Table 2, Fig.1) reduced to (1.54 ± 0.21602 SD, $n = 7$, Table 2, Fig. 1). After trainings (see the scheme of training in the Table 2 and Fig. 1) in LT group at 8th week BDNF value increased up to 2,7 (2.7057 ± 1.66047 SD, $n = 7$, Table 2, Fig.1). Thus, the obtained data indicate the significant role of exercises in the gain of BDNF level. On the other hand the increased level of BDNF may act as neuroprotector for recovery of numerous disturbances, such as reduction of the brain weight, memory loss and different degenerative processes. It may be concluded that the increase of BDNF in hippocampus may cause positive plastic changes and provide the prevention of the mentioned disturbances.

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The results of these studies indicate that in rats injection of the lead acetate leads to a reduction of brain-derived neurotrophic factor (BDNF), compared to those in the hippocampus of normal animals.

It is shown that prolonged physical exercise (daily for 8 weeks) led to a significant increase of the level of BDNF, pointing to his neuroprotective role in the process of restoring the lost body weight and various neurodegenerative disorders (loss of memory, decline of cerebral weight, etc.)

Մահամմադ Շահանդեհ

Երկարատև ֆիզիկական վարժությունների ազդեցությունը կապարի ացեթատի սրացած առնետների հիպոկամպի ուղեղածին նեյրոթրոֆիկ ֆակտորի մակարդակի վրա

Հայտնաբերված է, որ համեմատած սորմալ առնետների, կապարի ացեթատի սրացած կենդանիների շրջանում գրանցվում է հիպոկամպի ուղեղածին նեյրոթրոֆիկ ֆակտորի (ՀՈՒՆՖ - BDNF) անկում:

Ցույց է տրված, որ երկարատև ֆիզիկական վարժությունները (ամեն օր ութ շաբաթվա ընթացքում) առաջացնում են ՀՈՒՆՖ-ի մակարդակի զգալի աճ: Դա կարող է վկայել մարմնի քաշի վերականգնման գործընթացում, ինչպես նաև տարբեր նեյրոդեգեներատիվ շեղումների դեպքում (հիշողության կորուստ, ուղեղի կշռի նվազում եւ այլն) նրա նյարդապաշտպանիչ դերի մասին:

Могаммад Шаанде

Влияние длительных физических упражнений на уровни нейротрофического фактора мозгового происхождения в гиппокампе крыс, подвергнутых воздействию ацетата свинца

Выявлено, что инъекция ацетата свинца у крыс приводит к снижению уровня нейротрофического фактора мозгового происхождения (НФМП – BDNF) по сравнению с таковым в гиппокампе нормальных животных.

Показано значительное повышение уровня НФМП в результате длительных физических упражнений (ежедневно в течение 8 недель), что указывает на его нейропротекторную роль в процессе восстановления утраченного веса тела и

устранения различных нейродегенеративных отклонений (потеря памяти, снижение мозгового веса и др.).

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