Early Exercise Promotes Positive Hippocampal Plasticity and Improves Spatial Memory in the Adult Life of Rats

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ABSTRACT: There is a great deal of evidence showing the capacity of physical exercise to enhance cognitive function, reduce anxiety and depression, and protect the brain against neurodegenerative disorders. Although the effects of exercise are well documented in the mature brain, the influence of exercise in the developing brain has been poorly explored. Therefore, we investigated the morphological and functional hippocampal changes in adult rats submitted to daily treadmill exercise during the adolescent period. Male Wistar rats aged 21 postnatal days old (P21) were divided into two groups: exercise and control. Animals in the exercise group were submitted to daily exercise on the treadmill between P21 and P60. Running time and speed gradually increased over this period, reaching a maximum of 18 m/min for 60 min. After the aerobic exercise program (P60), histological and behavioral (water maze) analyses were performed. The results show that early-life exercise increased mossy fibers density and hippocampal expression of brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B, improved spatial learning and memory, and enhanced capacity to evoke spatial memories in later stages (when measured at P96). It is important to point out that while physical exercise induces hippocampal plasticity, degenerative effects could appear in undue conditions of physical or psychological stress. In this regard, we also showed that the exercise protocol used here did not induce inflammatory response and degenerating neurons in the hippocampal formation of developing rats. Our findings demonstrate that physical exercise during postnatal development results in positive changes for the hippocampal formation, both in structure and function.

KEY WORDS: exercise; brain; development; plasticity; hippocampus; memory

INTRODUCTION

Structural and functional changes occur in the brain throughout lifespan. The ability of brain to change its structure and function is a process known as plasticity. Brain plasticity allows the central nervous system to acquire new information and learn skills, to reorganize neuronal networks in response to environmental stimulation, and to recover from brain injuries. Basic mechanisms that are involved in plasticity include anatomical, neurochemical, and electrophysiological manifestations (e.g., neurogenesis, programmed cell death, neurotransmitter release and long-term potentiation (LTP)). In general, these changes are adaptive and beneficial, but can also trigger negative alterations for the brain in some situations (Trojan and Pokorny, 1999; Johnston, 2009).

Clinical and animal studies have frequently described the influence of physical exercise on the adult brain plasticity (Cotman and Berchtold, 2002; Vaynman and Gomez-Pinilla, 2005). Exercise improves cognitive functions (Kramer et al., 2006; Kashihara et al., 2009), reduces anxiety and depression (Martinsen, 2008), and protects the brain against neurodegenerative disorders (Goodwin et al., 2008; Rolland et al., 2008; Honea et al., 2009). In addition, studies aimed to understand the neurobiological bases of these benefits have demonstrated that exercise modifies neuronal activity (Vanderwolf, 1969; Czurko
et al., 1999; Van Praag et al., 1999), enhances neurotrophic factor expression (Neeeper et al., 1995; Gomez-Pinilla et al., 1997), formation of synapses (Dietrich et al., 2008), growth of blood vessels (Van der Borght et al., 2009), and cell proliferation (Van Praag et al., 1999). Many of these alterations have been observed in hippocampal formation, a brain region linked to learning, memory, and emotional processes (Sanders et al., 2003; Kesner et al., 2004) and highly susceptible to damage in neurodegenerative disease (Harry and D’Hellencourt, 2003). Although the effects of physical exercise in the mature brain are well documented, its influence in the developing brain has been little explored.

Brain development is a complex process and stimuli during this period could determine brain’s functional integrity in adulthood. Events (learning and experience) that occur during early postnatal development may modulate the functional maturation of the brain and result in the development of a more complex neural circuitry (Linkenhoker et al., 2005). In humans, many reports have shown that exercise induces significant cognitive improvement throughout brain development (Sibley and Etnier, 2003; Hillman et al., 2005). A meta-analysis conducted on 16 studies found a positive relationship between physical activity and learning and intelligence scores (i.e., perceptual skills, intelligence quotient, achievement, and math and verbal tests) in children from elementary to high school age (Sibley and Etnier, 2003). In addition, it has been observed that aerobic exercise in childhood might increase the resilience of the brain in later life. Indeed, a positive correlation between physical activity in the age range 15–25 yr and information processing speed in older men (62–85 yr) has been described (Dik et al., 2003).

Since brain plasticity decreases with age (Akopian and Walsh, 2006; Lynch et al., 2006), it is very important to assess whether exercise may alter plasticity during early life and to determine the basic mechanism of such effects. The aim of the present study was to examine the influence of an aerobic exercise program undertaken during postnatal brain development. For this purpose, we evaluated the hippocampal plasticity of adult rats submitted to daily treadmill exercise during the adolescent period.

**MATERIAL AND METHODS**

**Exercise Paradigm**

Male Wistar rats were used in this study. The colony room was maintained at 21°C ± 2°C with a 12-h light/dark schedule, and ad libitum food and water throughout the experiments. Rats were bred in our laboratory, and the date of birth was considered day 0. The pups were housed with their mother in individual cages until weaning at postnatal day 21 (P21). The rats were then divided into two groups: exercise (n = 27) and control (n = 27) groups. Animals in the exercise group were familiarized with the apparatus for three days by placing them on a treadmill (Columbus instruments) for 5 min/day at speed of 8 m/min at 0% degree incline. Electric shocks were used sparingly to motivate the rats to run. To provide a measure of trainability, we rated each animal’s treadmill performance on scale of 1–5 according to the following anchors [1, refused to run; 2, below average runner (sporadic, stop and go, wrong direction); 3, average runner; 4, above average runner (consistent runner occasionally fell back on the treadmill); 5, good runner (consistently stayed at the front of the treadmill)] (Dishman et al., 1988; Arida et al., 2007). Animals with a mean rating of 3 or higher were included in the exercise group. If any animal was excluded from the exercise group it would not form the control group. This procedure was used to exclude possible differences in stress levels between animals. Subsequently, selected animals were submitted to a physical exercise program during the adolescent period, as previously described by Gomes da Silva et al. (2010). In brief, animals in the exercise group were submitted to treadmill exercise from P21 to P60. Each training session started with a 5-min warm-up at 8–10 m/min. Running time and speed were gradually increased, reaching a maximum 18 m/min for 60 min. Animals in the control group were transferred to the experimental room and handled in the same way as animals in the exercise group (privation of water and food during treadmill exercise). All experimental protocols were approved by the ethics committee of the Universidade Federal de São Paulo (UNIFESP), and all efforts were made to minimize animal suffering in accordance with the proposals of International Ethical Guideline for Biomedical Research (CIOMS, 1985).

**Histological Methods**

**Tissue preparation**

At P60, 14 animals from both the exercise and control groups (seven from each group) were deeply anesthetized (Tionembutal, 50 mg/kg, i.p.) and perfused transcardially with solution of 0.01 M phosphate-buffered saline (PBS), followed by solution containing 4% formaldehyde in 0.1 M phosphate-buffered (PB), pH 7.4. Animals from the exercise group were killed 1 h after the last exercise session. After perfusion, the brains were removed immediately from the skull and postfixed in 4% paraformaldehyde in PB for 24 h. The brains were then cut coronally with a vibratome (Leica) in 50-μm thick slices and stored at −20°C in the biological tissue bank in our laboratory (for preservation of tissue). To inhibit the formation of ice crystals that damage the structure of cells, the slices were maintained in an antifreeze solution containing 30% of sucrose, 1% of polyvinylpyrrolidone 40 (PVP-40), and 30% of ethylene glycol in PB (pH 7.2).

**Immunofluorescence**

Hippocampal slices (bregma −2.8/−3.3 mm; Paxinos and Watson, 1996), previously stored in the tissue bank, were selected to observe whether the protocol of physical exercise during development promotes inflammatory response in the hippocampal formation of rats. For this, the slices were rinsed in PBS and preincubated for 20 min in PBS solution containing 0.01% of saponin and 1% of bovine albumin. After this
procedure, the slices were incubated for 48 h with the respective primary antibodies [interleukin 6 (IL6; 1:100; IBL), interleukin 10 (IL10; 1:100; R&D), and tumor necrosis factor alpha (TNFα; 1:100; IBL)] previously diluted in solution of 1% albumin and 0.01% of saponin in PBS. For each primary antibody, we used two slices of each animal from both groups (exercise and control). To validate the test, we also incubated in primary antibodies hippocampal slices from an animal with 540 days of life (18 months). These slices were previously stored in the tissue bank (from another project) and were used in this study as positive controls. Subsequently, all slices were rinsed in PBS containing 0.01% of saponin and 1% of albumin and incubated for 30 min with secondary antibodies (1:200) conjugated to AlexaFluor® 488 or 564 diluted in PBS. Finally, the slices were washed in PBS, mounted on slides, and coverslipped with Vectashield (Merk). Following this, regions of Ammon’s horn (subregions: CA1 and CA3) and dentate gyrus of the hippocampal formation from studied groups were analyzed in a confocal laser scanning system from BioRad 1024 UV attached to a Zeiss Axiovert 100 microscope using a 40×1.2 NA PlanApochromatic water immersion lens.

**Fluoro-Jade B**

A sequence of three hippocampal slices per animal (bregma −2.8/−3.3 mm; Paxinos and Watson, 1996), previously stored in the tissue bank, was selected to observe the immunohistochemical staining of Fluoro-Jade B (FJB), a derivative of fluorescein anionic tribasic that selectively labels degenerating neurons (Schmued and Hopkins, 2000). For this, slices from exercise and control groups were mounted on gelatin-coated slides and incubated in a sequence of solutions containing 1% of sodium hydroxide in 80% of ethanol for 5 min, 70% of ethanol for 2 min, distilled water for 2 min, and 0.06% of potassium permanganate for 10 min. After these procedures, the slides were rinsed in distilled water and transferred to a stock solution of 0.01% FJB (Chemicon) in 0.01% acetic acid for 20 min. As a positive control, we added to the immunohistochemical procedure a slice of the hippocampal region (from another project) of an animal injected with 350 mg/kg of pilocarpine (a potent cholinergic agonist that induces status epilepticus and leads to severe widespread cell loss in several brain areas). Then, hippocampal slices were rinsed in distilled water, mounted on slides, coverslipped, and analyzed by confocal microscopy, as described above.

**Immunohistochemistry**

Hippocampal slices from exercise and control groups (bregma −2.8/−3.6 mm; Paxinos and Watson, 1996), previously stored in the tissue bank, were selected to analyze the density of neuronal cells in the hippocampal formation. Three slices per animal were pretreated with 3% of H2O2 for 10 min to block endogenous peroxidase activity, rinsed in PBS, preincubated for 45 min in PBS containing 10% of normal serum and 0.2% of Triton X-100, and then incubated in primary antibody against the neuron-specific nuclear protein (NeuN; 1:1000; Chemicon) at 4°C overnight. Paired slices of each group were processed in the same vial to minimize the differences during the immunohistochemical procedure. Slices were then rinsed in PBS, incubated in biotinylated antirabbit IgG (1:200; Vector) in PBS for 2 h at room temperature, rinsed in PBS, incubated in avidin–biotin peroxidase complex (ABC; Vector) for 1 h, washed several times in PBS, and then revealed in a solution containing 0.075% of diaminobenzidine and 0.002% of H2O2. After this sequence of procedures, the slices were finally washed in PBS, mounted on gelatin-coated slides, dehydrated, and coverslipped with Entellan (Merk). Subsequently, regions of Ammon’s horn (subregions: CA1 and CA3) and dentate gyrus of the hippocampal formation of each animal were digitized with a bright-field microscope (Nikon Eclipse 6600) for quantitative analysis.

**Neo-Timm**

Neo-Timm is a modification of the traditional Timm’s method introduced to improve the specific staining of zinc, and thereby produce a more distinct visualization of the mossy fibers (axons of granule cells) (Babb et al., 1991). Animals from the exercise and control groups (five from each group) were deeply anesthetized (Pentobarbital, 75 mg/kg, i.p.) and perfused transcardially with Millonig’s buffer solution (MB) containing 16% of sodium phosphate monobasic, 0.02% of calcium chloride, and 4% of sodium hydroxide, followed by solution of 0.1% sodium sulfide in MB, solution of 3% glutaraldehyde in PB, and solution of 0.1% sodium sulfide in MB, pH 7.4. After perfusion, the brains were carefully removed from the skull and postfixed for 24 h in solution containing 3% of glutaraldehyde in PB. The brains were then cut coronally with a vibratome (Leica) in 50-μm thick slices and mounted on gelatin-coated slides for revelation in the darkroom. Slides were revealed in solution containing 50% of Araric gum, 11.7% of citrate buffer, 1% of hydroquinone, and 0.15% of silver nitrate at room temperature for 30 min. Hippocampal slices were then dehydrated, coverslipped with Canada balsam, and imaged with a brightfield microscope (Nikon Eclipse 6600) for quantitative analysis.

**Quantitative analysis**

To analyze the NeuN and Neo-Timm staining, we used the method described by Scorza et al. (2010). The hippocampal formation of each animal was scanned with a video camera (Sony) connected to a brightfield microscope (Nikon Eclipse E600) (Supporting Information 1A,D). The images were then processed in RGB format with three-color frequency bands (red, green, and blue), each ranging from 0 (highest luminosity) to 255 (lowest luminosity). Subsequently, the images were compressed to grayscale (Supporting Information 1B,E) to obtain the corresponding histograms (frequency band mean). Afterwards, to observe the transition between the background and the staining, the derived of histogram vector values was obtained by equation:

\[ \text{Histogram vector values} = \frac{\text{Histogram values}}{\text{Total histogram values}} \times 255 \]
A trend line using the histogram derived values was performed to identify the start of the luminous intensity zone of neuronal cells or mossy fibers. The significant pixels were then converted into binary matrix (black and white) (Supporting Information 1C,F) and quantified by the black pixels sum per area (i.e., density of neurons and mossy fibers). The quantification of pixels was carried out by software that allows matrix manipulations (Matlab) and in images with the same resolution. The data were plotted in percentage of neurons and mossy fibers (CTL group = 100%).

**Methods of Protein Immunodetection**

**Tissue preparation**

At P60, the hippocampal formation of animals from the exercise and control groups (five of each group) was removed immediately after decapitation and homogenized in 0.01 M Tris hydrochloride (pH 7.6) containing 5.8% of sodium chloride, 10% of glycerol, 1% of Nonidet P40 (NP-40), 0.4% of ethylenediamine tetraacetic acid (EDTA), and protease inhibitors. Animals from the exercise group were killed 1 h after the last exercise session. Samples were sonicated, and protein concentration was determined by Lowry’s Method (Lowry et al., 1951) and samples were stored at −80°C.

**Enzyme-linked immunosorbent assay (ELISA)**

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that has been shown to mediate the positive effects of exercise on synaptic plasticity and cognitive function (Vaynman et al., 2004). Hippocampal BDNF expression assays were performed using the ELISA kit E-max (Promega) according to manufacturer’s recommendations. Samples from exercise and control groups, previously stored at −80°C, were centrifuged for 5 min at 14,000 rpm at 4°C and the supernatant transferred to a 96-well plate (Corning Costar) coated with anti-BDNF (1:1,000) then incubated for 2 h at room temperature. After this period, the plate was washed with Tris-buffered saline Tween-20 (TBS-T) and incubated with the following antibodies: antihuman (1:500) for 2 h and conjugate anti-IgY HRP (1:200) for 1 h. After these procedures, color reaction with tetramethyl benzidine was quantified in a plate reader at 450 nm (Quick Elisa). Values were reported as relative expression of total hippocampal protein.

**Immunoblotting**

The immunoblotting was performed to determine the expression of BDNF receptors, tropomyosin-related kinase B (TrkB), and p75 neurotrophin (p75ntr), in the hippocampal formation of animals from the exercise and control groups. A standard curve was done to determine the linear range of the method. In this line, 40 μg of proteins of samples previously stored at −80°C were applied to Tricine/SDS/Polyacrylamide gel (7.5 cm × 5.0 cm; 10% separating gel; 2% stacking gel). The gels were blotted in 25 mM Tris, 192 mM glycine, 20% (by vol) methanol pH 8.3 onto 0.2-μm cellulose nitrate sheets (Millipore). The blots were incubated with the respective primary antibodies [TrkB (1:300; Santa Cruz Biotechnology) and p75ntr (1:300; Santa Cruz Biotechnology)] at 4°C overnight. Peroxidase-conjugated goat antimouse IgG (Vector) was used according to the manufacturer's instructions. The immunodetection was performed using chemiluminescence detection system (Millipore) with exposure to X-ray films (Hyperfilm, GE). The incubation with anti-βIII-tubulin immunoglobulins (1:12,000, Abcam) was performed as internal control. The molecular weights of proteins TrkB, p75ntr, and βIII-tubulin (145, 75, and 50 kDa, respectively) were determined by running a prestained protein ladder (Amersham Biosciences). The band densities on immunoblots were measured by densitometry. All values were reported as relative to the expression of βIII-tubulin.

**Behavioral Analysis**

To determine the learning and memory of animals from the exercise and control groups (ten from each group) the water maze test, similar to that described by Morris (1984), was used. The water maze consisted of a black circular pool (200 cm in diameter) conceptually divided in four equal imaginary quadrants (quadrants 1–4). The water temperature was maintained between 21°C and 25°C. A black circular platform (12 cm in diameter) was placed 1.5 cm under the water surface in the center of quadrant 3. On the walls of the experimental room were fixed objects (frames, pictures) to be used as reference points. Animals were placed in the water maze for 120 s to find the platform. Over five days (P61–P65), animals performed two trials per day (with a 10-min interval between trials). In each trial, animals began the test at different points (labeled N, S, E and W). A video-camera (Sony) fixed above the water maze recorded all the experiments. The time to reach the platform (latency), the swim path length, and the speed of each animal in the maze were analyzed by Ethovision program (Noldus).

A day after the end of the last session in the water maze (P66), the platform was removed for a 120-s probe trial. The time spent in each of four imaginary quadrants was recorded. The analysis of time spent in quadrant 3 was performed to ensure that the animal used the reference points of the experimental room to find the submerged platform (D’Hooge and De Deyn, 2001; Stafstrom, 2002). Thirty days after this test (P96), animals from the exercise and control groups were submitted to a “retest.” In the retest, the platform was replaced in the center of quadrant 3. From the starting point N, animals performed a single trial (120 s) to find it. The retest was used to assess the long-term memory of the animals studied.

**Statistical Analysis**

Statistical analysis was conducted by Student’s t-test or analysis of variance (ANOVA) for repeated measures (ANOVA).
Values were considered significant when $P < 0.05$. Data are presented as mean and standard error of the mean (±standard error of mean (SEM)).

RESULTS

Inflammatory Response and Degeneration of Hippocampal Neurons

To assess whether the physical exercise protocol during the adolescent period of rats could promote negative effects in the hippocampal formation, we used inflammatory and degenerating neuron markers. The pro- (IL6 and TNFα) and antiinflammatory (IL10) response in positive control was observed in regions of Ammon's horn and the hilus of the dentate gyrus (Fig. 1). The FJB histochemical staining in positive control was observed in neurons located in pyramidal cell layer of CA1 and CA3 and in the hilus of the dentate gyrus (Fig. 1). In control and exercise groups, no inflammatory response and degenerating neurons were found in the studied hippocampal regions (Fig. 1).

Staining Density of Neurons and Mossy Fibers in the Hippocampal Formation

The NeuN and Neo-Timm markers were used to analyze the staining density of neurons and mossy fibers in the hippocampal formation of developing rats submitted to physical exercise. The NeuN immunoreactivity was observed within or in the vicinity of the pyramidal cell layer of CA1 and CA3 and in the granule-cell layer of the DG (Fig. 2). No significant differences in neuronal density were detected between the exercise (CA1 = 108% ± 8.7%, $t = 0.7124, df = 12, P = 0.4898$; CA3 = 104.6% ± 5.1%, $t = 0.6633, df = 12, P = 0.5197$; DG = 103.2% ± 5.2%, $t = 0.4069, df = 12, P = 0.6913$) and control groups (CA1 = 100% ± 7.1%; CA3 = 100% ± 4.7%; DG = 100% ± 5.9%) (Fig. 2). The Neo-Timm staining was observed throughout the stratum lucidum of CA3 and hilus of the dentate gyrus of both groups (Fig. 3). Quantitative analyses revealed that the density of mossy fibers by Neo-Timm staining was significantly higher in the hippocampal formation of the exercise group (119.6% ± 5.4%, $t = 3.123, df = 8, P = 0.0142$) when compared with the control group (100% ± 3.2%) (Fig. 3).

Hippocampal BDNF Expression and Its Receptors (TrkB and p75ntr)

BDNF has been shown to mediate positive effects of exercise on synaptic plasticity and cognitive function (Vaynman et al., 2004). Therefore, we investigated BDNF expression and its receptors in the hippocampal formation of rats submitted to physical exercise during development. A significant increase in hippocampal BDNF expression (129.3% ± 8.1%, $t = 3.557, df = 8, P = 0.0074$) and TrkB (160.8% ± 21.5%, $t = 2.464, df = 8, P = 0.039$) was noted in the exercise group when compared with the control group (BDNF = 100% ± 1.5%; TrkB = 100% ± 12.1%) (Figs. 4 and 5A). No significant difference in p75ntr expression was detected between the studied groups (exercise = 121.2% ± 44.5% vs. control = 100% ± 26.7%, $t = 0.4085, df = 8, P = 0.6936$) (Fig. 5B).

Learning and Memory

Learning and memory of animals from the exercise and control groups were analyzed in the water maze for five days. ANOVA showed that the latency ($F_{(1,72)} = 22.16; P = 0.0002$) and the swim path length ($F_{(1,72)} = 14.92; P = 0.0011$) in the exercise group were significantly lower than in the control group (Figs. 6A,B). These results demonstrate that developing rats submitted to exercise presented a better performance in the water maze when compared with the control rats.

To verify whether the results described above were influenced by the physical conditioning of exercised animals during the development, the speed of swimming in the water maze was examined. ANOVA showed no significant difference in mean speed of animals during five days of water maze between the studied groups ($F_{(1,72)} = 0.21; P = 0.6505$) (Fig. 6C).

At P66, the platform was removed from the water maze to evaluate the time spent in each of four imaginary quadrants. The results showed that both exercise (quadrant 1 = 26.2 ± 2.2 s; quadrant 2 = 17.6 ± 1.2 s; quadrant 3 = 54.3 ± 2.9 s; quadrant 4 = 21 ± 3.5 s) and control groups (quadrant 1 = 29.7 ± 1.4 s; quadrant 2 = 17.7 ± 2.1 s; quadrant 3 = 51.8 ± 4 s; quadrant 4 = 20.3 ± 1.7 s) presented a preference for the quadrant where the platform was previously located ($P < 0.05$) (Fig. 7). The preference for the platform quadrant indicates that animals: (a) used the reference points of the experimental room to find the submerged platform; (b) retained environmental information (spatial memory). This information was important to ensure that the next outcomes would not be influenced by previous problems of learning.

At P96, the platform was replaced in the water maze to analyze the long-term memory of the animals studied. The results showed that the latency in finding the platform was significantly lower in the exercise group (19.8 ± 4.3 s, $t = 3.048, df = 18, P = 0.0069$) than in the control group (52.7 ± 9.9 s) (Fig. 8), suggesting an enhancement of the long-term spatial memory in animals submitted to physical exercise during development.

DISCUSSION

The present study demonstrated that an aerobic exercise program undertaken during postnatal brain development increased staining density of mossy fibers and hippocampal expression of BDNF and its receptor TrkB, improved spatial learning and memory. It is important to point out that while physical

Hippocampus
exercise induces hippocampal plasticity, degenerative effects could appear in undue conditions of physical or psychological stress. Forced treadmill running is a type of training that could chronically activate different levels of stress response. Moreover, it has been shown that treadmill exercise can alter expression in the brain of inflammatory cytokines (Colbert et al., 2001;
Carmichael et al., 2005, 2010, Chennaoui et al., 2008) and low molecular weight proteins, known to affect the integrity of the blood–brain barrier and induce cell death during development (Hagberg and Mallard, 2005; Deverman and Patterson, 2009). In this regard, we showed that the exercise protocol used in this study did not induce inflammatory response or degenerating neurons in the hippocampal formation of developing rats.

**FIGURE 2.** NeuN immunoreactivity and neuronal percentage (%) in regions of CA1, CA3, and DG of rats from exercise group (EX) and control group (CTL). Scale bar = 100 µm.

**FIGURE 3.** Neo-Timm staining in regions of CA3 and hilus of the dentate gyrus and mossy fibers staining percentage (%) in rats from the exercise group (EX) and control group (CTL). An increase in density of mossy fibers was detected in the hippocampal formation of exercised rats during development (*P < 0.01). Scale bar = 150 µm.
Human and animal studies have demonstrated that exercise in infancy and adolescence can enhance brain health and plasticity (Sibley and Etnier, 2003; Hillman et al., 2005; Uysal et al., 2005; Gomes da Silva et al., 2010; Silva et al., 2010). It is important to note that neurogenesis induced by early-life exercise could have a significant impact on brain structure and functional development. New cell formation in the hippocampal formation is most prevalent in young rats, and an increased cell proliferation in the dentate gyrus has been observed in 4-week-old rats trained for five days compared with animals sub-
mitted to exercise at 8 and 62 weeks old (Kim et al., 2004). Thus, exercise-induced hippocampal neurogenesis has been suggested to enhance learning and memory capability (Snyder et al., 2005). In an elegant study, Uysal et al. (2005) reported that rats trained during development presented a significant increase in density of hippocampal cells using Nissl staining as well as a better spatial memory in the Morris water maze test in adulthood. In our study, treadmill exercise in developing rats improved spatial memory in the Morris water maze, but no difference was detected in density of hippocampal neurons. A possible explanation for these results could be related to staining technique performed. We used a specific immunohistochemical procedure for neuronal cells (NeuN staining), whereas Uysal et al. (2005) performed a technique that stains neuronal and glial cells (Nissl staining). The exercise protocol could also influence to these divergent findings. In the study by Uysal et al. (2005) animals ran over a period of 8 weeks at speed of 8 m/min, 30 min daily, 5 days per week. In our exercise protocol, animals ran over a period of 6 weeks at a greater intensity (up to 18 m/min over 60 min, 7 days per week). Although low (Uysal et al., 2005) and progressive intensity (our study) of physical exercise during postnatal development have been shown to induce positive changes in spatial memory of rats examined in adult life, changes in neuronal density is a subject that deserves more attention.

A variety of potential mechanisms could cause exercise-induced increases mossy fibers. Tong et al. (2001) examined the hippocampal expression of approximately 5,000 genes in rats submitted to 3 weeks of physical exercise and reported changes in a large number of gene transcripts, many of which are known to be associated with neuronal activity, synaptic structure, and neuronal plasticity. Growth-related genes like neurotrophins have been considered the most likely candidates in mediating the effects of exercise on plasticity (Cotman and Berchtold, 2002; Vaynman and Gomez-Pinilla, 2005). Previous studies have demonstrated that a few days of exercise result in a significant upregulation of several neurotrophins, including nerve growth factor (NGF) (Neeper et al., 1996), fibroblast growth factor 2 (FGF-2) (Gomez-Pinilla et al., 1997), and BDNF (Neeper et al., 1995, 1996; Russo-Neustadt et al., 1999). In particular, exercise-induced upregulation of BDNF appears to be more robust and long lasting compared with the other neurotrophins. Furthermore, BDNF has been shown to play an important role in mossy fiber outgrowth (Rabacchi et al., 1999). For instance, application of BDNF to cultured rat dentate granule cell explants resulted in marked increases in axon number and extension (Lowenstein and Arsenault, 1996). In addition, BDNF knockout in mice significantly reduced hippocampal mossy fiber density in our study might contribute, at least in part, to better cognitive performance observed in exercised rats during development.

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Hippocampus for people, particularly children and teenagers. Moreover, these public policies aimed at stimulating physical exercise programs changes for the hippocampal formation, both structure and neuroplastic processes. These stimuli occurring during early postnatal brain development significantly increased hippocampal expression of BDNF and its receptor TrkB. The importance of exercise-induced increases in BDNF and TrkB expression indicate its potential role in modulation of synaptic plasticity and cognitive function (Vaynman et al., 2004). For instance, in Morris water maze tests, rats submitted to 1 week of voluntary exercise are significantly better at locating the hidden platform than control rats. Significantly, this benefit was eliminated when BDNF-TrkB signaling was inhibited. In their investigation, animals with free access to running wheels but injected with a TrkB-IgG chimera to block the action of BDNF through the TrkB receptor showed no improvement in cognitive performance over control animals (Vaynman et al., 2004). Furthermore, the TrkB-IgG chimera eliminated exercise-driven increases in cAMP response-element-binding protein (CREB) (Vaynman et al., 2004), a molecule that plays a critical role in the formation of long-term memory (Abel and Kandel, 1998).

In our study, no significant difference in hippocampal p75ntr expression was detected in exercised rats during development when compared with unexercised rats. Although p75ntr expression decreases dramatically by adulthood, it is widely expressed during the developmental stages (Chao, 2003). Interestingly, the activation of the p75ntr receptor facilitates apoptosis during development and after injury in the central nervous system (Chen et al., 2009). However, the activation of p75ntr by BDNF induces cell death only in the absence of TrkB signaling or when this is decreased (Davey and Davies, 1998).

Previous investigations have shown that experience and learning can modulate the functional maturation of the brain by neuroplastic processes. These stimuli occurring during early postnatal brain development may result in the development of more complex neural circuitry (Linkenhoker et al., 2005). In the present study, we demonstrated that an aerobic exercise program during the adolescent period promotes hippocampal plasticity and improves spatial memory in the adult life of rats. Another important finding in our investigation was that early-life exercise enhanced ability to evoke the spatial memories in later life (when measured at P96), supporting previous findings in humans, which show a correlation between physical activity in childhood and cognitive benefits throughout life (Dik et al., 2003). Based on these observations, we can conclude that physical exercise during postnatal development results in positive changes for the hippocampal formation, both structure and function. This information is relevant for the development of public policies aimed at stimulating physical exercise programs for people, particularly children and teenagers. Moreover, these findings can also have a great therapeutic value for some neurological disorders that emerge during infancy and adolescence (Arida et al., 2010; Gorczynski and Faulkner, 2010).

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EXERCISE PROMOTES HIPPOCAMPAL PLASTICITY AND IMPROVES MEMORY


