Behavioural Brain Research 308 (2016) 64–74

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Distinct neuroplasticity processes are induced by different periods of acrobatic exercise training

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HIGHLIGHTS

- Short and long-term acrobatic exercise induce distinct plasticity in motor areas.
- Motor skill learning changes synaptic and structural proteins in brain motor areas.
- Plasticity changes correlate with acquisition and consolidation phases.

ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 11 April 2016
Accepted 15 April 2016
Available online 26 April 2016

Keywords:
Acrobatic exercise
Early growth response 1
Synaptic plasticity
Motor areas
Synaptic proteins
Structural proteins

ABSTRACT

Short and long-term physical exercise induce physiological and structural changes in brain motor areas. The relationship between changes of structural and synaptic proteins in brain motor areas and acrobatic exercise is less understood. Our aim was to evaluate the expression of synapsin I (SYS), synaptophysin (SYP), microtubule-associated protein 2 (MAP2), neurofilament (NF), and a marker for recent neuronal activity (Egr-1) in the motor cortex, striatum and cerebellum of adult rats subjected to acrobatic exercise (AE, for 1–4 weeks). We used adult Wistar rats, divided into 4 groups based on duration of acrobatic training, namely 1 week (AE1, n = 15), 2 weeks (AE2, n = 15), 4 weeks (AE4, n = 15), and sedentary (SED, n = 15). In AE groups, the rats covered 5 times a circuit that was composed of obstacles, three times a week. The protein levels were analyzed by immunoblotting and immunohistochemistry. The results revealed that short-term AE (AE1 and AE2) induced MAP2 decreases and NF, SYP and Egr-1 increases in the motor cortex; an increase of MAP2, SYS and SYP in the dorsolateral striatum, whereas the dorsomedial striatum showed increased NF, SYS, SYP and Egr-1. Granular cerebellar layer showed increased NF and Egr-1, with increased NF and SYP in the molecular layer. Long-term AE (AE4) promoted an increase of MAP2, SYP and Egr-1 in motor cortex; MAP2, SYS and SYP in the dorsomedial striatum; and NF and Egr-1 in the cerebellar granular layer. In conclusion, our data suggest that different durations of AE induce distinct plastic responses among distinct cortical and subcortical circuits.

1. Introduction

Motor skill learning is essential for environmental adaptations during life. Right from birth to old age, we continue to learn new and different motor skills that enable us to function optimally in all aspects of life. Exercise practice is critical to motor skill acquisition and brings numerous benefits to the central nervous system (CNS) of humans [1,2] and animals [3–5]. Exercise accelerates cellular and molecular cascades [6], induces the expression of genes associated with plasticity [7], promotes neurogenesis [8], changes glutamate receptors and their subunits [9], and increases vascularization and

Abbreviations: CNS, central nervous system; AE, acrobatic exercise; SED, sedentary rats; SYS, synapsin I; SYP, synaptophysin; MAP-2, microtubule associated protein-2; NFs, neurofilaments; IEGs, immediaty early genes; Egr-1, early growth response gene 1; LTP, long term potentiation.

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http://dx.doi.org/10.1016/j.bbr.2016.04.029
0166-4328/

brain metabolism [8]. As a result, these changes promote improvements in learning, memory and plasticity of the CNS [6,10,11].

Several studies have shown that certain forms of plasticity induced by exercise depend on neuronal protein synthesis [5,12], such as synaptic vesicle proteins like synapsin I (SYS) [5,13,14] and synaptophysin (SYP) that are involved in vesicle formation, efficiency of neurotransmitter release and are important for the maintenance of plastic changes in CNS [4,10,15]. In addition, some structural proteins such as microtubule-associated protein-2 (MAP2) and neurofilaments (NF) have also shown plastic effects and can also be changed with acrobatic or treadmill exercise and/or enriched environments [16,17]. These findings suggest that motor skill learning may reorganize the synaptic and dendritic structures in the motor areas leading to persistent alterations in neuronal activity. Current hypothesis suggests that protein synthesis is essential for synaptic plasticity and for memory consolidation [18]. The identification of synthesized proteins for synaptic plasticity has revealed that immediate-early genes (IEGs) such as activity-regulated cytoskeleton-associated protein (Arc, also known as activity-regulated gene 3.1, Arc3.1) and early growth response gene 1 (Egr-1, also called zinc finger binding protein clone 268, Zif268) contribute to structural and functional modifications of synapses [19,20].

Studies using Egr-1 knockout mice have shown that Egr-1 is required for the expression of late-phase hippocampal LTP and memory consolidation [21]. In addition, Egr-1 increases in the sensorymotor cortex, hippocampus, and striatum of animals exposed to an enriched environment [22]. Therefore, the induction of Arc and Egr-1 after behavioral and electrical stimulation provides a useful way to detect neural networks undergoing neuronal activity through cellular imaging of those two markers [23–25]. It is known that different types of exercise promote different responses in brain regions [17,26,27]. In addition to the type of activity, some studies have focused the CNS and behavior changes of animals in relation to short and long periods of training [10,17,28–31]. Treadmill exercise revelead an increase of SYP after 7 days, SYS expression after 3 and 7 days, and NF after 3 days of training in the striatum. In the cerebellum, increase of SYS was observed after 7 and 15 days and of NF after 3 days of training. On the other hand, the motor cortex showed decreased levels of expression of NF proteins after 3 days of training, followed by an increase after 15 days, and no change in the synaptic protein expression [32]. Thus, these results suggest that moderate treadmill exercise can promote distinct changes in structural and synaptic proteins in different brain regions of rats in different periods of training [32]. Another study using voluntary exercise showed increased SYS in the hippocampus after a short period of training (3 and 7 days) [14]. A short period of acrobatic exercise changed SYP protein expression in the motor cortex, however it did not change MAP2, regardless of the difficulty and duration of training [33]. Furthermore, exercise is also related to the changes of neurotrophic factors. Complex motor learning and moderate physical activity with little learning produce different effects on the pattern of expression of BDNF and its receptor, and may have implications for neural plasticity arising from such experience [31].

Thus, physical exercise induces plasticity in the CNS of animals and humans, and type, duration, and frequency at which it occurs are all able to modify those changes. However, the intrinsic plasticity mechanisms that the acrobatic exercise (AE) generates during short and long periods of training are still unclear. In this study we associated the expression of Egr-1 to the plastic mechanisms, as analyzed by the expression of structural and synaptic proteins in motor regions of the rat brain, at different times of AE.

2. Material and methods

2.1. Animals

Male Wistar rats, 2 months old, provided by the Animal Facility of the Institute of Biomedical Sciences of the University of São Paulo—USP, were kept in a room with constant temperature of 23 °C and light/dark cycle artificially controlled 12/12 h, with free access to food and water. The animals were randomly divided into 2 groups: acrobatic exercise (AE, n = 45) and control-sedentary (SED, n = 15). The AE group was divided into 3 groups with different training periods: 1 week (AE1, n = 15), 2 weeks (AE2, n = 15) and 4 weeks (AE4, n = 15). This study was conducted in accordance with the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of São Paulo (protocol 152/2010).

2.2. Acrobatic exercise protocol

Acrobatic training was conducted in the active period of the animal [34] and consisted in crossing a circuit composed of the following obstacles: see-saw, vertical barriers, parallel rope bridge, parallel beams of roundwood, bridges of roundwood and rope. The circuit is based on previous studies [17,35] and was located at a height of 1.50 m from the ground. The training was performed on alternate days, three times per week and 5 trials each day. The trial started in the small plataform before the see-saw and the obstacle sequence was cited above. The new trial started after the previous, without an inter-trial interval. Animals received small manual stimuli when necessary to continue to the next obstacle, and it was more frequent in the beginning of training. We registered the time spent by each animal to complete each trial on the circuit. After the end of the 5 trials of each animal in the circuit, all obstacles and platforms were cleaned with 70% ethanol [17].

2.3. Immunohistochemistry

The acrobatic exercise and control animals (n = 5 in each group) were intraperitoneally anesthetized with ketamine (100 mg/kg) and xylazine (25 mg/kg) and submitted to transcardiac perfusion with saline 0.9%, followed by a fixative solution consisting of 2% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB, pH 7.4). The animals were submitted to this procedure after one hour of the end of training. After perfusion, the brains were collected and stored in 2% paraformaldehyde for 4 h. After this period, the material was transferred to sucrose 30% in PB 0.1 M for cryoprotection and stored at 4 °C during at least 48 h. The brains were cut at a thickness of 30 μm on a sliding freezing microtome (Leica SM 2000R). The immunostaining for SYS, SYP, MAP2, NF and Egr-1 was previously described [17,32]. A 1:1000 dilution was used for the rabbit polyclonal anti-Egr-1 antiserum (Santa Cruz Biotechnology, Inc. CA, USA), rabbit polyclonal anti-SYS antiserum (Chemicon, Temecula, CA, EUA), rabbit polyclonal anti-SYP antiserum (DakoCytomation, Glostrup, Dinamarca), mouse monoclonal anti-MAP2 antiserum (Chemicon, Temecula, CA, EUA) and mouse monoclonal anti-PAN, NF antiserum (Zymed Laboratories, San Francisco, CA, EUA). The PAN antibody recognizes a homologous region in the three neurofilaments (NF68, NF160 and NF200), which makes it impossible to distinguish them with the technique of immunohistochemistry, but only when analyzed using immunoblotting.

The qualitative analysis of the material and the measurements by densitometry were performed using an optical microscope (E1000, Nikon) coupled to a digital camera and Nikon Imaging Software ACT-U.