REVISTA NEUROCIÊNCIAS

Original

Effects of physical exercises on the neurogenesis of the brain of adolescent rat

Efeitos dos exercícios físicos sobre a neurogênese do cérebro de ratos adolescentes

Efeitos dos ejercicios físicos sobre la neurogênese do cérebro de ratos adolescentes

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Resumo
Introdução. A neurogênese do cérebro de ratos jovens pode ser afetada por diversos fatores em seu desenvolvimento, sendo a prática de exercícios físicos um fator importante na neurogênese do tecido nervoso. **Objetivo.** Investigar os efeitos de diferentes protocolos de exercícios físicos de média e alta intensidade na neurogênese do cérebro de ratos adolescentes. **Método.** O estudo experimental, onde foram utilizados três grupos de animais: um grupo controle (RC), um grupo que realizou exercício físico de média intensidade (R1) e um grupo que realizou exercício físico de alta intensidade (R2). Cada grupo com 8 animais, totalizando 24 animais. O programa de exercício físico progressivo resistido na escada vertical foi realizado durante 4 semanas, 5 dias por semana com uma duração variando entre 30 e 45 minutos. Após o término dos experimentos, os animais foram eutanasiados para retirada do encéfalo e posterior processamento, coloração pelo método de Nissl e análise histomorfométrica por contagem de neurônios no giro denteado do hipocampo e zona subventricular. **Resultados**. Os dados demonstraram que não houve diferença significante na área e altura do ápice do giro denteado do hipocampo entre os grupos (p=0,2474 e p=0,3337; respectivamente). Os resultados do comprimento do ápice do giro denteado do hipocampo demonstraram diferenças significantes (p=0,0172). **Conclusão**. A prática de exercícios físicos resistidos progressivos de média e alta intensidade não modificou a morfologia e densidade neuronal no giro denteado do hipocampo e zona subventricular de ratos jovens.
Unitermos. Exercício; Cérebro; Neurogênese

Abstract
Introduction. The neurogenesis of the brain of young rats can be affected by several factors in their development, with the practice of physical exercises being an important factor in the neurogenesis of nervous tissue. **Objective**. To investigate the effects of different physical exercise protocols of medium and high intensity on neurogenesis in the brain of adolescent rats. **Method**. The experimental study, where three groups of animals were used: a control group (RC), a group that performed medium-intensity physical exercise (R1) and a group that performed high-intensity physical exercise (R2). Each group with 8 animals, totaling 24

animals. The progressive resisted physical exercise program on the vertical ladder was carried out for 4 weeks, 5 days a week with a duration varying between 30 and 45 minutes. After the end of the experiments, the animals were euthanized for removal of the brain and subsequent processing, staining using the Nissl method and histomorphometric analysis by counting neurons in the dentate gyrus of the hippocampus and subventricular zone. **Results**. The data demonstrated that there was no significant difference in the area and height of the apex of the hippocampal dentate gyrus between the groups (p=0.2474 and p=0.3337; respectively). The results of the length of the apex of the hippocampal dentate gyrus showed significant differences (p=0.0172). **Conclusion**. The practice of medium and high intensity progressive resisted physical exercises did not modify the morphology and neuronal density in the hippocampal dentate gyrus and subventricular zone of young rats.
Keywords. Exercise; Brain; Neurogenesis.

Resumen

Introducción. La neurogénesis del cerebro de ratas jóvenes puede verse afectada por varios factores en su desarrollo, siendo la práctica de ejercicios físicos un factor importante en la neurogénesis del tejido nervioso. **Objetivo**. Investigar los efectos de diferentes protocolos de ejercicio físico de media y alta intensidad sobre la neurogénesis en el cerebro de ratas adolescentes. **Método**. El estudio experimental, donde se utilizaron tres grupos de animales: un grupo control (RC), un grupo que realizaba ejercicio físico de intensidad media (R1) y un grupo que realizaba ejercicio físico de alta intensidad (R2). Cada grupo con 8 animales, totalizando 24 animales. El programa de ejercicio físico resistido progresivo en escalera vertical se realizó durante 4 semanas, 5 días a la semana con una duración variable entre 30 y 45 minutos. Una vez finalizados los experimentos, los animales fueron sacrificados para la extracción del cerebro y posterior procesamiento, tinción mediante el método de Nissl y análisis histomorfométrico mediante conteo de neuronas en la circunvolución dentada del hipocampo y zona subventricular. **Resultados**. Los datos demostraron que no hubo diferencias significativas en el área y la altura del vértice de la circunvolución dentada del hipocampo entre los grupos (p=0,2474 y p=0,3337, respectivamente). Los resultados de la longitud del ápice de la circunvolución dentada del hipocampo mostraron diferencias significativas (p=0,0172). **Conclusión**. La práctica de ejercicios físicos resistidos progresivos de media y alta intensidad no modificó la morfología y la densidad neuronal en el giro dentado del hipocampo y la zona subventricular de ratas jóvenes.
Palabras clave. Ejercicio; Cerebro; Neurogénesis

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INTRODUCTION

The practice of physical activity in humans has been encouraged in the modern world as a strategy to prevent chronic non-communicable diseases such as diabetes, heart disease and cerebrovascular diseases[1,2]. In this sense, taking into account the proper care and clinical condition, the change in lifestyle, with the increase in the time the individual is active, should be encouraged by health professionals regardless of age group[3].

Specifically, such behavior should be even more motivated in the pediatric and hebiátric population, given that these stages of life comprise greater gains in human growth, development and maturation[4]. However, according to the same authors, such practice must occur consciously, taking into account the limits and possibilities of the type, duration and intensity of the proposed physical exercise.

The participation of children and adolescents in competitive activities has been increasingly precocious. An example of this trend, at the national level, can be found in the study[5] in which the authors observed an important precocity in the search for physical exercises aimed at the practice of futsal and handball in 14 sports federations in the country, Brazil. Such a scenario may contribute to the exposure of this population to high-intensity physical exercises, corroborating the need to understand the impacts of these practices.

For a long time, it was believed that the adult brain was incapable of performing neurogenesis, however, throughout history, evidence has emerged that neurogenesis occurs even in adulthood, mainly in the hippocampus and subventricular zone of the wall of the lateral ventricles[6].

This neurogenesis is regulated by several factors, such as age, inflammation, stress, diet, and the endocrine system. Physical activity also acts by inducing neurogenesis in young adult and elderly rats, because the body performs endocrine and neurohumoral metabolic adaptations, such as the angiogenesis of brain tissue, favoring the supply of nutrients, oxygen and neurotrophins[6].

In addition to the effect on the proliferation of neurons, physical exercise increases the density of dendritic spines and reduces oxidative stress, increasing neuron viability.

It is already established that exercise exercise is beneficial to the nervous system[6], however, works[7,8] showed hypogonadism in the trained group compared to the control group and also changes in the musculoskeletal system. This raises the question whether, during development, physical exercise in the high-intensity modality could have an adverse effect on other body systems, such as the nervous system.

The study aims to investigate the effects of different protocols of medium and high intensity physical exercises on the neurogenesis of adolescent rats, since this is a critical period of development.

METHOD

The work is a prospective experimental study suitable for the described objectives, in a controlled environment (laboratory). Three groups of animals were used: a control group and two intervention groups, namely: a control group (CR), a group that performed medium-intensity physical exercise (R1) and a group that performed high-intensity physical exercise (R2). Each group with 8 animals, totaling 24 animals.

Sample

Twenty-four Wistar rats (Rattus norvegicus), males, 30 days old and weighing approximately 100 grams. The entire project was submitted to the Ethics Committee Involving the Use of Animals (CEUA) of the Federal University of São João del -Rei. The puberty stage is considered the second stage of a rodent's life. The average age at which rats reach maturity is 50 days old Sengupta[9], so the age of 30 days for the beginning of the experiments was chosen (Table 1).

Table 1. Distribution of animals in the experimental groups.

Groups	**Treatment**	**Number of Animals**
RC	Sedentary/Control	N=8
R1	Medium Itnesity Physical Exercise	N=8
R2	High Intensity Physical Exercise	N=8

Procedure

Physical exercises

Physical exercise was performed for 4 weeks on the vertical ladder, following the modified protocol[10]. In the medium intensity physical exercise program (R1 group) the animals performed 8 climbing movements to reach the housing chamber. In the first week, 25% of the animal's body weight was used; in the second week, a load of 50% was used; in the third week 75%; in the fourth week 80%. The high-intensity physical exercise program (group R2) was also modified[10] and consisted of performing 8 climbs to reach the housing chamber. The animals were subjected to progressive loads where the first two climbs were carried out with a load of 50% of the animal's total body weight, in the third and fourth climbs 75% of body weight was used, in the fifth and sixth climbs 90% of the body weight was used. and in the last two climbs 100% of the animal's total weight.

In both protocols, the maximum heart rate (HRmax) of the animals was evaluated daily to confirm that the training is using 80 to 95% of the HRmax. of the animals. The interval between each ascent was of 60 seconds for the animal to rest in the housing chamber.

After the end of the physical training program, the animals were euthanized by means of in-depth anesthesia (100% isoflurane soaked in cotton). The brains of the animals were removed for processing, Nissl staining and histomorphometric analysis.

Nerve tissue histochemistry

The brains of the animals were removed after euthanasia, stored for 24 hours in paraformaldehyde, followed by 70%. Then, the brains were cut into 1mm slices for histological processing and 5UM sections in the microtome. Sections were histochemically stained using the Nissl method[11]. After staining, the stained slides were photographed for histomorphometric analysis.

For this, the image J program was used, where the following parameters were evaluated: neuronal density (cell count) at the apex of the dentate gyrus and subventricular zone of the lateral ventricles (Figure 1) and morphometric measurement of height, length (one) and area (a^2) from the apex of the dentate gyrus of the hippocampus (Figure 2).

Figure 1. Photomicrograph of the apex of the dentate gyrus of the hippocampus. In yellow, the area; in green, the height and in red, the length. 100x magnification.

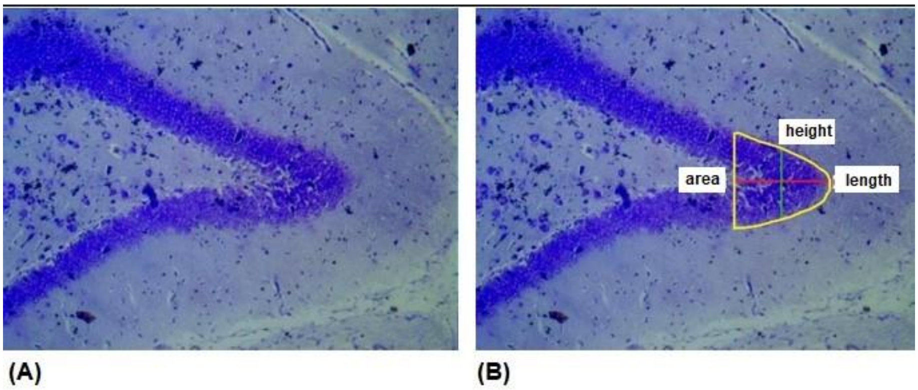
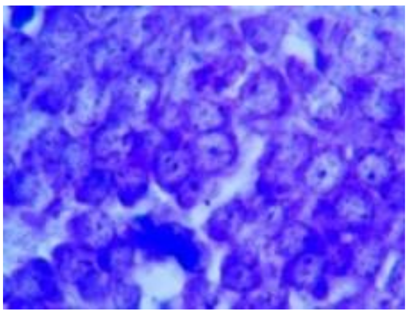


Figure 2. Photomicrograph of the apex of the dentate gyrus of the hippocampus for cell counting. 1000X magnification.



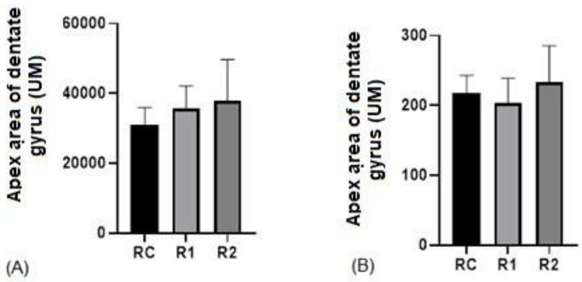
Statistical analysis

For the comparative analysis between the means of the groups, the analysis of variance test (ANOVA), One -Way and post Tukey test were used to compare the means between the three groups, considering the significance level of $p<0.05$. The results were shown as mean ± standard error of the mean (SEM).

RESULTS

The data showed that there was no significant difference in the area of the apex of the dentate gyrus of the hippocampus between the groups ($p=0.2474$), where the RC group had a mean of 30919±1780 UM, in the R1 group it was 35702±2254, and in the R2 group it was equal to 37899±4163 (Figure 3A).

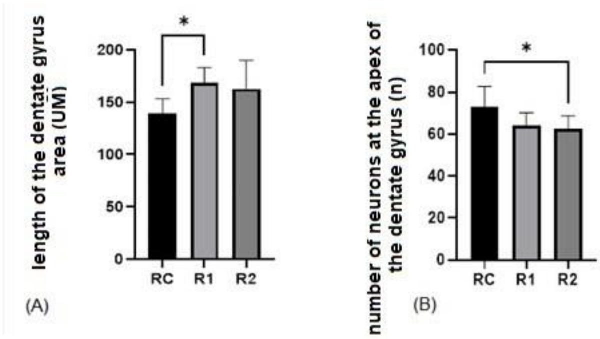
Figure 3. Graph of mean apex area and height of the apex of the dentate gyrus of the hippocampus. (A) Area of the apex of the dentate gyrus. (B) Height of the apex. of the dentate gyrus.



The results of the average height of the apex of the dentate gyrus of the hippocampus between the groups also did not show significant differences ($p=0.3337$). The mean value in the RC group was 217.8±8.979, in the R1 group it was 204.2±12.23, and in the R2 group it was 233.7±18.32 (Figure 3B).

The results on the length of the apex of the dentate gyrus of the hippocampus showed significant differences (p=0.0172). The RC group had a mean value equal to 139±5.09, R1 equal to 168.3±5.207, and R2 equal to 162.8±9.652 (Figure 4A).

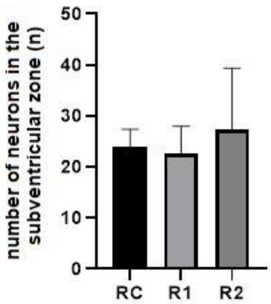
Figure 4. Mean length and number of neurons at the apex of the dentate gyrus of the hippocampus. (A) Length of apex of dentate gyrus. (B) Number of neurons at the apex of the dentate gyrus.



The density of neurons at the apex of the dentate gyrus of the hippocampus also showed significant differences between the RC and R2 groups (p=0.0218). The mean values for the number of neurons in the RC group were 73.15±3.385, in the R1 group it was 64.10±2.183, and in the R2 group it was 62.69±2.169 (Figure 4B).

The density of neurons in the subventricular zone of the thalamus did not show significant differences between groups (p=0.5366). The mean values for the number of neurons in the RC group were 23.86±1.335, in the R1 group it was 22.71±1.985, and in the R2 group it was 27.33±4.917 (Figure 5).

Figure 5. Number of neurons in the Subventricular Zone of the Thalamus.



DISCUSSION

The results demonstrate that resistance and progressive physical exercises of medium and high intensity do not generate major changes in the dentate gyrus and subventricular zone of the brain of young rats. The literature is scarce regarding the application of this type of exercise in the central nervous system.

Studies show that physical exercises are able to promote and modulate neurogenesis hippocampus, synaptic plasticity and learning, altering the morphology of neurons, especially in animals that performed aerobic exercises and had brain injuries[12-16]. Such effects result, in part, from the increase in vascularization and blood flow and, consequently, from the increase in the supply of oxygen, nutrients, glucose and neurotrophins[17,18].

The performance of neurotrophins, such as Brain Derived Neurotrophic Factor (BDNF), Vascular Endothelial Growth Factor (Vegf), Insulin-Like Growth Factor (Igf-1) and also Growth Hormone (GH), are expressed in aerobic protocols[18], but little is known about their production in resistance protocols.

BDNF is capable of increasing neuronal survival and neurogenesis, in addition to increasing resistance to injury[19-21]. While Vegf has angiogenic, neurogenic and neuroprotective functions, and promotes improvements in cognitive function, having its action triggered by tissue hypoxia. Studies demonstrate its relationship with neurogenesis as new cells in the dentate gyrus of the hippocampus are located closer to blood vessels. Studies have also shown that neurogenesis resulting from physical exercise in mice is dependent on Vegf[22-25].

The highest expression of neurotrophic factors such as BDNF and Vegf occurs after low and medium intensity protocols compared to strenuous exercise protocols[26]. Very high-intensity exercise releases more glucocorticoids and the hippocampus is quite sensitive to stress, despite stress even in lower-intensity protocols. However, exercises exert a protective effect against stress in the brain and hippocampus and generate lower expression of glucocorticoid receptors[27].

Progressive resistance exercise, however, does not seem to be able to promote the same effects due to its differences in intensity, duration and required structures. Shorter protocols that demand more from the musculoskeletal system, such as those used in the present work, may not be able to produce in sufficient quantities the substances that act on the central nervous system promoting neuroprotection, neuromodulation, neurogenesis and angiogenesis.

Therefore, research comparing strength protocols and aerobic protocols is necessary to elucidate the effects of each on the central nervous system.

CONCLUSION

Therefore, it is concluded that progressive resistance physical exercises of high and medium intensity are not capable of generating large and significant changes in the morphology and neuron count of the dentate gyrus of the hippocampus and subventricular zone in the brain of young rats.

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