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Impact of neonatal asphyxia and hind limb immobilization on musculoskeletal tissues and S1 map organization: Implications for cerebral palsy

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Abstract

Cerebral palsy (CP) is a complex disorder of locomotion, posture and movements resulting from pre-, peri- or postnatal damage to the developing brain. In a previous study (Strata, F., Coq, J.O., Byl, N.N., Merzenich, M.M., 2004. Comparison between sensorimotor restriction and anoxia on gait and motor cortex organization: implications for a rodent model of cerebral palsy. Neuroscience 129, 141–156.), CP-like movement disorders were more reliably reproduced in rats by hind limb sensorimotor restriction (disuse) during development rather than perinatal asphyxia (PA). To gain new insights into the underpinning mechanisms of CP symptoms we investigated the long-term effects of PA and disuse on the hind limb musculoskeletal histology and topographical organization in the primary somatosensory cortex (S1) of adult rats. Developmental disuse (i.e. hind limb immobilization) associated with PA induced muscle fiber atrophy, extracellular matrix changes in the muscle, and mild to moderate ankle and knee joint degeneration at levels greater than disuse alone. Sensorimotor restricted rats with or without PA exhibited a topographical disorganization of the S1 cortical hind limb representation with abnormally large, multiple and overlapping receptive fields. This disorganization was enhanced when disuse and PA were associated. Altered cortical neuronal properties included increased cortical responsiveness and a decrease in neuronal selectivity to afferent inputs. These data support previous observations that asphyxia per se can generate the substrate for peripheral tissue and brain damage, which are worsened by aberrant sensorimotor experience during maturation, and could explain the disabling movement disorders observed in children with CP.

Keywords: Perinatal asphyxia; Developmental disuse; Movement disorders; Hind paw; Muscle atrophy; Joint degeneration; Primary somatosensory cortex; Electrophysiological mapping

Introduction

Perinatal asphyxia (PA) remains a major cause of neonatal mortality and of permanent neurodevelopmental disability in children, including cerebral palsy (CP), seizure disorders and mental retardation in later life (Hill and Volpe, 1989; Vannucci et al., 1999). According to several studies, preterm birth, asphyxia before, during and after birth, and fetal and/or maternal infections entail a higher risk for CP (Hill and Volpe, 1989; Nelson and Grether, 1999; Haynes et al., 2005; Blomgren and Hagberg, 2006). Several animal models based on PA have reproduced brain damage found in patients with CP, such as periventricular white matter injury (e.g. Olivier et al., 2005; Blomgren and Hagberg, 2006). Only a few studies using

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PA have reported spasticity in relation to degraded locomotion in monkeys (Myers, 1975) and rabbits (Derrick et al., 2004; Drobyshevsky et al., 2007), while hypertonic spasticity has been commonly described in animal models of disuse (e.g. Canu and Falempin, 1996; Bouet et al., 2003; Strata et al., 2004).

Normal infants produce a large and rich repertoire of spontaneous movements from early fetal life until the end of the first half of a year of life. In contrast, children with CP display scarce, monotonous and stereotypical patterns of cramped-synchronized spontaneous movements that lack complexity, variation, and fluency (Prechtl, 1997; Hadders-Algra, 2004; Einspieler and Prechtl, 2005). Deficits in these spontaneous movements could account for musculoskeletal tissue changes found in these children. Indeed, varying degrees of atrophy and hypertrophy of muscle fibers (Lindboe and Platou, 1982; Romanini et al., 1989; Rose et al., 1994; Marbini et al., 2002) and increased fat and connective tissue within muscles (Castle et al., 1979; Jarvinen et al., 2002) have been reported in children with spastic CP. These muscle changes could be responsible for abnormal forces on bones and joints resulting in secondary bone malformations (Banks, 1972; Gormley, 2001) and/or articular cartilage degenerative changes (Banks, 1972; Lundy et al., 1998). Moreover, musculoskeletal changes contribute to provide abnormal sensory inputs to the brain, resulting in repetitive, aberrant sensory feedback, deleterious somatosensory and motor cortical reorganization, and ultimately in degraded motor function. A recent study in humans has provided evidence of somatosensory cortex reorganization following perinatal brain injury and of the effects of motor impairments on tactile discrimination abilities of infants with CP (Clayton et al., 2003).

Recently, Strata et al. (2004) developed a rodent model to reproduce the motor deficits observed in children with CP. Rats exposed to PA exhibited subtle motor behavioral anomalies and alterations of the representation of hind limb movements in the primary motor cortex (M1). While PA alone did not induce spasticity or degraded motor function in adult rats, hind limb immobilization (i.e. disuse) during development with or without PA, resulted in increased muscular tone at rest and during active flexion or extension, abnormal walking patterns in open-field, on a suspended bar, or on a rota-rod. These restrained rats also displayed a degraded M1 representation of hind limb movements.

Most of the studies on animal models of CP focus on brain damage and/or motor deficits (e.g. Bernert et al., 2003; Derrick et al., 2004; Drobyshevsky et al., 2007; Kohlhauser et al., 1999, 2000; Olivier et al., 2005; Van de Berg et al., 2000, 2003; see Vannucci et al., 1999 for review), but not on sensory deficits and peripheral tissues changes, even though somatosensory inputs and musculoskeletal integrity are essential components of motor function, control and development. As part of a broad effort to understand the role of early brain injuries and/or disuse on musculoskeletal system and brain network development, the present study examines the hind limb muscle and joint histology and the topographical organization of the primary somatosensory cortex (S1) in sensorimotor restricted rats with or without exposure to PA. Our results show that PA alone induces almost no effects on both peripheral tissues and S1 hind limb maps compared to control rats. In contrast, the sensorimotor restriction alone had deleterious effects on musculoskeletal histology and S1 map organization. Interestingly, the combination of PA and hind limb immobilization had the most deleterious impacts. These results contribute to gain new insights into the generation of movement disorders in human cerebral palsy.

Materials and methods

Subjects

Twenty eight newborn Sprague–Dawley rats from either sex were randomly assigned to 4 groups: 1) controls (CONT, $n=7$); 2) asphyxiated at birth (PA, $n=7$); 3) sensorimotor restricted during development (SR, $n=6$); and 4) asphyxiated at birth and sensorimotor restricted (PA+SR, $n=8$). All rats had water and food ad libitum, and were maintained in a 12-h light–dark cycle. The floor of all cages was covered with sawdust. All experiments were carried out in accordance with the guidelines laid down by the NIH and all animal use was approved by the Committee on Animal Research at the University of California at San Francisco.

Neonatal asphyxia

Pups from different litters experienced two episodes of asphyxia (PA) for 12 min each at the day of birth (P0) and on P1. The pups were placed on a thermal blanket (37.5 °C) in a plexiglas airtight chamber with two valves (Vetequip, Pleasanton, CA). Atmospheric nitrogen (N_2) gas was passed through the inlet valve until complete asphyxia occurred (Buwalda et al., 1995). Lowering the atmospheric oxygen concentration in the chamber resulted in hyperactivity of the pups, followed by a loss of movement, sporadic gasping and a change of skin color from pink to bluish. After each episode of asphyxia, pups were removed from the chamber to be kept in normal atmospheric conditions until they recovered their original skin color, normal breathing and postural reflexes. They were then returned to their mother. Atmospheric air was used for control and SR rats.

Sensorimotor restriction

Rats belonging to SR and PA+SR groups were restrained for 16h per day from P2 to P28. Pups' feet were gently bound together with medical tape, and their hind limbs were immobilized in an extended position with a cast made of hand-moldable epoxy putty stick (see Supplemental Fig. 1), which allowed only limited movements around the hip joint (see Fig. 1 in Strata et al., 2004, for more details). Casts did not prevent pups to urinate, defecate and to receive maternal cares. After casting, pups were returned to their mother and unrestrained littermates. The restrained rats were allowed to move freely for 8h per day. The casting was simulated in unrestricted rats without taping their hind limbs, so that all rats received similar handling. The size of the casts was adapted to the growth of the rats from P2 to P28. Body growth of restrained pups was significantly lower than that of unrestricted rats from the same litters (see Strata