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Neuropathology and behavioral impairments after three types of global ischemia surgery in *Meriones unguiculatus*: Evidence in motor cortex, hippocampal CA1 region and the neostriatum

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ABSTRACT

The effects of three types of global ischemia by occlusion of carotid artery on motor and exploratory behaviors of Gerbils were evaluated by the Activity Cage and Rota rod tests. Animals were divided based on two surgical criteria: unilateral (UNI) or bilateral (BIL) carotid occlusion, with (REP) or without (OCL) reperfusion; and their behavior was evaluated on the fourth (4) or sixth (6) day. There was reduction of cell number in striatum, motor cortex M1 area, and hippocampal CA1 area in all groups in comparison to control animals. For M1 area and striatum, the largest reduction was observed in UNI6, UNI4, and BIL4 groups. Neuronal loss was also observed in CA1 area of BIL4 rodents. There was a decrease in crossings and rearings in all groups in activity cage test, compared to control. Reperfusion, unilateral and bilateral occlusion groups showed decrease in crossings. Only the BIL4 showed a decrease of rearing. In the Rota rod test, except the UNIOCL6, the groups showed a decrease in the balance in comparison to control. Both groups with REP4 showed a major decrease in balance. These findings suggest that both unilateral and bilateral carotid occlusions with reperfusion produce impairments of motor and exploratory behavior.

1. Introduction

Cerebral ischemia or stroke, one of the leading causes of death and long-term disability in aging populations, often results in irreversible brain damage and subsequent loss of neuronal function [1]. Stroke is the third cause of death and the primary cause of disability in many countries [2]. In both humans and animals, ischemia produces neuronal damage in vulnerable areas of the brain including hippocampus, striatum, and cerebral cortex. Considering the important role of these structures in the control of different types of motor behavior, it is expected that cerebral ischemia will produce morphological and functional changes that can be evaluated by behavioral models [1]. Many of these studies have shown sensorimotor abnormalities in ischemic rats, leading to changes in exploratory behaviors, and also cognitive and neurobehavioral deficits [3].

Experimental models have been used in order to study the basic mechanisms of neuronal death after transitory cerebral ischemia [4]; the pathophysiology and therapeutic potential of the reactions and current alterations induced by ischemia and cerebral reperfusion [5]. The three main models used for in vivo studies are: (a) focal ischemia, (b) global ischemia and (c) hypoxic ischemia [1].

In the early eighties, a model of global ischemia in gerbils was described with the transitory occlusion of both common carotids arteries or only one artery, which can induce a global cerebral ischemia, when the animals have an incomplete polygon of Willis [2]. The global lesions are generally of short duration, usually ranging from 3 to 30 min. The main characteristic of this model is the significant delay between the time of the lesion and cellular death, which generally occurs anywhere from 12 h to several days. This delay depends on the cell population and lesion duration [6,7]. In this sense, the behavioral and morphologic responses depend on the time when the evaluation begins, probably because the affected structures react differently to ischemia and repair mechanisms [8]. In the bilateral (BIL) model, it is necessary to accomplish reperfusion (REP). Moreover, in the unilateral (UNI) model surgery can be completed with or without reperfusion (OCL).

Interestingly, there is evidence that neurons in the CA1 area of the hippocampus had their peak of neuronal death 4 days post-ischemia [1], although we cannot rule out the possibility of neuronal damage in later periods. In fact, it was demonstrated that unilateral carotid occlusion causes significant damage in neostriatum and motor cortex six days after the occlusion followed by reperfusion [6].

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Since the cellular and behavioral modifications can be affected by the type of surgery (OCL or REP), and by UNI or BIL arterial occlusion, and considering the influence of the duration of carotid occlusion before the evaluation of post-ischemia neuronal effect, this study aimed at the analysis of the effects of different methodological approaches of global ischemia on motor performance and exploratory behavior in Mongolian gerbils evaluated by the Activity Cage and Rota Rod tests after four and six days of ischemic procedure.

2. Materials and methods

2.1. Animals

Ninety six male naive Mongolian gerbils (*Meriones unguiculatus*; *Rodentia; Gerbillinae*) weighing 60–80 g were used. The animals were housed in a colony room with food and water ad libitum. They were maintained under controlled temperature (23 ± 1 °C) and lighting conditions (07:00–19:00 h). The experiments were performed between 9:00 and 14:00 h in compliance with the recommendations of the Committee for Ethics in Animal Experimentation of the Ribeirão Preto School of Medicine of the University of São Paulo (CETEA-FMRP-USP) (proc. 029/2007), which are based on the Brazilian College of Ethics in Animal Research (COBEA).

2.2. Proceedure

The animals were assigned to the following groups (each group had 12 animals): Control group – naive animals i.e., animals not subjected to experimental surgery. Sham group – animals submitted to a ventral incision in the neck without occlusion of the carotid artery.

The experimental groups animals submitted to experimental surgery, assigned to three subgroups as follows: UNI4REP and UNI6REP – left common carotid artery was exposed and occluded, with suture, for a period of 10 min, after that the suture was released to allow cerebral reperfusion.

UNI4OCL and UNI6OCL-left common carotid artery was exposed and occluded, and the suture thread was tied, leading to definitive occlusion of the artery as described elsewhere [6].

BIL4 and BIL6-both carotid arteries were exposed and occluded for a period of 5 min and the suture was released to allow cerebral reperfusion.

In these groups, behavioral and morphologic evaluation was performed four (4) or six (6) days after surgical procedure.

2.3. Surgical procedures

All the animals in the experimental groups and the sham group were intramuscularly anesthetized with Zoletil (5 mg/kg), receiving a ventral incision in the neck. Then, their subcutaneous and muscular tissues were pushed back for the occlusion of the carotid artery. The rectal temperature was maintained at 37 ± 0.5 °C with a heat 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 were allowed an average of 50 min recovery time after surgery. Animals from UNI groups that were used in the present study showed the following clinical signs of ischemia: unilateral ptosis and withdrawal of the paw. As BIL groups did not show these signs, only after morphological confirmatory analysis, their respective data were included in the current study.

2.4. Behavioral tests

2.4.1. Activity cage test

One day before surgery all the animals were submitted to a 5-minute session of habituation in the Activity Cage (EP 149 model, Insight LTDA, Brazil) test apparatus, which consisted of an enclosed square area of 60 cm in diameter, elevated 50 cm off the floor. The cage was fitted with four parallel horizontal infrared beams, with 16 sensors each, 2 and 6 cm off the floor. Interruption of a beam generated an electrical impulse, which was subsequently processed and sent to a computer. The experimental room was illuminated with a 40-W fluorescent lamp (350 lx at the arena floor level).

The animals were individually placed in the middle of the activity cage test and 15 min-sessions were recorded four days after surgery for the UNI4OCL, UNI4REP and BIL4 groups, or six days after surgery, for the UNI6OCL, UNI6REP and BIL6 groups. The following behavioral responses were analyzed: number of crossings (i.e. number of sensors traversed into 3 × 3 cm sections) and number of rearings (standing on hind legs in the middle of the activity cage test or against the walls).

2.4.2. Rota rod test

The Rota rod test has a cylindrical surface moving around its axis at an acceleration of 5.5 rpm. Immediately after the activity cage test all animals were placed, one by one, in the Rota rod apparatus (EFF 412 model, Insight LTDA, Brazil). The experiment started only when the animals were stable for three seconds with all four paws in the cylinder. The animals have to balance on the moving cylinder, and the test was interrupted any time the animal fell off it. The time of permanence on the cylinder in movement was recorded and the data presented were equivalent to the mean of permanence of three attempts to balance in the apparatus. The system measures the time each animal remains on the apparatus by a microprocessor circuit. The Rota rod is widely used to assess motor behavior in experiments with focal or global brain injuries in rats and mice [3,9]. The test was performed the same way as in a previous study from our laboratory [6].

2.4.3. Histology

Immediately after the end of 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 solution (pH 7.4). The brains were removed and fixed in the same fixative for 4 h, at 4 °C. After being dehydrated with graded concentration of alcohol the brains were embedded in paraffin. Coronal sections (10 μm) of the striatum, M1 area of motor cortex and CA1 area of the hippocampus were made using a microtome (Jung Histocut, Leica model 820-II Germany) and stained with hematoxylin and eosin for light microscope examinations. Morphological analysis was performed by the Image J 1.37 V software (National Institutes of Health, U.S.A), by counting the number of neurons nuclei. The morphological analysis of UNI group brains was performed in the brain hemisphere ipsilateral to carotid occlusion. As for the BIL groups, although lesions were found in both hemispheres, for an equivalent comparison with the UNI groups, only the left hemisphere was analyzed. Histology slides were always evaluated in the established stereotactic coordinates for the same structure.

2.4.4. Statistical analysis

The results are reported as mean ± S.E.M. Data were submitted to one-way analysis of variance (ANOVA). Student Newman Keuls post hoc comparisons were carried out if significant overall F values were obtained. Significance was set at $p<0.05$. For comparisons between the control group and the sham group groups it was used Student's *t* test.

3. Results

Comparisons between the control group and sham groups revealed no significant differences for crossings ($t = 0.7689$; $p = 0.22$) and rearings ($t = 0.7542$; $p = 0.20$) in activity cage as well as Rota rod test ($t = 0.8892$; $p = 0.1965$). The morphological evaluation followed the same direction for the CA1 area of the hippocampus ($t = 0.504$;