Research article

Continuous and not continuous 2-week treadmill training enhances the performance in the passive avoidance test in ischemic gerbils


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ABSTRACT

This study aims to investigate the frequency and total duration effects of the 2-week treadmill training after experimental ischemic stroke in the passive avoidance test. We performed bilateral occlusion of common external carotid arteries, for five minutes, in Mongolian gerbils. The training groups were: continuous training for twelve consecutive days or not continuous training for six non-consecutive days. The groups remained in the treadmill for 15 min, with the speed set at 10 m/min, and the training started 24 h after the stroke. In the Shuttle Box, each animal had ten trials during the Learning Session (LS), which occurred 24 h before the stroke. The Retention Test (RT) occurred 24 h after the stroke and started on the second, third, seventh and twelfth day after LS. After the experiments, the brains were perfused, and coronal sections of the CA1 area of the hippocampus were cut and stained with hematoxylin and eosin. ANOVA on Ranks was used for Behavioral data analysis and morphological data by percentage. Ischemic training groups showed preservation in neuron density in the CA1 area of the hippocampus, when compared to the control groups. Animals subjected to continuous training, showed a higher latency in the RT when compared to ischemic animals in both weeks [(2nd, H = 39.81; P < 0.05), (3rd, H = 38.08; P < 0.05), (7th, H = 44.17; P < 0.05), and (12th, H = 39.55; P < 0.05)]. Animals in the not continuous training showed higher latency in the RT, in the second week only [(2nd, H = 39.81; P < 0.05), (3rd, H = 38.08; P < 0.05), (7th, H = 44.17; P < 0.05), and (12th, H = 39.55; P < 0.05)]. These findings suggest that improvement of memory after stroke after treadmill training is dependent on the frequency and total duration of training.

1. Introduction

Brain ischemia causes disability among adults, since it makes the total functional recovery always a challenge [1,2]. The obstruction of one or more blood vessels that irrigate the brain characterizes the ischemic strokes [3]. After ischemic stroke, motor, learning, memory and other deficits arise [4] and are due to neuronal death that occurs after necrosis and apoptosis of the areas submitted to ischemia [5].

Physical exercise is widely used in experimental and clinical studies since evidence suggests that it can prevent brain atrophy and improve cognitive performance in healthy subjects and subjects with degenerative diseases [6]. Physical exercise can prevent neuronal death and thus promote the recovery of lost functions [7]. The parameters such as frequency of exercise, intensity, duration, and specificity are a challenge for proposing a protocol of physical exercises to prevent damage or restore the brain function after ischemia [1,2]. In rats, forced treadmill exercise decreases the size of damaged areas compared to sedentary ischemic controls [8,9].

Acute treadmill protocols, with a duration of 5 consecutive days, using a speed of 10 meters/min, lasting 15 min beginning 24 h after ischemic lesion, preserves the motor behavior in ischemic Gerbils [10,11]. On the other hand, the earlier onset of exercise might increase the injured area in rats [12]. Among the parameters for the prescription of physical exercise after ischemia, the start time appears to be in agreement. Twenty four hours post-ischemic lesions interventions [13,14] are more effective than those performed after 12 h, 3, and 7 days [15,16] in Gerbils and rats.

The mechanisms by which physical activity changes the brain...
functions have not yet been completely elucidated [17]. Exercise might activate the cellular and molecular pathways that contribute to promoting neuroprotection. Several authors have shown that after ischemia, physical exercise decreases apoptotic neuronal death in diverse brain areas, such as the motor cortex and the striatum [18–20].

Another brain area susceptible to global ischemic injury is the hippocampus, especially the CA1 region [11]. In this area, the neurons show delayed neuronal death [21], thus learning and memory impairments last longer than the motor impairments, because death of neurons due to ischemia and recovery are slower in this area.

Animal models that evaluate the functional performance of the hippocampus of ischemic animals are suitable for testing chronic treatment for post-brain ischemia. The Passive Avoidance Test (PAT) by the Step-Through or Shuttle Box is a widely used model for memory evaluation in Gerbils and Wistar rats [22,23]. This model has been shown to detect learning and memory impairments in ischemic Gerbils [24].

The acute evaluation (5-day post-brain ischemia) of the Gerbils motor behavior in a recent study from our laboratory showed that a continuous treadmill exercise protocol maintains more neurons and improves behavioral performance when compared to a non continuous protocol [11].

To advance the understanding of how the frequency of physical exercise may interfere with the behavioral performance after brain ischemia, this study proposes to use the passive avoidance test to analyze two 2-week protocols consisting of continuous and not continuous exercise, and their repercussion in the neurons of the hippocampal CA1 area.

2. Materials and methods

2.1. Animals

We used a total of 159 male Mongolian gerbils (Meriones unguiculatus, Rodentia, Gerbillinae), weighing between 50 and 70 g. The animals were housed in the Laboratory of Neuropsicobiology and Motor Behavior of the Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP) in a temperature controlled room (23°C ± 1°C) with a light/dark cycle of 12/12 h. The animas were kept in polypropylene colony cages lined with sawdust. Experiments were performed in compliance with recommendations of the Committee on Ethics in Animal Experimentation of the FMRP-USP (Protocol number 068/2008), based on the National Council for Animal Experimentation Control (CONCEA) guidelines.

2.2. Surgery

Each animal was anesthetized intramuscularly with Zoletil (5 mg/kg). After anesthesia has been established, a trichotomy was performed followed by a ventral incision in the neck. The subcutaneous and muscular tissues were removed and the right and left common external carotid arteries were exposed and occluded for 5 min through a suture. During this period, the region was irrigated with sterile sodium chloride to prevent tissue dehydration. For the sham-operated groups (Sham and Sham Stimulated), we stopped the surgery before occlusion of the arteries. At the end of surgery, each animal received an intramuscular injection of 120,000 IU of penicillin G benzathine (Fontoura-Wyeth-Brazil), and the body temperature was maintained with a heat lamp until the gerbil regained consciousness [11].

2.3. Memory test

We used a Shuttle Box containing two compartments, one clear and the other dark (each measuring 16.5 × 16.0 × 14.7 cm), separated by an automatic door. The floor of the dark compartment is made of iron grids connected to a shock generator.

Each animal had ten trials during the Learning Session (LS), and the maximum determined latency time was 300 s. The animals start the test in the clear compartment and are allowed to move freely. After 5 s, the door that prevents the passage to the dark compartment opens. As soon as the animals move into the dark compartment, the door closes and they receive a shock to the legs for 3 s (0.4 mA). Immediately after the shock, the animals are relocated to the clear compartment for further exposure to the passive avoidance test. Every time the animals return to the dark compartment, they receive the shock again. The Retention Test (RT) started on the second, third, seventh and twelfth day after LS, and 24 h after the experimental stroke. In RT, each animal was relocated to the clear compartment, and the latency time of entrance into the dark compartment was measured [23].

2.4. Treadmill training

We used a motorized treadmill for rodents (Isight Ltda, Ribeirão Preto, Brazil). The training intensity was determined by the speed of the treadmill that was maintained at 10 m/min, with 0% grade of inclination, and no speed increase in all experiment. This speed is considered as a moderate forced exercise [1]. The total duration was set at fifteen minutes for each animal [10]. The continuous protocol was defined by daily stimulation of the animals, whereas the not continuous protocol was defined by one-day stimulation followed by another day without it [11]. The total duration of both protocols is twelve days after the brain ischemia and start 24 h after the experimental surgery.

2.5. Protocols and groups

The animals were distributed in the following experimental groups and protocols:

Home Cage Control Group (HC): naïve animals exposed to the RT on the second, third, seventh and twelfth days after LS. 
Control Group (C): naïve animals submitted to LS, and RT on the second, third, seventh and twelfth days after LS.
Continuous Stimulated Control Group (CSC): naïve animals, submitted to LS; RT on the second, third, seventh and twelfth days after LS, and motor stimulation in the second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth and eleventh days.
Not Continuous Stimulated Control Group (NCSC): naïve animals, submitted to LS, RT on the second, third, seventh and twelfth days after LS and; motor stimulation on the third, fifth, seventh, ninth, eleventh days.
Sham Group (S): animals submitted to the surgery without the production of brain ischemia 24 h after LS and, RT on the second, third, seventh and twelfth day after LS.
Continuous Stimulated Sham Group (CSS): animals submitted to the surgery without the production of brain ischemia 24 h after LS, RT on the second, third, seventh and twelfth day after LS, and motor stimulation in the second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth and eleventh days after sham surgery.
Not Continuous Stimulated Sham Group (NCSS): animals submitted to the surgery without the production of brain ischemia 24 h after LS, RT on the second, third, seventh and twelfth day after LS, and motor stimulation on the third, fifth, seventh, ninth, eleventh days after sham surgery.
Ischemic Group (I): animals submitted to the surgery for the production of brain ischemia 24 h after LS, and RT on the second, third, seventh and twelfth day after LS.
Continuous Stimulated Ischemic Group (CSI): animals submitted to the surgery for the production of brain ischemia 24 h after LS, RT on the second, third, seventh and twelfth day after LS, and motor stimulation in the second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth and eleventh days after surgery.
Not Continuous Stimulated Ischemic Group (NCSI): animals submitted to the surgery for the production of brain ischemia 24 h after LS,
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