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Influence of treadmill training on motor performance and organization of exploratory behavior in Meriones unguiculatus with unilateral ischemic stroke: histological correlates in hippocampal CA1 region and the neostriatum.

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Abstract

This study examined the effects of motor stimulation via treadmill on the behavior of male gerbils after external carotid ischemic brain lesion. The animals were assigned to five groups; Ischemic with no stimulation (SIG), Ischemic with stimulation (SIG 12/24/48/72 hrs after surgery), Non-Ischemic with no stimulation (CC), Non-Ischemic with stimulation (CE) and Sham, surgery without occlusion with no stimulation (SH). All the animals were tested in the Open-field (OF) and Rotarod (RR), four days after surgery in order to evaluate exploratory behaviors and motor performance. Data were submitted to one-way variance (ANOVA) and Dunnett’s post hoc comparisons. SIG and SIG 12 groups showed a significant decrease in motor response (crossing) when compared to the control group (CC) (F = 20.65, P <0.05) in the OF. SIG12 group showed an increase in grooming behavior (F = 23.136, P <0.05) and all ischemia groups (SIG, SIG12/24/48/72) spent less time on the RR (F = 10.40, P <0.05), when compared to the control group (CC). Histological analyses show extensive lesions in the hippocampus and neostriatum for all groups with ischemia (SIG, SIG12/24/48/72), which are structures involved in the organization of motor behavior. Interestingly, the most pronounced damage was found in animals submitted to motor stimulation 12 hours after ischemia which can be correlated to the increased number of grooming behavior showed by them in the OF. These findings suggest that motor stimulation through treadmill training improve motor behavior after ischemia, except when it starts 12 hours after surgery.

Keywords: Motor recovery; Treadmill training; Global Ischemia; Open-field test; Rotarod test.
Cerebral ischemia or stroke, one of the leading causes of death and long-term disability in aged populations, often results in irreversible brain damage and subsequent loss of neuronal function [1]. In cerebral ischemia, cerebral blood flow (CBF) is reduced and ischemic tissue damage occurs in various regions of the brain [2]. The model of cerebral ischemia after unilateral common carotid artery (CCA) occlusion in gerbils resembles carotid occlusion in humans. Few studies have shown developing signs of severe cerebral ischemia, which is considered to be evidence of neuronal damage, ≤5 minutes after unilateral CCA occlusion [3 e 4], while others show how, after 5 minutes of ischemia, lesions were present in many regions of the hippocampus, cerebral cortex and striatum [4 e 5].

It has been suggested that physical exercise may ameliorate neurological impairment by preventing neuronal loss following many brain insults [6]. In fact, a recent report showed that treadmill training overcomes ischemia neuronal death in gerbils [7]. On the other hand, few studies with laboratory animals have indicated that the excess use of the injured limb, induced by immobilization of the intact one, may increase the extent of the cerebral injury [8 e 9].

Another form of motor stimulation is the treadmill training used in many studies, however, there is a great variability in starting times, intensity and length of exposure to the apparatus [7,10,11,12,13].

This study analyzes the effect of motor stimulation using a treadmill on motor performance and exploratory behavior in Mongolian gerbils evaluated by the Open Field and Rotarod tests. Motor stimulation was done at different starting times after ischemia in order to investigate possible interactions.

Eighty male naive Mongolian gerbils (Meriones unguiculatus; Rodentia; Gerbilidae) weighing 60-80 g were used. The animals were housed in a colony room with food and water ad libitum. They were maintained under the controlled temperature (23 ± 1 °C) and lighting conditions (07:00–19:00 h) and tested during the light phase of the cycle.

The animals were assigned to the following groups (each group had 12 animals): SIG- animals submitted to experimental surgery but not to motor stimulation. SIG12 – Animals submitted to surgery and motor stimulation on the electric treadmill 12 hours after ischemia for three consecutive days, in a total of 4 sessions. SIG24 - Animals
submitted to surgery and motor stimulation in the electric treadmill 24 hours after ischemia for three consecutive days, in a total of 3 sessions. SIG48 - Animals submitted to surgery and motor stimulation in the electric treadmill 48 hours after ischemia for two consecutive days, in a total of 2 sessions. SIG72 - Animals submitted to surgery and motor stimulation in the electric treadmill 72 hours after ischemia for one day, one session.

Control group (CC) - naive animals i.e., animals not subjected to experimental surgery and not stimulated. Stimulated Control group (CE) - animals not subjected to experimental surgery but subjected to motor stimulation. Sham group (SH) - animals submitted to a ventral incision in the neck without occlusion of the carotid artery and not stimulated.

The animals assigned to groups SIG, SIG 12/24/48/72 were intramuscularly anesthetized with zoletil (5 mg/kg), receiving a ventral incision the neck. The subcutaneous and muscle tissues were pushed back and the left external common carotid artery was exposed and occluded, with suture, for a period of 10 minutes. During this procedure, the surgical field was irrigated with sterile sodium chloride in order to prevent tissue dehydration. After that, the suture thread was tied, leading to definitive occlusion of the artery as described elsewhere [14]. The rectal temperature was maintained at 37 ± 0.5 °C with a heating lamp until the gerbil regained consciousness. At the end of surgery, each animal received an intramuscular injection of 120,000 IU penicillin G benzathine (Fontoura-Wyeth-Brazil). The animals took an average of 50 minutes to recovery from surgery and only those that showed clinical signs of ischemia were used in the studied.

Motor stimulation consisted of 15-min sessions once a day and the exercise workload was set at a speed of 10 m/min, with 0% grade of inclination. This protocol was modified from a previous one used for gerbils [13]. One day before surgery all the animals were submitted to a 5-min session of habituation in the arena (Open Field test - OF), which consisted of an enclosed circular area of 60 cm in diameter, divided in twelve sections, elevated 50 cm off the floor. The experimental room was illuminated with a 40-W fluorescent lamp (350 lux at the arena floor level). Four days after surgery, the animals were individually placed in the middle of the OF arena and the 15-min
sessions were recorded. The following behavioral responses were analyzed: number of crossings (i.e. number of floor sections traversed) and number of groomings (cleaning the head and/or the body with the forelegs for more than 10 seconds).

Immediately after the OF test all the animals were placed in a rotarod apparatus. The rotarod (RR) has a cylindrical surface moving around its axis at an acceleration of 5.5 rpm. The animals have to balance on the moving cylinder, and the test is interrupted any time the animal falls off of it. The system provides the measurement of time of each animal’s permanence on the apparatus by means of a microprocessor circuit. The Rotarod is widely used to assess motor behavior in experiments with focal or global brain injuries in rats and mice [15, 16].

Histology - Right after the behavioral experiments all the animals were anesthetized with zoletil (10 mg/kg) and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate-buffered saline (pH 7.4). The brains were removed and fixed in the same fixative for 4 h, at 4ºC. After dehydration with graded concentration of alcohol, the brains were embedded in paraffin. Coronal sections (10 μm) of the motor cortex, striatum and hippocampus were made using a microtome (Leica) and stained with hematoxylin and eosin for light microscope examinations. Morphological analysis was performed by the ImageJ 1.37V software (National Institutes of Health, U.S.A)

The results are reported as mean ± S.E.M. Data were submitted to one-way variance (ANOVA). Dunnett’s post hoc comparisons were carried out if significant overall F values were obtained. Significance was set at p<0.05.

Histological analyses show damage in the hippocampus and striatum, for the following groups: SIG, SIG 12, SIG 24, SIG 48 and SIG 72. There was a 60% of reduction in number of CA1 pyramidal cells of the hippocampus and increased number of picnotic nuclei in the SIG 12 group when compared to SIG24 (Fig. 1), which is a particularly vulnerable region affected by the ischemic model. In the same way, the SIG 12 group presented an impressive neuronal cell loss and increased number of picnotic nuclei in the striatum when compared to the SIG24 group (Fig. 2).

Treadmill training induced a consistent locomotor effect in SIG24, SIG 48, SIG72 groups since there was no significant difference between these groups and the control group (CC) both for number of crossings (F7, 79 = 20.65) and grooming (F7, 79
The post hoc analyses revealed that SIG and SIG12 groups had a decrease in number of crossings, and SIG12 group had an increase in grooming behavior, as compared to the control group (CC) (Fig. 3). For the Rotarod test all ischemic groups showed a decrease in time spent on the moving cylinder, when compared to the control group CC \([F(7, 79) = 10.40; p < 0.05]\) (Fig. 4).

It has been well established that ischemia causes oxygen and glucose deprivation, resulting in tissue infarct and neuronal death [17]. The present work shows behavioral and morphological evidence that the unilateral encephalic ischemia causes extensive lesions in striatum and hippocampus, which are structures involved in the organization of motor behavior [7,13]. There is evidence that physical activity is related to greater expression and release of neurotrophic factors related to survival, differentiation and alteration of neuronal synapses [18,19]. In addition, it has been well documented that physical exercise exerts neuroprotective effects on the injured hippocampus of Mongolian gerbil [7,13]. Previous studies have used chronic stimulation of gerbils 24 hours after the establishment of brain ischemia. In the present work, we decided to test different starting times of motor stimulation after ischemia (12, 24, 48, 72 hours), using high velocity (10 m/min), shorter duration (15 minutes) and lower frequency (a maximum of 4 days) in order to obtain motor stimulation-induced responses during a critical post-ischemic period.

The present data obtained on the rotarod test illustrates a significant decrease in motor and balance ability in ischemic and non-trained animals which are in agreement with the literature [15, 16]. However, the trained animals also demonstrated a poor motor ability when compared to the control group, which can be related to the short stimulation time and to the high motor demand of the test. These behavioral modifications are widely justified by the process of ischemic injury presently used, affecting brain regions such as the hippocampus and striatum [20].

On the other hand, data obtained in the open-field test were different from those obtained in the rotarod test. According to previous reports, in the open-field test, the animals show spontaneous motor behaviors [22], in contrast with the rotarod test, in which the animals show forced motor behaviors induced by the imminence of falling [23]. The behavior data from the circular arena show that treadmill training improves the motor control for groups in which the motor stimulation started at 24, 48, 72 hours
after ischemia. Two behavior parameters in the circular arena were used for that evaluation: the number of crossings; parameter that represents the main motor response during the organization of the exploratory behavior, and the grooming response; which is related to a complex structure supraspinal.

Treadmill training was effective in increasing crossing responses in the ischemic animal group with stimulation. In line with these results are the previous studies suggesting that the treadmill training improves memory by suppressing ischemia-induced apoptosis [7]. The same pattern is seen in grooming behavior of all experimental groups, except when motor stimulation started 12h after encephalic ischemia (SIG12).

Starting motor stimulation 12 hours after brain ischemia induced a behavioral profile quite different from the other groups. The most significant effect induced by this starting time of treadmill training was a decrease in the number of crossings in the OF similar to the non-stimulated ischemic group (SIG). The increase in grooming behavior seen in the SIG12 groups is a very interesting finding. Grooming responses can be elicited by stressor stimuli [24] and by thalamic lesions [25]. A recent report showed evidence that this behavior appears to be dissociated from a purely locomotor activation, with various other components being involved [26]. The increased grooming motor response observed in this study may suggest a significant lesion of neuronal networks involving structures related to behavioral homeostasis in order to inhibit this stereotyped behavior [27]. Increased grooming has been detected in decerebrated rats [28] indicating an important role played by the integrity of these structures, at high levels of the neuroaxis, in the control of this type of motor behavior. Similarly, our histological data show an increased neuronal rarefaction in neostriatum and hippocampus for this experimental group (SIG12).

The present work shows that treadmill training is effective for the rearrangement of the motor behavior. However, starting this training too early may cause increase in the damage of the cortical extension and subtelencephalic structures, with serious risk of interfering with recovery of neuronal function.
REFERENCES


Figure Captions

Figure 1– Photomicrographs of coronal sections of the hippocampus, of Mongolian gerbil submitted to chronic and unilateral occlusion of the external carotid artery, followed by 12 h (SIG12) and 24 h (SIG24) motor stimulation in treadmill apparatus. Note a severe reduction (60%) in the number of CA1 pyramidal cells and increased number of picnotic nuclei in SIG12 group when compared to SIG24 group. Black arrows point out pyramidal cells; arrowheads picnotic nuclei. The scale bar in B represents 37 µm in SIG12 and SIG24.

Figure 2– Photomicrographs of coronal sections of the neostriatum, of Mongolian gerbil submitted to chronic and unilateral occlusion of the external carotid artery, followed by 12 h (SIG12) and 24 h (SIG24) motor stimulation in treadmill apparatus. Note the important local neuronal cell loss (148 neuronal cells against to 217, respectively) and increased number of picnotic nuclei in SIG12 group when compared to SIG24 group. Black arrows point out pyramidal cells; arrowheads picnotic nuclei. The scale bar in B represents 37 µm in SIG12 and SIG24.

Figure 3– Behavioral responses of Mongolian gerbils in the open-field test. A- Number of crossings and B frequency of grooming behavior. The motor responses are reported as mean ±SEM. * statistically significant differences, in comparison to the control group, according to the one-way ANOVA, followed by Dunnett’s test (p<0.05). N=10 for both groups. CC = control group, CE = stimulated control group, SH= sham group, SIG = ischemia group, SIG12 / 24 / 48 / 72 = ischemic group submitted to motor stimulation starting at 12 h, 24 h, 48 h, and 72h after the carotid artery occlusion, respectively.

Figure 4– Time of permanence on the rotarod test moving cylinder. Data are reported as mean ±SEM. * statistically significant differences, in comparison to the control group, according to the one-way ANOVA, followed by Dunnett’s test (p<0.05). N=10 for both groups. CC = control group, CE = stimulated control group, SH= sham group, SIG = ischemia group, SIG12 / 24 / 48 / 72 = ischemic group submitted to motor stimulation starting at 12 h, 24 h, 48 h, and 72h after the carotid artery occlusion, respectively.
Figure 2 colored
Figure 3
Figure 4

The figure shows a bar graph comparing different treatments labeled as CC, CE, SH, SIG, SIG12, SIG24, SIG48, and SIG72. The y-axis represents the rotarod performance, with values ranging from 0 to 25. The graph displays the performance differences among the treatments, with asterisks indicating significant differences compared to the control group (CC).