Title

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AEROBIC EXERCISE IN ADOLESCENCE RESULTS IN AN INCREASE OF NEURONAL AND NON-NEURONAL CELLS AND IN mTOR OVEREXPRESSION IN THE CEREBRAL CORTEX OF RATS

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Abstract—Better cognitive performance and greater cortical and hippocampal volume have been observed in individuals who undertook aerobic exercise during childhood and adolescence. One possible explanation for these beneficial effects is that juvenile physical exercise enables better neural development and hence more cells and neuronal circuitries. It is probable that such effects occur through intracellular signaling proteins associated with cell growth, proliferation and survival. Based on this information, we evaluated the number of neuronal and non-neuronal cells using isotropic fractionation and the expression and activation of intracellular proteins (ERK, CREB, Akt, mTOR and p70S6K) in the cerebral cortex and hippocampal formation of the rats submitted to a physical exercise program on a treadmill during adolescence. Results showed that physical exercise increases the number of neuronal and non-neuronal cortical cells and hippocampal neuronal cells in adolescent rats. Moreover, mTOR overexpression was found in the cortical region of exercised adolescent rats. These findings indicate a significant cellular proliferative effect of aerobic exercise on the cerebral cortex in postnatal development.

Key words: physical exercise, adolescent, brain, cerebral cortex, hippocampus, mTOR.

INTRODUCTION

Brain development is a highly complex process that begins in the prenatal period and ends gradually in late adolescence and early adulthood (Winick and Noble, 1965; Dobbing and Sands, 1973; Lenroot and Giedd, 2006; Belsky and de Haan, 2011; Kolb et al., 2014; Houston et al., 2014). During the postnatal period of brain development, several morphological and functional changes occur in the neurons, synapses, and neural circuits (Spear, 2000; Zhang and Poo, 2001): new neurons are produced while others are eliminated (Spalding et al., 2013); some dendrites are branched while others are retracted (Wong and Ghosh, 2002); and new synapses are formed while others are removed (Cohen-Cory, 2002). This dynamic remodeling of the brain in postnatal development also happens for non-neuronal cells which form the axonal myelin sheath and the blood–brain barrier, and also provide neuroprotection (Privat, 1975; Bandeira et al., 2009; Bergles and Richardson, 2015; Bilimoria and Stevens, 2015). Thus, changes in cortical thickness have been observed throughout the childhood and adolescent period (e.g., gray matter reduction and white matter increment) (Gogtay et al., 2004; Moura et al., 2016). These changes have been directly linked to a complex architecture of glia, vasculature, and neurons with dendritic and synaptic processes (Sigaard et al., 2016). In view of these neuronal and non-neuronal changes, the developing brain may be more vulnerable at this time to environmental stimuli.

Human and animal studies have reported that environmental stimuli, such as physical exercise, can produce significant effects on the brain during postnatal development (Sibley and Etnier, 2003; Gomez-Pinilla and Hillman, 2013; de Almeida et al., 2013; Gomes da Silva and Arida, 2015). Exercise during childhood and adolescence is able to improve reading comprehension (Hillman et al., 2009), elevate language processing (Scudder et al., 2014), enhance attention and results in tasks (Buck et al., 2008), improve arithmetic problem-

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Abbreviations: Akt, protein kinase B; CREB, cAMP response element-binding protein; DAPI, 4′-6-diamidino-2-phenylindole dihydrochloride; ERK, extracellular signal-regulated protein kinase; mTOR, mammalian target of rapamycin; p70S6K, p70 ribosomal protein S6 kinase.

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solving skills (Moore et al., 2014), increase learning and memory (Herting and Nagel, 2012), enhance intelligence scores (Aberg et al., 2009), and improve academic achievement (Bunketorp Käll et al., 2015; Donnelly et al., 2016). Studies aimed at understanding the neurological bases of these benefits have demonstrated that juvenile exercise increase cortical activity (Li et al., 2014; Krafft et al., 2014), modifies neuronal activity (Moore et al., 2014; Scudder et al., 2014), improves the efficiency of networks underlying cognitive control (Voss et al., 2011), enhances white matter integrity (Chaddock-Heyman et al., 2014; Schaeffer et al., 2014), and presents a positive relationship to the microstructure of gray matter (Chaddock-Heyman et al., 2014). These alterations have been detected in the cortical (e.g. frontal, temporal, parietal and occipital cortex) and subcortical (e.g. hippocampal formation) regions of children and adolescents who undertook aerobic exercise.

One possible explanation for these beneficial effects is that exercise enables better neural development and hence results in more neuronal and non-neuronal cells. Indeed, higher neurogenesis has been found in the hippocampal formation of physically active rats (Kim et al., 2004; Lou et al., 2008; de Almeida et al., 2013;). For instance, adolescent rats submitted to one week of treadmill exercise presented a significant increase of BrdU/NeuN-double-labeled cells in the hippocampal region of the dentate gyrus (Lou et al., 2008). Moreover, moderate exercise improves spatial memory in mice (So et al., 2017) and rats (Inoue et al., 2015). The importance of this finding is that new neuron formation induced by juvenile exercise may have a significant impact on the cytoarchitecture of the brain in postnatal development.

Although the cell effects of early exercise described above are widely documented for the hippocampal formation, their influence on the cerebral cortex has so far been poorly explored. Some studies have reported neurogenesis in the neocortex of adult mice (Magavi et al., 2000) and primates (Gould et al., 1999). In view of these data, we evaluated the absolute number of neuronal and non-neuronal cells in the cerebral cortex and hippocampal formation of rats submitted to an aerobic exercise program during the adolescent period (P21-P60). In addition, taking into account the promising influence of exercise on intracellular signaling proteins associated with cell growth, proliferation and survival (Molteni et al., 2002; Fang et al., 2013; Bechara et al., 2014; Lin et al., 2015), we also analyzed the cortical and hippocampal expression and activation of the following proteins: extracellular signal-regulated protein kinase (ERK), cAMP response element-binding protein (CREB), kinase B (Akt), mammalian target of rapamycin (mTOR), and p70 ribosomal protein S6 kinase (p70S6K).

The signaling pathways of MAPK/ERK and CREB have been shown to be involved with cell proliferation and synaptic plasticity (Minichiello, 2009; Sun et al., 2015). These findings are interesting, since the CREB pathway is necessary for the formation of several types of memory (Silva et al., 1998). Akt is a serine/threonine kinase that plays a critical role in modulating cell growth and survival (Muslin and DeBosch, 2006). It is important to emphasize that Akt is expressed throughout the body, but its expression in the nervous system increases dramatically during stress or cellular injury, showing its wide role in the nervous system also involving protection, maintenance of cellular integrity and survival (Chong et al., 2005). The intracellular activation of mTOR is involved in several physiological functions including cell survival, proliferation and differentiation through the regulation of protein synthesis in different cell types (Lee, 2015). In animals, five days of forced exercise promoted a 70% increase in BDNF levels in the brain. This fact is associated with increased activation of BDNF receptors and consequently increased mTORC1 signaling in the hippocampus (Fang et al., 2013). When activated, mTOR phosphorylates p70S6K promoting the initiation of translation of synaptic protein synthesis (Hoeffer and Klann, 2010). P70S6K provides neuroprotection by combating apoptosis (Chong et al., 2012; Shang et al., 2012).

In this way, our hypothesis was that the cortical and hippocampal cell effects induced by juvenile exercise could be accompanied by alterations in the expression or activation of proteins linked to cell growth, proliferation and survival (ERK, CREB, Akt, mTOR and p70S6K).

EXPERIMENTAL PROCEDURES

Exercise paradigm

Twenty-four male Wistar rats at postnatal day 21 (P21) were used in present study. The colony room was maintained at 21 ± 2 °C with a 12-h light/dark schedule, and *ad libitum* food and water throughout the experiments. To provide a measure of trainability, animals were familiarized with the equipment for three days (P21-P23) by placing them in Columbus Instruments treadmill for 5 min/day at speed of 8 m/min. The electric shocks (2 mA) were used sparingly to motivate the rats to run. Then, we rated each animal's treadmill performance on a scale of 1–5 [1 – refused to run; 2 – below average runner (sporadic, stop and go, wrong direction); 3 – average runner; 4 – above average runner (consistent runner that occasionally fell back on the treadmill); 5 – good runner (consistent runner that stayed at the front of the treadmill during the whole session)] (Dishman et al., 1988). Rats with a mean rating of 1 or 2 would have been excluded from the present study in order to avoid different stress levels among animals (Arida et al., 2011). However, as observed in previous studies (Gomes da Silva et al., 2011; Gomes da Silva et al., 2012) young animals submitted to physical training were good runners, and none of them had to be excluded. After this, the rats were randomly distributed into two groups: control (n = 12) and exercise (n = 12). Rats from the exercise group were submitted to forced aerobic exercise program on a treadmill during the adolescent period (for review of adolescent period in rats: Spear, 2000). This type of exercise was chosen because sustained aerobic exercise has been efficient in the neurogenic response (Arida et al., 2011; Nokia et al., 2016). The exercise session started with a 3-min warm-up at 8 m/min. From P21 to P60, running time and speed were

gradually increased, reaching a maximum of 18 m/min for 60 min, as described by Gomes da Silva et al., 2010. Rats from the control group were transferred to the experimental room and kept on a stopped treadmill under the same conditions as the exercise group (same amount of time and same circadian periods). All experimental protocols were approved by the Ethics Committee of the Hospital Israelita Albert Einstein (SGPP protocol # 1920-13) and all efforts were made to minimize animal suffering in accordance with the proposals of the International Ethical Guidelines for Biomedical Research (CIOMS, 1985).

Cell counting

The total number of neuronal and non-neuronal cells in the cerebral cortex and hippocampal formation was investigated using the isotropic fractionator method (Herculano-Houzel and Lent, 2005). The isotropic fractionator method is a simple, quick and inexpensive technique that estimates the total number of cells in a given tissue. For your application, it is not necessary to use any specific software, but the same principles employed in the stereological analysis (Herculano-Houzel et al., 2015). This method replicates approximately all the outcomes produced by the unbiased stereology technique, which makes it valid for determining the absolute number of neuronal and non-neuronal cells (Bahney and von Bartheld, 2014), both in healthy and injured brains (Repetto et al., 2016). For this, the brains of both groups (n = 6 in each group) were removed immediately after decapitation and placed in a solution containing 4% formaldehyde in 0.1 M phosphate-buffered saline (pH 7.40) for one week. Subsequently, the cerebral cortex (all cortical tissue) and hippocampal formation (Ammon's horn and dentate gyrus) were dissected by means of consistent anatomical landmarks. The cerebral cortex comprised all regions dorsolateral to the olfactory tract, excluding the hippocampal formation, and was dissected from each hemisphere by peeling it away from the striatum and other subcortical structures (adapted from Herculano-Houzel and Lent, 2005). The tissues were chemomechanically dissociated in a saline solution (0.1% Triton X-100) and kept by agitation to achieve an isotropic suspension of isolated nuclei. The total number of cells was estimated by determining the number of nuclei stained with the fluorescent DNA marker (DAPI), using a hemocytometer for quantification. Then, the samples were incubated with a primary antibody against the neuron-specific nuclear protein (NeuN; 1:200; Chemicon) at 4 °C overnight, and subsequently, the samples were incubated with a secondary antibody (1:300 conjugated to AlexaFluor® 555) for 2 h. The neuronal cells in each sample was estimated by counting NeuN-labeled nuclei in at least 500 DAPI-stained nuclei and the number of non-neuronal nuclei was obtained by subtraction (Gomes da Silva et al., 2016).

Intracellular proteins analysis

We used MAGPIX® technology to evaluate the cortical and hippocampal expression and activation of the following intracellular proteins: Akt, mTOR, p70S6K, ERK and CREB. MAGPIX® technology involves a unique process that blends latex microspheres with two fluorophores. By using precise proportions of two fluorophores, 100 different sets of microspheres can be created, each with a signature based on a "color code" that can be identified by the MAGPIX® instrument. Milliplex kits with the proteins of interest are developed with these microspheres and with capture antibodies specific for each analyte. MAGPIX® detects magnetic analytes using an LED / CCD camera. From this signal, the reading is performed on the Luminex 200® equipment with xPONENT® software version 4.2. For this, the cortices and hippocampus of rats from the exercise (n = 6) and control (n = 6) groups were 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 and commercial kits of protease (Cat# M222-1 ml; Amresco) and phosphatase (Cat# B15001-A and B; Biotool) inhibitors. Animals from the exercise group were killed 1 h after the last exercise session. Animals from the control group were killed 1 h after the last stay on the treadmill (in the same period of exercised animals). Samples were sonicated and stored at -80 °C. Intracellular signaling kits were then used: Akt total and phosphorylated (Cat# 48-618MAG; EMD Millipore), ERK total and phosphorylated (Cat# 48-619MAG; EMD Millipore), mTOR total (Cat# 46-685AMAG; EMD Millipore) and phosphorylated (Ser2448) (Cat# 46-686AMAG; EMD Millipore), p70S6K total (Cat# 46-630MAG; EMD Millipore) and phosphorylated (Thr412) (Cat# 46-629AMAG; EMD Millipore), and CREB total (Cat# 46-632MAG; EMD Millipore) and phosphorylated (Ser133) (Cat# 46-631AMAG; EMD Millipore), according to the manufacturer's specifications. In brief, 200 μl of assay buffer was added into each well of a 96-well plate, sealed and mixed for 10 min at room temperature (20–25 °C). After removal of the assay buffer, 25 μL of standard or control solution was added into the appropriate wells. Thereafter, 25 μL of assay buffer, samples (12,5 μg of protein/well) and a solution containing premixed beads were added to each well and incubated overnight. On the following day, the plate was washed twice and incubated with 25 μL of detection antibodies for 1 h at room temperature. Subsequently, 25 μL of streptavidin–phycoerythrin was added into each well containing the detection antibody and incubated for a further 30 min. The plate was then washed twice and incubated with 125 μL of drive fluid solution for 5 min. Finally, the plate was placed in the MAGIPIX® equipment in order for the reading of microspheres by Luminex technology.

Statistical analysis

Statistical analysis between the control and exercise groups was conducted by Mann–Whitney non-parametric test. The extreme value test (Grubbs) was used to remove outlier values, particularly protein expression and activation data. All values were

considered significant when $p < 0.05$. Data are presented as mean and standard error of the mean (±SEM).

RESULTS

Total number of neuronal and non-neuronal cells

The absolute cell composition of neuronal and non-neuronal cells was investigated in the cerebral cortex and hippocampal formation of the animals from the exercise and control groups. A significant increase in the number of neuronal and non-neuronal ($U = 0.0$, $p = 0.006$ for both) cells was found in the cerebral cortex of rats from the exercise group when compared with rats from the control group (Fig. 1). In the hippocampal formation, a significant increase in the number of neuronal cells was found in exercise group animals compared with the control group animals ($U = 0.0$, $p = 0.004$) (Fig. 1). However, no difference was observed in the hippocampal number of non-neuronal cells between groups ($U = 11.0$, $p = 0.262$).

Expression and activation of intracellular signaling proteins

The expression and activation of intracellular signaling proteins (ERK, CREB, Akt, mTOR and p70S6K) were also investigated in the cerebral cortex and hippocampal formation of rats from the exercise and control groups. Considering the total amount of each protein, no significant difference between studied groups was observed in the cortical (Fig. 2A) and hippocampal (Fig. 2C) expression of ERK ($U = 12.0$, $p = 0.917$ and $U = 11.0$, $p = 0.465$; respectively), CREB ($U = 8.0$; $p = 0.347$ and $U = 18.0$; $p = 1.0$), Akt ($U = 17.0$; $p = 0.873$ and $U = 8.0$; $p = 0.347$) and p70S6K ($U = 13.0$; $p = 0.715$ for both brain structures). However, a significant increase in total mTOR expression was noted in the cerebral cortex of rats from the exercise group when compared with those from the control group ($U = 0.0$, $p = 0.006$) (Fig. 2A). No similar effect was found in the hippocampal formation for total mTOR ($U = 16.0$, $p = 0.749$) (Fig. 2C).

To determine the activation of signaling proteins, we calculated the phosphorylated/total ratio. In the cortex, it was not detected significant alteration in their activation between studied groups (p-ERK/t-ERK $U = 9.9$, $p = 0.465$; p-CREB/t-CREB $U = 8.0$, $p = 0.624$; p-Akt/t-Akt $U = 11.0$, $p = 0.465$; p-mTOR/t-mTOR $U = 14.0$, $p = 0.855$; p-p70S6K/t-p70S6K $U = 10.0$, $p = 0.361$) (Fig. 2B). In the hippocampal formation, as well, no significant was found in the activation of the studied proteins (p-ERK/t-ERK $U = 12.0$, $p = 0.917$; p-CREB/t-CREB $U = 12.0$, $p = 0.337$; p-Akt/Akt $U = 5.0$, $p = 0.117$; p-mTOR/mTOR $U = 11.0$, $p = 0.465$; p-p70S6K/p70S6K $U = 8.0$, $p = 0.347$) (Fig. 2D).

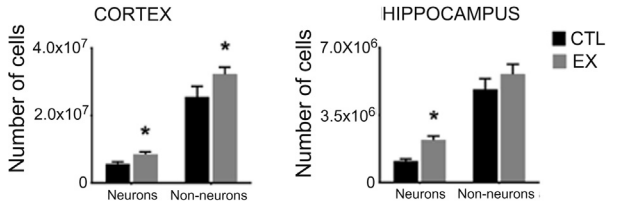

Fig. 1. Total number of neuronal and non-neuronal cells in the cerebral cortex and hippocampal formation of rats from control (CTL, $n = 6$) and exercise (EX, $n = 6$) groups. *Significant difference between groups (Mann-Whitney test; $p \leq 0.05$).

DISCUSSION

The present study investigated the hypothesis that early physical activity could increase the number of brain cells and alter the expression and activation of intracellular signaling proteins linked to cell growth, proliferation and survival. To do this, we evaluated the number of neuronal and non-neuronal cells and the expression and activation of ERK, CREB, Akt, mTOR and p70S6K in the cerebral cortex and hippocampal formation of rats submitted to an aerobic exercise program during the adolescent period (P21-P60). Results showed that physical exercise increases the number of cortical neuronal and non-neuronal cells and hippocampal neuronal cells in adolescent rats. Moreover, mTOR overexpression was detected in the cortex of exercised adolescent rats, although not in the hippocampal formation. These data indicate a significant cellular proliferative effect of aerobic exercise on the cerebral cortex in postnatal development.

As mentioned, it was found that aerobic exercise in adolescence results in more neuronal and non-neuronal cells in the cerebral cortex and more neuronal cells in the hippocampal formation of rats. These findings reinforce our initial hypothesis that exercise may be able to increase the number of brain cells. From these results we can suggest that these exercise-induced changes may be related to two phenomena that are characteristic of the brain in postnatal development: neurogenesis, i.e. the formation of new neurons from a precursor pool situated in specialized niches, and neuronal death, resulting in the elimination of a certain proportion of neurons from the mature population.

It is known that physical exercise during postnatal development of the brain increases hippocampal cell proliferation (Kim et al., 2004; de Almeida et al., 2013). An increase in the proliferation of new cells has been observed in the dentate gyrus of rats submitted to 5 days of aerobic exercise, when compared with control rats (Kim et al., 2004). Our data (increased number of neuronal cells) corroborate the findings of those studies showing an exercise-induced neuronal proliferative effect during postnatal hippocampal development (Kim et al., 2004; de Almeida et al., 2013). These results are promising, since this increase in the number of neuronal cells induced by early exercise can have a significant impact on the structure and function of the developing brain. It has been noted that physically active children, besides having greater hippocampal volume, also show better cognitive performance in mnemonic tests (Chaddock et al., 2010; Kobilo et al., 2011). This beneficial effect of exercise has also been found in pre-adolescents and adolescents (Herting and Nagel, 2012).

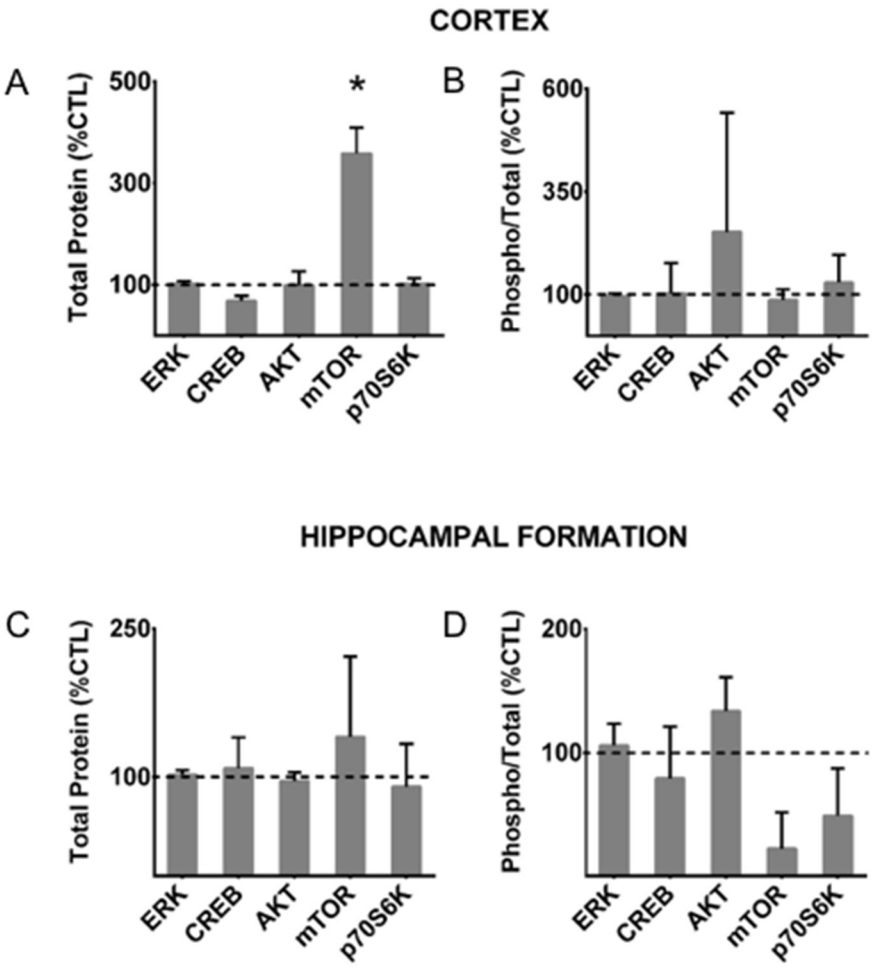

Fig. 2. Percentage (%) of total protein (A and C) and phosphorylated/total (activation) (B and D) in the cerebral cortex (A and B) and hippocampal formation (C and D) of rats from the exercise group (Cortex: ERK – CTL $n = 6$, EX $n = 6$; CREB – CTL $n = 5$, EX $n = 4$; AKT – CTL $n = 5$, EX $n = 6$; mTOR – CTL $n = 6$, EX $n = 5$; p70S6K – CTL $n = 5$, EX $n = 6$; Hippocampal formation: ERK – CTL $n = 5$, EX $n = 5$; CREB – CTL $n = 6$, EX $n = 6$; AKT – CTL $n = 5$, EX $n = 5$; mTOR – CTL $n = 6$, EX $n = 5$; p70S6K – CTL $n = 5$, EX $n = 5$). Data were normalized to the control group (100%). *Significant difference between groups (Mann-Whitney test; $p \leq 0.05$).

Although neurogenesis in the hippocampal formation is largely accepted, and studies have shown that chronic exercise increases the volume of the motor cortex (Kleim et al., 2002; Ding et al., 2006), new neuronal formation in the postnatal cerebral cortex is still very controversial. For example, Noctor et al. (2007) observed that cortical neurogenesis ends after the first postnatal week, whereas Bandeira et al. (2009), using the same cell count method used in our study (isotropic fractionator), found an exponential increase of neurons in the cerebral cortex of rats between P15 and P25. Moreover, the authors noted that the number of cortical neurons doubles from P15 to adulthood (P70) (Bandeira et al., 2009). Despite the controversy (Feliciano and Bordey, 2013), our findings suggest that physical exercise may have either stimulated the production of cortical neurons within existing proliferative niches in cortex (Sequerra et al., 2013), or reactivated neurogenesis from parenchymal glia (Péron and Berninger, 2015). Furthermore, the cortical proliferative effect may be associated with an increase in neuronal progenitor cells, since a significant increase in progenitors has been observed in the visual cortex of young rats with free access to a running wheel over 10 days (Ehninger and Kempermann, 2003).

In contrast, no changes were found in the number of non-neuronal cells in the hippocampus of the animals. This finding is different from those showing alterations in the hippocampal glial cells (Fahimi et al., 2016). However, a significant increase in the cortical number of non-neuronal cells in rats from the exercise group was found. It is conceivable that this result is related to the process of formation of new glial and endothelial cells (cortical gliogenesis and angiogenesis), since postnatal brain development has been characterized by a notable increase (90%) in these non-neuronal cells in the cerebral cortex (Bandeira et al., 2009). In support of this finding, physical exercise has also been seen to increase the number of microglia in the visual cortex of rats (Ehninger and Kempermann, 2003).

Another explanation for our results concerning neuronal cells may be related to exercise-induced changes during the neuron death period of development. It is known that cell death selectively removes unnecessary neurons or neuronal branches and excessive connections. This process occurs as neuronal complexity increases and the neurons begin to exhibit increased synaptic surface, thereby ensuring the formation of appropriate neuronal circuits (Low and Cheng, 2006). Using the isotropic fractionator method, Morterá and Herculano-Houzel (2012) noted a reduction in the total number of neurons in different brain areas of rats throughout late adolescence, adulthood and old age. These data indicate that neuronal elimination begins between adolescence and early adulthood. Based on this information, we reason that undertaking aerobic exercise during the adolescent period may slow down or even delay this downregulating cell number process. Maybe this effect induced by early exercise occurs by means of intracellular signaling proteins related to cell growth, proliferation and survival.

In our study, a mTOR overexpression was found in the cerebral cortex of rats from the exercise group when compared with rats from the control group. Our results accord, at least in part, with findings by Fang et al. (2013), showing that 5 days of treadmill exercise increases mTOR levels in rats without stress. The importance of this finding is that mTOR appears to play an important role in the developing brain, promoting cell

proliferation, differentiation and survival (Lee, 2015). Furthermore, mTOR dysregulation during brain development can cause serious neurological disorders (Lee, 2015). With regard to our findings, it is possible that the increased number of cortical cells found in exercised rats may be related to overexpression of mTOR.

In our study, no change in the expression and/or activation of ERK, CREB, Akt and p70S6K was detected in the cerebral cortex and hippocampal formation of adolescent rats submitted to aerobic exercise. These results differ from previous studies showing changes in these intracellular signaling proteins in response to aerobic exercise (Molteni et al., 2002; Chen and Russo-Neustadt, 2005; Bruel-Jungerman et al., 2009; Gomez-Pinilla et al., 2011; Zhao et al., 2011; Fang et al., 2013; Lin et al., 2015). For example, Gomez-Pinilla et al. (2011) observed increased levels of hippocampal pCREB in rats after 7 days of free access to running wheel. Chen and Russo-Neustadt (2005) and Bruel-Jungerman et al. (2009) reported hippocampal Akt activation in rats after both running wheel and treadmill exercise. Similarly, Fang et al. (2013) noted a significant increase of p70S6K expression in rats submitted to treadmill exercise over 5 days. These divergent data may have arisen because of the training protocol or the age at which the rats were investigated. Indeed, our training protocol consisted of 40 consecutive days of treadmill running, while other studies used 5–7 days of voluntary running wheel (Gomez-Pinilla et al., 2011; Fang et al., 2013.). In relation to age, we investigated rats during the adolescent period (from P21 to P60), while some studies used adult rats (Chen and Russo-Neustadt, 2005; Gomez-Pinilla et al., 2011; Fang et al., 2013). However, it is important to note that similar results to ours were found by Shen et al. (2001), showing no change in ERK and pERK/ERK expression between exercised and control rats, especially when brain tissue collection was performed between 0 and 3 days after the end of the training protocol (as in our study).

Even in view of the divergent data described above, we observed that juvenile aerobic exercise results in more neuronal and non-neuronal cells and in overexpression of at least one important signaling protein associated with cell proliferation (mTOR) in the brain during postnatal development, particularly in the cerebral cortex. Nevertheless, additional studies (e.g. BrDU labeling) are needed to better elucidate the mechanisms involved in the increase of cortical and hippocampal neuronal cells induced by physical exercise in the postnatal period.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest. All authors read and approved the final manuscript.

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