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Characterization of a cerebral palsy-like model in rats: Analysis of gait pattern and of brain and spinal cord motor areas

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ABSTRACT

In an attempt to propose an animal model that reproduces in rats the phenotype of cerebral palsy, this study evaluated the effects of maternal exposure to bacterial endotoxin associated with perinatal asphyxia and sensorimotor restriction on gait pattern, brain and spinal cord morphology. Two experimental groups were used: Control Group (CTG) – offspring of rats injected with saline during pregnancy and Cerebral Palsy Group (CPG) – offspring of rats injected with lipopolysaccharide during pregnancy, submitted to perinatal asphyxia and sensorimotor restriction for 30 days. At 29 days of age, the CPG exhibited coordination between limbs, weight-supported dorsal steps or weight-supported plantar steps with paw rotation. At 45 days of age, CPG exhibited plantar stepping with the paw rotated in the balance phase. An increase in the number of glial cells in the primary somatosensory cortex and dorsal striatum were observed in the CPG, but the corpus callosum thickness and cross-sectional area of lateral ventricle were similar between studied groups. No changes were found in the number of motoneurons, glial cells and soma area of the motoneurons in the ventral horn of spinal cord. The combination of insults in the pre, peri and postnatal periods produced changes in hindlimbs gait pattern of animals similar to those observed in diplegic patients, but motor impairments were attenuated over time. Besides, the greater number of glial cells observed seems to be related to the formation of a glial scar in important sensorimotor brain areas.

1. Introduction

Cerebral palsy (CP) is a complex locomotion and posture disorder, which results from pre, peri or postnatal insults to the developing brain (Coq et al., 2008). Despite the improvements in prenatal care and parturition, this clinical condition affects 1 per 500 live births and its incidence remains stable in the last years (Badawi and Keogh, 2013). The most frequently neurological findings observed in CP include hemorrhage with intraventricular extension, white matter lesions around the ventricles (known as periventricular leukomalacia, PVL) and damages in cortex, basal ganglia, thalamus, hippocampus, cerebellum and brainstem (Folkerth, 2005). Though CP results from a not progressive upper motor neuron injury, a number of neural and mechanical aspects interact in the development of this disorder (Graham and Selber, 2003; Derrick et al., 2004). The delayed motor development, the abnormal muscle tone, the postural changes and musculoskeletal deformities are CP comorbidities that can contribute to the remodeling of sensory and motor cortical maps (Strata et al., 2004; Krigger, 2006; Coq et al., 2008).

Asphyxia at birth is traditionally considered to be a major cause of CP, because alters the electrolytic gradient of the cells and leads to the release of excitatory amino acids to the extracellular space (Kraus and Acheen, 1999; Wu and Colford, 2000; Yoon et al., 2003; Schwab et al., 2006). Such changes result in excitotoxicity, necrosis and neuronal

Abbreviations: BBB, Basso Beattie and Bresnahan rating; CP, cerebral palsy; CPG, cerebral palsy group; CTG, control group; G17, gestation day 17; IL1-β, interleukin 1-beta; i.p., intraperitoneal; LPS, lipopolysaccharide; PVL, periventricular leukomalacia; P0, postnatal day 0; P2, postnatal day 2; P28, postnatal day 28; P29, postnatal day 29; P30, postnatal day 30; P45, postnatal day 45; P48, postnatal day 48; P49, postnatal day 49; TNF-α, tumor necrosis factor-alpha

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death (Barks and Silverstein, 1992). However, current studies indicate that asphyxia is related only to a part of CP cases, indicating that infections and inflammations during pregnancy may also play an important role in the genesis of this disorder (Yoon et al., 2003).

In the experimental context, several CP models are being developed based on a combination of aggressions in embryonic and postnatal periods (Larouche et al., 2005; Meireles et al., 2017). It seems that the prenatal exposure of rat pups to the lipopolysaccharide (LPS), a structural constituent of gram-negative bacteria, promotes a sensitization of the immature brain to a subsequent asphyxia (Cai et al., 2001). Moreover, LPS activates the maternal immune system and leads to interleukin 1-beta (IL1-β) and tumor necrosis factor-alpha (TNF-α) production (Kopp and Medzhitov, 1999; Cai et al., 2001). These inflammatory cytokines may induce oligodendrocytes apoptosis and myelin degeneration, impairing the corticospinal tract development and causing injuries in the cerebral cortex and hippocampus (Damman et al., 2001; Clowry et al., 2014). Another strategy employed to reproduce the CP phenotype at experimental level is the hindlimb immobilization, also known as sensorimotor restriction. This intervention, alone or in association with perinatal anoxia, is able to mimic the lack of movement caused by the spasticity (Strata et al., 2004; Marcuzzo et al., 2010). The sensorimotor restriction also produced long-lasting deficits such as reduced body growth rate, abnormal gait patterns and primary motor cortex disorganization (Coq et al., 2008).

In a previous study, Stigger et al. (2011) showed that the association of prenatal LPS injections, asphyxia and sensorimotor restriction causes a greater impairment in motor function (evaluated by the rotarod, horizontal ladder and narrow suspended bar tests) as compared with the use of these factors alone. In addition, the combination of these interventions produced more evident changes on soleus and tibial anterior muscle morphology compared to the group exposed only to the sensorimotor restriction. Other studies also confirm that prenatal LPS injections, asphyxia and sensorimotor restriction used in association during the early stages of development produce motor, cognitive and muscular deficits similar to those observed in CP patients (Marques et al., 2014; Popik et al., 2016; Meireles et al., 2017). However, the repercussions of these aggressions during embryonic and postnatal periods over the gait pattern and morphology of nervous system structures have still not been described. Thus, the present study aimed to verify the effects of LPS administration combined with asphyxia and sensorimotor restriction on specific aspects of locomotion, besides characterizing possible morphological changes on the primary somatosensory cortex, striatum, white matter and spinal cord in rats. The association of these three interventions was adopted in order to reproduce the phenotype observed in severely compromised CP patients, which present serious functional and brain damages.

2. Materials and methods

2.1. General

Experimental procedures were approved by the Research Ethics Committee of the Universidade Estadual do Oeste do Paraná (UNIOESTE, Nr. 24/16). Initially, 42 female and 10 male *Wistar* rats with approximately 250–300 g in body weight were obtained from a local breeding colony (Biotério Central da UNIOESTE, Brazil). Animals were maintained in standard boxes, in a temperature-controlled environment (20 ± 1 °C), under a 12:12 h light/dark cycle, with food and water available *ad libitum*. All tests were performed during the light phase of the photo cycle.

The estrous cycle from females was checked daily by the observation of vaginal smear. Once the proestrus phase was detected, the females were individually mated with a sexually experienced male. The first day of pregnancy was confirmed by the presence of sperm in the vaginal smear up to 12 h after mating.

2.2. Experimental procedure

Pregnant rats remained under standard conditions until the 17th day of gestation (G17). In this day, part of the female began to receive intraperitoneal (i.p.) injections of LPS (n = 27; 200 μg/kg diluted in 100 μL of sterile saline; 0127:B8–L3129, Sigma, USA), while the remainder of the females began to receive injections of saline solution (i.p., n = 15, vehicle). The administration of vehicle or LPS continued to be performed every 12 h until the end of the gestation (Girard et al., 2009).

After birth, pups were divided in two experimental groups: 1) Control − offspring from mothers injected with saline solution during pregnancy (CTG) and 2) Cerebral Palsy − offspring from mothers injected with LPS during pregnancy, submitted to perinatal asphyxia and sensorimotor restriction (CPG). Only male pups were the subjects of this study, since the brain development in females suffers influence from sex hormones (Simerly, 1998). Thus, pups were culled in 8 per litter when sexual differentiation was evident, with the preferential removal of females.

The procedure used for asphyxia was the same proposed by Stigger et al. (2011). CPG pups were placed in a closed chamber with a flow of 9 L/minute of 100% N_2, during 20 min, in the day of birth (considered the postnatal day 0, P0). It is known that newborn rats are capable to maintain their body temperature 3.1–6 °C below the normoxic body temperature (Gordon and Fogelson, 1991). This ability allows pups to prevent the primary damage caused by asphyxia to the brain (Zhao et al., 1996; Colbourne et al., 1997; Bona et al., 1998; Ginsberg, 1998) and the secondary free-radical delivery (Palmer, 1997; Maier et al., 2001). Thus, the temperature inside the chamber was maintained at 37 °C ± 1 °C, which is sufficient to produce a rectal temperature of 37.05 °C ± 0.05 °C in newborn rats (Rogaslka and Caputa, 2005). The CPG animals were observed during the entire asphyxia process, having been evidenced that pups became cyanotic and evolved in a short period of time from hyperpnea to the third gasping phase (evidencing their hypoxic state). Although all CPG pups have survived the procedure, part of them has entered the stage of terminal apnea (having been necessary to perform cardiopulmonary resuscitation). The CTG animals underwent the same procedure, but the chamber remained open with normal atmospheric airflow.

From the second postnatal day until the thirtieth (P2 to P30), CPG animals were also exposed to 16 h of sensorimotor restriction per day. For this, pup's hindlimbs were immobilized using epoxy resin support and tape (Stigger et al., 2011). This restriction kept the hip, knee and ankle of the animals in an extended position. As described by Marcuzzo et al. (2008), this restriction was well tolerated by the pups and it does not prevent micturition and defecation. Throughout the period that sensorimotor restriction was performed on the CPG animals, the CTG animals were also removed from the housing box and the experimenters manipulated these animals as if they were to put the microporous tapes and the epoxy mold. This procedure was adopted to avoid possible differences in the development of litters, since it is known that early-life environmental events, such as the handling procedure, can induce long-lasting alterations upon behavioral and neuroendocrine systems (Raineki et al., 2009).

2.3. Gait pattern analysis

Animals hindlimb motor function was assessed at 29 and 45 days after birth (P29 and P45 respectively, *n = 12* per group) using an adapted open-field. Such apparatus had 60 × 60 × 60 cm, with the front wall made of glass and the opposite one made of mirror. All animals were placed separately in this apparatus during 5 min, without the sensorimotor restriction, for the observation of gait pattern. Each motor test was recorded with a digital camera (Kodak EasyShare C182, Rochester, New York, EUA). The walk analysis was subsequently carried out by a trained evaluator using Basso, Beattie and Bresnahan