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Research paper

Early environmental enrichment affects neurobehavioral development and prevents brain damage in rats submitted to neonatal hypoxia-ischemia

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HIGHLIGHTS

- Early environmental enrichment has affected developmental milestones.
- Daily performance of reflexes was earlier improved in enriched rats.
- Hypoxia-ischemia caused atrophy of striatum, corpus callosum and neocortex.
- Early stimulation prevented the tissue damage on corpus callosum and neocortex.
- Hypoxia-ischemia did not affect the sensorimotor development in neonate rats.

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ABSTRACT

Our previous results demonstrated improved cognition in adolescent rats housed in environmental enrichment (EE) that underwent neonatal hypoxia-ischemia (HI). The aim of this study was to investigate the effects of early EE on neurobehavioral development and brain damage in rats submitted to neonatal HI. Wistar rats were submitted to the HI procedure on the 7th postnatal day (PND) and housed in an enriched environment (8th–20th PND). The maturation of physical characteristics and the neurological reflexes were evaluated and the volume of striatum, corpus callosum and neocortex was measured. Data analysis demonstrated a clear effect of EE on neurobehavioral development; also, daily performance was improved in enriched rats on righting, negative geotaxis and cliff aversion reflex. HI caused a transient motor deficit on gait latency. Brain atrophy was found in HI animals and this damage was partially prevented by the EE. In conclusion, early EE stimulated neurobehavioral development in neonate rats and also protects the neocortex and the corpus callosum from atrophy following HI. These findings reinforce the potential of EE as a strategy for rehabilitation following neonatal HI and provide scientific support to the use of this therapeutic strategy in the treatment of neonatal brain injuries in humans.

1. Introduction

The observation of children through reflex assessment and motor developmental milestones are tools used to evaluate neurobehavioral development which relates to neurological status assessed by the maturation of motor coordination and cognitive aspects [1,2]. It has been also established that impaired development may represent a predictive factor of behavioral modifications in adulthood [3,4] and through the evaluation of neonatal reflexes, it is possible to identify the persistence or absence of reflexes and detect developmental delay. Reflexes are automatic responses to a stimulation form and constitute the basis for motor skills. The primitive reflex activity is the first form of integration between human beings and the environment; and based on the dynamical systems theory, the motor development is the product of the refining and remodeling pre-existing patterns and it is dependent of the interaction of neural maturation and self-organizing properties of

Abbreviations: HI, hypoxia-ischemia; EE, environmental enrichment; SE, standard environment.

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the sensorimotor system [5,6]. Likewise in humans, rat development is considered a sign of brain maturation [7] and follows a sequence of the appearance of reflexes and maturation of motor skills [3]. Consequently, there are many factors that may damage normal development in both humans and rodents such as visual and hearing impairment, growth disorders resulting from perinatal and postnatal asphyxia, and environmental factors [8–13].

Perinatal asphyxia represents a major cause of brain damage in term newborn infants and is frequently associated with neurodevelopmental disabilities [14,15]. Experimental models of hypoxia-ischemia (HI) aim to reproduce neuropathological and functional characteristics found in humans such as white matter damage and neuronal loss, motor and cognitive deficits [16–19].

Currently, a non-pharmacological therapy has been used for brain damage using stimulation through interactions with the environment known as environmental enrichment (EE) [20–22]. Some studies have demonstrated that late enrichment (starting two weeks after the neonatal HI) improves memory and preserves dendritic spine density in the hippocampus of rats submitted to HI [23,24]. Only one study evaluated the impact of early EE housing in rats submitted to HI; the authors found improvement in working memory with no effects on tissue atrophy in the hippocampus and striatum [25]. We hypothesized that if EE recovers functional deficits such as learning and memory, it could also act on the onset of neurobehavioral developmental milestones. This issue deserves attention, especially because, in clinical cases, early intervention is critical for successful therapy and leads to improvement in cognitive and motor outcomes in cases of perinatal disorders [2,19]. The novelty of this study lies on investigating the effects of early enrichment on neurological reflexes following HI. Thus, the present study aimed to evaluate the role of early EE as a therapeutic intervention in rats submitted to neonatal HI. We investigated: (a) the maturation of physical characteristics and neurological reflexes; and (b) the volume of the striatum, corpus callosum and neocortex of rats undergoing neonatal HI followed by an early EE protocol.

2. Materials and methods

2.1. Animals

Pregnant Wistar rats obtained from the Central Animal House of the Institute of Basic Health Sciences were maintained under standard laboratory conditions. Wistar pup rats from 6 litters were randomly assigned to four groups: control maintained in a standard environment (CTSE: n = 6 males and 5 females); CT maintained in EE (CTEE: n = 6 males and 6 females); HI maintained in SE (HISE: n = 5 males and 6 females); and HI maintained in EE (HIEE: n = 6 males and 6 females). It has already been reported that sex does not influence the appearance of reflexes or the extent of brain damage after HI; therefore, male and female rats were randomly distributed among groups [15,25]. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institute of Health (USA) and with the Federation of Brazilian Societies for Experimental Biology. This project was approved by the Ethics Committee at the Universidade Federal do Rio Grande do Sul (n. 23260).

2.2. Hypoxia-ischemia

On postnatal day (PND) 7, to produce unilateral brain injury, we used the Rice-Vannucci model [16,26], which consists of a permanent left common carotid artery occlusion associated with hypoxia (O_2 level at 8%) for 90 min [17].

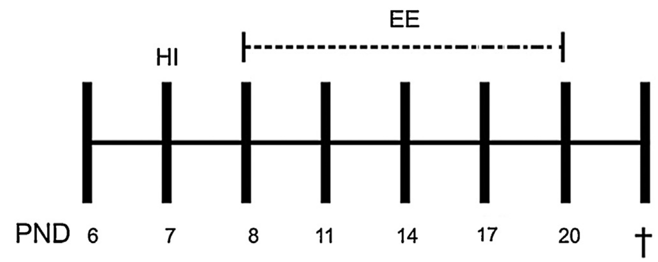

Fig. 1. Timeline of experimental procedures. Examinations of neurobehavioral development began 24 h before hypoxic-ischemic insult, 24 h after and were carried out every 3 days until PND 20. One day following the behavioral testing, animals were killed for morphological analysis (†).

2.3. Environmental enrichment

The early EE procedure used in this study was previously described by Pereira et al. [25]. The early continuous housing consists of free exploration by the dam and pups into the EE cage. The enriched environment consisted of a cage (40 × 40 × 20 cm) with three floors, a ramp, and a running wheel. So in volume the EE cage was larger than standard cages. We randomly used 7–10 objects with different shapes and textures (Lego pieces, soft toys, hairbrush, baby rattle, plastic toys, and rough objects). Objects were changed once a week to keep the novelty factor. Stimulated CT and HI-animals with your dams were housed in groups of 8 per cage, from PND 8–20, in EE cages, while non-stimulated litters were housed in standard cages.

2.4. Neurobehavioral development evaluation

Neurobehavioral development was assessed 24 h before and 24hr after HI and also on the PNDs 11, 14, 17 and 20 (Fig. 1). Physical characteristics such as eye opening, incisor tooth eruption and ear unfolding were observed and the day of appearance was recorded [18,27]. Also, the following signs and reflexes were tested: (1) *Righting reflex*: rats were placed in the supine position and time to turn over to the prone position was recorded [18]; (2) *Negative geotaxis*: animals were placed head down on an inclined board (45°) of 30 cm. The day on which they began to turn around and climb up the board with their forelimbs reaching the upper board was observed [28]. In cases where the animal did not turn around and climb up the board within 30 s, the maximal time was assigned. Time to reach the upper board's end was registered daily from the first negative geotaxis appearance day; (3) *Sensory reflexes*: the ears and eyelids were gently touched with a cotton swab and first day ear twitch reflex and eyelid contraction was recorded [18,28]; (4) *Limb placement*: each animal was suspended and the back of its forepaw and hindpaw were approached to the edge of the bench in order to touch it. The first day on which the rat lifted and placed its paws on Table was recorded [18]; (5) *Limb grasp*: the fore- and hindlimbs were touched with a thin rod, and the first day of grasping onto the rod was recorded [18]; (6) *Gait*: animals were placed in the center of a circle of paper (13 cm in diameter) and the day when they began to move off the circle with both forelimbs was recorded [18]. The maximum time was assigned (30 s) when the animal failed to leave the circle. Time to move off the circle was recorded daily from the first gait appearance day; (7) *Auditory startle*: startle response to a clapping sound was observed [18]; (8) *Cliff aversion reflex*: animals were placed with their forepaws overhanging the board's edge. Time required to turn more than 90° from the edge was recorded within a maximum observation time of 30 s [18].