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Research report

Behavioral benefits of maternal swimming are counteracted by neonatal hypoxia-ischemia in the offspring

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HIGHLIGHTS

- Rats subjected to neonatal hypoxia-ischemia increased motor activity in the adulthood.
- Maternal swimming improved object recognition memory in adult male offspring.
- Neonatal hypoxia-ischemia counteracted memory benefits of maternal exercise.
- Memory effects in prenatal exercised-rats were BDNF-independent.

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ABSTRACT

Hypoxia-ischemia (HI) represents one of the most common causes of neonatal encephalopathy. The central nervous system injury comprises several mechanisms, including inflammatory, excitotoxicity, and redox homeostasis unbalance leading to cell death and cognitive impairment. Exercise during pregnancy is a potential therapeutic tool due to benefits offered to mother and fetus. Swimming during pregnancy elicits a strong metabolic programming in the offspring's brain, evidenced by increased antioxidant enzymes, mitochondrial biogenesis, and neurogenesis. This article aims to evaluate whether the benefits of maternal exercise are able to prevent behavioral brain injury caused by neonatal HI. Female adult Wistar rats swam before and during pregnancy (30 min/day, 5 days/week, 4 weeks). At 7th day after birth, the offspring was submitted to HI protocol and, in adulthood (60th day), it performed the behavioral tests. It was observed an increase in motor activity in the open field test in HI-rats, which was not prevented by maternal exercise. The rats subjected to maternal swimming presented an improved long-term memory in the object recognition task, which was totally reversed by neonatal HI encephalopathy. BDNF brain levels were not altered; suggesting that HI or maternal exercise effects were BDNF-independent. In summary, our data suggest a beneficial long-term effect of maternal swimming, despite not being robust enough to protect from HI injury.

1. Introduction

Neonatal hypoxia-ischemia (HI) results in a harmful encephalopathy, causing memory impairment and reduced cognitive skills [1–4], with an occurrence of approximately 1–6 per 1000 newborns [5]. The behavioral changes can be directly related to decreased volume and atrophy of certain brain structures such as the hippocampus [3,6–8], allied to the metabolic insult evidenced by excitotoxicity, oxidative stress, and inflammatory mechanisms [9]. The animal model of Levine [10] adapted by Rice et al. [11] is the most widely used to study the effects of HI injury during the perinatal period. Signaling neurotrophic

Abbreviations: BDNF, Brain-derived neurotrophic factor; CHI, Control + hypoxia-ischemia; CNS, Central nervous system; CS, Control + sham; EHI, Maternal exercise + hypoxia-ischemia; ES, Maternal exercise + sham; HI, Hypoxia-ischemia; ME, Maternal exercise; PND, Postnatal day.

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factors are upregulated in response to HI-related injury in order to restores normal cell function or induces metabolic adaptations. Brain-derived neurotrophic factor (BDNF) has been reported as one of these potent brain defense indicators [12], although its neuroprotective effects have not been proven in human beings [13].

The complex pathophysiology of HI-encephalopathy and the lack of effective drugs for the treatment of these consequences, require the development of neuroprotective therapeutic strategies. Aerobic physical activity has been recognized as an important therapeutic option, assisting the treatment of chronic diseases [14–16]. In addition, aerobic exercise stimulates antioxidant capacity in some central nervous system (CNS) areas [14,17], improves behavioral parameters in animal models and induces hippocampal neurogenesis [14,18,19]. Moreover, BDNF serum levels and the volume of the anterior hippocampus are increased by aerobic exercise, and a relationship between both features has been demonstrated in humans [20]. Animal models submitted to voluntary wheel running displayed increased hippocampus cell proliferation and BDNF levels, with consequent improved learning memory [21–23]. Choi [24] showed that rats subjected to Levine-Rice model of HI that performed moderate exercise on a treadmill exhibited a decrease in DNA fragmentation. Fascinatingly, a better post-ischemic recovery was observed in mouse that underwent treadmill [24,25].

Recently, Akhavan et al. [26] reported that maternal voluntary wheel running exercise during pregnancy prevented hippocampal neurons loss elicited by postnatal HI. Data published by our group have demonstrated promising evidences of metabolic programming in the young offspring from dams submitted to maternal swimming, concerning antioxidant status and mitochondriogenesis in brain [27]. Cerebellum, parietal cortex, and hippocampus from pups presented enhanced activity of enzymatic and non-enzymatic antioxidants, followed by increased mitochondrion mass and membrane potential, evidences of mitochondria biogenesis [27]. A similar maternal swimming model has shown considerable improvement in Morris water maze performance as well as an increased number of neurons in hippocampus of rat pups [28,29]. In face of this innovative strategy, the main objective of this study was to evaluate whether the benefits provided to the offspring by maternal exercise during pregnancy were able to overcome the deleterious effect of neonatal HI in the offspring. In order to accomplish this goal, we evaluated the motor activity and object recognition memory in adulthood, as well as the concentration of mature BDNF in cerebellum, parietal cortex, hippocampus, and striatum of Wistar rats.

2. Materials and methods

2.1. Animals

Adult female (30 animals) and male (15 animals) Wistar rats were obtained from the Central Animal House of Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. The offspring was kept with its mother in a single box until weaning at 21 days. Animals were maintained on a 12/12-h light/dark cycle in an air-conditioned colony room at a constant temperature (22 ± 1 °C), with free access to water and a 20% (w/w) protein commercial chow. Experiments were approved by the local animal ethics commission (Comissão de Ética no Uso de Animais/Universidade Federal do Rio Grande do Sul—CEUA/UFRGS) under the number 23670, and followed national animal rights regulations (Law 11.794/2008), the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996), and Directive 2010/63/EU. We further attest that all efforts were made to minimize the number of animals used and their suffering.

2.2. Experimental design

Female Wistar rats (approximate body weight = 250 g) were initially randomized into two groups: (1) sedentary control, females exposed to aquatic environment stress, without swimming; and (2) maternal exercise, females subjected to swimming protocol initiated one week previous to mating. During the mating, two females were housed with a male rat during 12 h. Pregnancy was confirmed by the presence of a vaginal plug or sperm in the vaginal fluid. The pregnant rats were submitted to the exercise protocol during the entire pregnancy. From the 20th day after the beginning of pregnancy, the rats were observed twice a day (9 h and 18 h), to verify the litter's birth (Fig. 1A). The day corresponding to the birth of offspring was defined as postnatal day (PND) 0. On PND7, the offspring was subjected to HI model. At this point, male pups were divided into the following four groups: (1) control + sham (CS), (2) control + hypoxia-ischemia (CHI), (3) maternal exercise + sham (ES), or (4) maternal exercise + hypoxia-ischemia (EHI). At the weaning day, the rats where housed according the gender (5 rats per cage) until behavioral tests, on PND60. Twenty-four hours after the end of memory tests, the animals were euthanized, and the brain was dissected into cerebellum, parietal cortex, hippocampus, and striatum. Samples were stored (−80 °C) until the determination of mature BDNF by immunoassay (Fig. 1B). In order to reduce the litter effect, one to two pups from each litter were used for the analysis (total = 86 animals).

2.2.1. Maternal swimming protocol

The maternal exercise protocol was adapted from Lee et al. [29], as described by Marcelino et al. [27]. The rats were divided into two groups: (1) control; and (2) maternal exercise. In the exercised group, female rats were submitted to swimming in a pool filled with 32 ± 1 °C water on 5 days/week for 4 weeks. Each swimming session lasted for 30 min, and always took place between 9 and 12 a.m. Each rat was isolated during swimming, which was conducted using an apparatus designed specifically for rat swimming. Within this apparatus, each room measures 30 × 30 × 90 cm (width × length × depth), preventing the animals from touching the bottom of the tank. The animals were left free to swim, without any extra weight, and were gently stimulated to swim when necessary. This protocol has moderate intensity exercise. Control rats were immersed in water, carefully dried, and returned to the housing boxes.

2.2.2. Model of hypoxia-ischemia (HI)

HI model was induced according to the method described by Levine [10] and further modified by Rice et al. [11]. At On PND7, a unilateral brain lesion was produced in the offspring. All pups subjected to the surgery were anesthetized with 4% halothane. The ventral surface of the neck (parallel and lateral to the trachea) was incised facilitating the access to the right carotid artery, which was isolated from the vague and occluded permanently with surgical thread. After surgery, animals were placed under a heating lamp for post-surgical with 15 min of recovery, and then were returned to their mothers. After 2 h of maternal care, the pups were exposed to 90 min of hypoxia atmosphere with a standard mixture of 8% oxygen and 92% nitrogen in a 1500 mL chamber partially immersed in a water bath at 37 °C [2,30]. Shortly after the hypoxic ischemic procedure, the animals returned to their mothers in their respective cages. Controls were sham-operated, i.e., subjected to the effect of anesthetic and suffered the same incision and isolation of the