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Research report

Beneficial effects of early environmental enrichment on motor development and spinal cord plasticity in a rat model of cerebral palsy

Marília Rossato Marques[a,b], Felipe Stigger[a,b], Ethiane Segabinazi[b], Otávio Américo Augustin[b], Sílvia Barbosa[b], Francele Valente Piazza[a,b], Matilde Achaval[a,b], Simone Marcuzzo[a,b,*]

[a] Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite 500, CEP: 90050-170 Porto Alegre, RS, Brazil
[b] Laboratório de Histofisiologia Comparada, Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite 500, CEP: 90050-170 Porto Alegre, RS, Brazil

HIGHLIGHTS

- A CP rat model experienced different early environments.
- The environmental enrichment prevented the motor deficits and can prevent histological changes.
- The early therapeutic intervention is important to prevent further motor impairments.

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ABSTRACT

Cerebral palsy (CP) results from nonprogressive lesions in the immature brain generating changes on the neuromuscular system. Environmental enrichment (EE) is a combination of stimuli that provides greater motivation and interest in novel movement exploration through the provision of various devices associated to enhanced social stimulation that would mimic the physiotherapy approach. The aim of this study was to verify whether EE is able to prevent the establishment of motor impairment in a CP rat model. The animals were divided in two groups: control animals (healthy) and animals submitted to a CP model. After this, the pups were exposed to two environments: enriched or standard, totaling four groups: Control group (without CP in a standard environment), CP group (CP model in a standard environment), EE group (without CP in an enriched environment) and CP–EE (CP model in an enriched environment). The experimental model was induced in pregnant Wistar rats by the association of maternal exposure to bacterial endotoxin, perinatal anoxia and sensorimotor restriction of the pups. The assessment of motor skills was held using the following tests: open field, rotarod, horizontal ladder, narrow suspended bar and stride length. The histological analysis evaluated the mean cross-sectional area (CSA) of the soleus muscle fibers, the mean CSA of motoneuronal somata and expression of synaptophysin in the ventral horn of the spinal cord. EE was able to prevent the motor deficits, however, it did not reverse the muscle atrophy observed in CP animals. Furthermore, there was an average increase in the mean area of motoneurons and an increase in the expression of synaptophysin in the ventral horn of the spinal cord of the CP–EE group in relation to CP animals reared in a standard environment. Hereupon, the stimulus increment provided by EE can prevent the onset of motor deficits and histological changes in a CP rat model.

1. Introduction

Cerebral palsy (CP) is a static encephalopathy resulting from nonprogressive lesions or anomalies in the immature brain [1,2] being the most common cause of physical disability affecting children [3]. Although the lesion is not progressive in CP, secondary motor impairment associated with abnormal motor patterns and postures [4] persists throughout the lifespan and interferes in children's normal development [5], contributing to activity limitations [6].

* Corresponding author at: Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Departamento de Ciências Morfológicas, Laboratório de Histofisiologia Comparada, Rua Sarmento Leite 500, 90050170 Porto Alegre, RS, Brazil. Tel.: +55 51 33083624; fax: +55 51 33083092..
E-mail address: simone.marcuzzo@ufrgs.br (S. Marcuzzo).

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The motor impairment is normally accompanied by morphologic, biochemical and physiological changes in the neuromuscular system [7,8]. These musculoskeletal challenges contribute to alterations in the gait pattern, such as slower speed, shorter stride length and more time spent in double support while walking [9]. Furthermore, children with CP have a reduction in movement efficiency, an increase in metabolic energy spent for walking [10–13] and a more sedentary lifestyle [14] that contributes to a decline in mobility-related activities [15]. Less mobility in CP is related to increased motor deficits and decreased physical conditioning [16].

Rehabilitation in CP disease consists of improving mobility, preventing deformity and helping children to learn the necessary skills for daily life. The therapy is often recommended throughout early childhood while the nervous and musculoskeletal systems are the most adaptable and the normal neuromotor development can be facilitated [16–18]. The therapy should support the development of cognitive, sensory, visual and musculoskeletal systems, involving play activities to enhance social integration [18].

In experimental studies, the environmental enrichment (EE) is used to provide physical activity, learning experiences, increased somatosensorial inputs and social interaction [19]. It induces plastic changes in the brain and recovery of sensorimotor function and memory impairment in several models of pathology [20–26]. The benefits observed in components of EE highlight their importance for the physical therapy and may strengthen the treatment of several neurological conditions including CP.

The CP rat model that consists of the association of maternal exposure to low doses of bacterial endotoxin, perinatal anoxia and sensorimotor restriction of the pups reproduces behavioral and damage characteristics that closely resemble the pattern described in CP [27]. Although there is a consensus that early intervention is more likely to improve the physical condition of patients with CP, the neurobiological mechanisms responsible for possible functional improvements are poorly understood. Therefore, the aim of this study was to investigate if the early exposure to an enriched environment could prevent the acquisition of the motor impairment induced in a rodent model of CP and investigate the biological substrate involved in it.

2. Materials and methods

All procedures were approved by the Ethical Committee at the Universidade Federal do Rio Grande do Sul (No. 23594). The animal care followed the recommendations of the Brazilian Society for Neuroscience, Committee of the School of Veterinary Surgery, University of Buenos Aires and International Brain Research Organization (IBRO) and are in compliance with the National Institute of Health's Guidelines for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985). Food and water were available ad libitum and the animals were maintained on a 12:12 h light/dark cycle in a temperature-controlled environment ($20 \pm 1\,^{\circ}C$), according to the Brazilian law that regulates animal use for didactic-scientific practice. All efforts were done to minimize animal suffering as well as to reduce the number of animals.

2.1. Experimental animals

The cerebral palsy model was induced as previously described [27], and consisted of the association of maternal exposure to low doses of bacterial endotoxin (lipopolysaccharide), perinatal anoxia and sensorimotor restriction of the pups. Pregnant Wistar rats ($n = 10$) were obtained from a local breeding colony (Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil) and prior to experiments, they were left undisturbed for 7 days. The animals were divided in two groups: control animals (healthy) and animals submitted to a CP model. After this, the pups were exposed to two environments: enriched or standard, totaling four groups: Control group (without CP in a standard environment), CP group (CP model in a standard environment), EE group (without CP in an enriched environment) and CP–EE (CP model in an enriched environment). Fig. 1 shows the time line of the experimental procedures. The CP model consisted in the association of maternal exposure to low doses of bacterial endotoxin (lipopolysaccharide), perinatal anoxia and sensorimotor restriction of the pups [27].

2.2. Environmental enrichment

The CT and CP groups were housed in standard laboratory cages (3 or 4 animals/cage) whereas the EE and CP–EE were placed in groups of eight animals in a complex enriched environment. This enriched environment consisted of a large cage ($50 \times 50 \times 50$ cm) with three floors, ramps, plastic tubes, one running wheel and several objects with different shapes and textures. The objects were rearranged every day and renewed every week to favor animals' exploratory behavior. The rat pup weights were evaluated in P1, P10, P20 and P29.

2.3. Motor skills assessment

At P29, the animals were submitted to motor skills evaluations. Spontaneous locomotor activities were evaluated using an open field, the motor balance and coordination was assessed using a rotarod and the hind-limb sensorimotor function was examined with the horizontal ladder and narrow suspended bar.

2.4. Open field test

The rats were evaluated in a 40 cm × 50 cm × 60 cm box, in which the floor was divided into 12 squares, and then filmed with a digital camcorder (DCR-SR47, Sony, Japan) for 3 min. The number of crossings from one square to another was counted.

2.5. Rotarod

The animals were placed in a rotarod (Insight, Brazil) with 60 mm diameter textured rod, 75 mm in length, rotating at a speed of 25 rpm. Each animal was tested 5 times with a 2 min interval between each trial and the maximum duration of the test was 3 min. The time spent by the animal on the rotarod was considered as the latency to fall.

2.6. Horizontal ladder and narrow suspended bar

The horizontal ladder is a ladder with 5 cm in width, with parallel metal rungs (2 cm a part) and the suspended bar is a rectangular bar with 2.5 cm in width. The apparatuses have 100 cm in length and were positioned 30 cm from the floor. Motor skills were assessed based on the rat's ability to walk on both these apparatuses. The animals were placed at one end of the ladder or bar and walked to the opposite end, entering in a darkened goal box [28]. The animals performed the test 3 times and each one was filmed with a digital camera (Sony; DCR-SR47, USA). Then the hind limb step errors were counted in each trial and the average values was considered as final value of errors. It was considered as an error when the hind limb of the animal slipped or was not placed on the bar or the metal rungs.

2.7. Gait testing

Walking pattern was evaluated at P29 and consisted of measuring the hind paw stride length (sum of the stance and the swing