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EXPERIENCE-DEPENDENT AMELIORATION OF MOTOR IMPAIRMENTS IN ADULTHOOD FOLLOWING NEONATAL MEDIAL FRONTAL CORTEX INJURY IN RATS IS ACCOMPANIED BY MOTOR MAP EXPANSION

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Abstract—One of the most common, and disruptive, neurological symptoms following neonatal brain injury is a motor impairment. Neonatal medial frontal cortical lesions in rats produce enduring motor impairments, and it is thought that lesion-induced abnormal cortical morphology and connectivity may underlie the motor deficits. In order to investigate the functional consequences of the lesion-induced anatomical abnormalities in adulthood, we used intracortical microstimulation to determine the neurophysiologic organization of motor maps within the lesion hemisphere. In addition, groups of neonatal lesion rats were given reach training or complex housing rehabilitation in adulthood and then mapped with intracortical microstimulation. The results demonstrate that neonatal medial frontal cortex lesions produce motor deficits in adulthood that are associated with abnormal motor maps. Further, adult behavioral treatment promoted partial recovery that was supported by reorganization of the motor maps whereby there were increases in the size of the forelimb motor maps. The experience-induced expansion of the forelimb motor maps in adulthood provides a neural mechanism for the experience-dependent improvements in motor performance.

Key words: neonatal brain injury, forelimb reaching, complex housing, intracortical microstimulation, sex, recovery.

Brain disease or injury during development can result in profound behavioral impairments that manifest and persist into adulthood. Motor impairments are one of the most common, and disruptive, neurological symptoms following perinatal cortical brain injury (Kennard, 1938a,b; Castro, 1972; Kolb and Whishaw, 1981; Villablanca et al., 1986; Finger et al., 2000), even if motor cortex areas are not directly damaged (Martin et al., 1993, 2000). Further, the severity of motor impairments is closely related to the stage of development when the injury occurred (Passingham et al., 1983). There is a critical period for optimal behavioral outcome after perinatal cortical injury in many species (Payne and Lomber, 2001), including humans (Anderson and Moore, 1995; Taylor and Alden, 1997), monkeys (Kennard, 1936; Goldman and Galkin, 1978; Passingham et al., 1983), cats (Villablanca et al., 1998), and rats (Kolb et al., 2000a,b). Rats incurring a cortical lesion within the second postnatal week present fewer and less severe behavioral impairments compared with similar injury *earlier* or *later* in development (Kolb and Whishaw, 1989; Kolb, 1995).

Damage to medial frontal cortex on postnatal day 10 shows sparing of cognitive behaviors (Nonneman and Corwin, 1981; Kolb and Gibb, 1993), yet conversely, these rats display persisting motor impairments marked by poor digit use and forelimb coordination, such as reaching and food manipulation (Kolb, 1995; Kolb et al., 1996, 1998a; Kolb and Cioe, 2000). There are direct corticospinal connections from medial frontal cortex (Nudo and Masterton, 1990), and the developing brain may be unable to compensate for their absence (Whishaw and Kolb, 1988). Brain damage that interferes with the normal pruning of exuberant corticospinal neurons has been shown to disrupt the localization of movement representations (Kennard, 1938a; Kartje-Tillotson et al., 1985, 1986, 1987) and motor behavior (Kennard, 1938a; Castro, 1972, 1975; Kolb et al., 2000a,b). Extensive anatomical analysis on rats with postnatal day 10 medial frontal cortex lesion has shown increased dopaminergic terminals in adjacent cortex (de Brabander et al., 1993; Kolb et al., 1994), an increase in dendritic arbor and spine density in the remaining cortex (Kolb et al., 1996, 1998a), anomalous thalamocortical connections (Kolb et al., 1994), yet an absence of thalamic degeneration in the dorsomedial nucleus (Vicedomini et al., 1984). These anatomical abnormalities may in turn manifest as a disruption in motor behaviors and cortical representations of movement.

Motor impairments resulting from cortical lesions can be rehabilitated in adulthood, at least to some extent with specific experiences such as skill reaching or general experiences such as complex housing (Gentile et al., 1987; Ohlsson and Johansson, 1995; Kolb et al., 1998b). However, the neurophysiologic mechanisms underlying the deficits, and those supporting the behavioral improvements, are unclear. The objective of the present study was to examine the effects of neonatal cortical injury on the neurophysiologic organization of microstimulation evoked forelimb movement representations. Intracortical microstimulation (ICMS) has been widely used to demonstrate lesion- (Kartje-Tillotson et al., 1985; Nudo and Milliken, 1996; Kleim et al., 2003b; Gharbawie et al., 2005) and experience-related changes in the neurophysiologic organization of motor representations (Neafsey et al., 1986;

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Abbreviations: ANOVA, analysis of variance; CFA, caudal forelimb area; Cg1, cingulate cortex, area 1; Cg3, cingulate cortex, area 3; Fr2, frontal cortex, area 2; ICMS, intracortical microstimulation; IL3, infralimbic cortex, area 3; RFA, rostral forelimb area.

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Neafsey, 1990; Nudo and Milliken, 1996; Nudo et al., 1996; Nudo, 1997; Kleim et al., 1998; Plautz et al., 2000). Here we assess how neonatal medial frontal cortex lesions affect the organization of forelimb movement representations. Further, we examine how behavioral rehabilitation in adulthood affects the organization of the motor representations.

EXPERIMENTAL PROCEDURES

Subjects and timeline

This study used 67 Long-Evans rats (52 males, 14 females) from six different litters obtained onsite from the Canadian Centre for Behavioral Neuroscience breeding colony. All procedures were approved by the University of Lethbridge animal care committee, and were in compliance with Canadian Council of Animal Care guidelines. Every effort was made to minimize the number of animals used and their suffering. On the tenth day after birth, rat pups were randomly allocated to either unilateral or bilateral aspiration removal of midline frontal cortex, or sham surgery. Weaning occurred on postnatal day 23 and consisted of pairing same sex siblings into standard laboratory caging. At 75 days after birth, rats in each lesion condition were randomly assigned into three main groups using a cross litter design. One group of rats (20 males) did not receive any behavioral rehabilitation. A second group of rats (13 males, 14 females) was trained on a skilled reaching task for 14 days. A third group of rats (14 males) was transferred to complex housing for 1 month. Male subjects were then mapped using ICMS, and the brain was harvested for histological analysis immediately after the mapping session.

Neonatal cortical lesions

At 10 days old, rat pups were removed from their home cage and cooled in a Thermatron™ chamber (−5 °C) for about 15 min until pups became immobile. Cryoanesthesia on postnatal day 10 does not interfere with cortical development (Kolb and Cioe, 2001). Pups were removed from the cooling chamber for surgery in random order. For unilateral medial frontal cortex lesions, the midline frontal plate was cut with iris scissors to create a window in the skull positioned over medial frontal cortex. With the aid of a surgical microscope, the lesion was intended (due to difficulty making lesions in neonates) to aspirate all layers of medial frontal cortex, including anterior cingulate (cingulate cortex, area 1 (Cg1), cingulate cortex, area 3 (Cg3)) and prelimbic (infralimbic cortex, area 3 (IL3)), as well as medial aspects of frontal cortex, area 2 (Fr2) (Zilles, 1985) through a glass pipette (1 mm tip) without damaging the underlying white matter. Following hemostasis, the wound was sutured with silk thread and the pups were placed on a heating pad. The same procedure was applied for bilateral lesions, removing medial frontal cortex in both hemispheres. For sham surgery, the skin was incised along midline of the scalp and then sutured. Pups were warmed to normal body temperature before being returned to their respective mothers. Three control rats that did not receive sham procedures were also included.

Housing conditions

All rats were housed and maintained in groups of two or three in standard laboratory cages unless otherwise stated. At postnatal day 75, a group of male rats (six control, five unilateral, and five bilateral) was transferred to a different room and placed in complex housing. The complex housing environment was constructed from four wire mesh cages. Each cage had dimensions 92 cm in length, 38 cm in width, and 36 cm in height yielding a total volume of 125,856 $cm^3 \times 4 = 503{,}424$ cm^3 (Petcetera, Richmond, British Columbia, Canada). Three of these cages were stacked vertically and interconnecting wire mesh ladders were installed to allow access to each level. A fourth cage was connected on the bottom level via two tunnels constructed from PVC piping (9 cm diameter). At least 50 toy objects of a variety of textures, shapes and sizes were randomly placed throughout the four cages, and exchanged with novel toys weekly. To promote exploration and climbing throughout the complex housing environment, food was available on the top level, and water was available on the ground level *ad libitum*.

Reach training

Beginning on postnatal day 75 (±5 days), groups of males (five control, five unilateral, and six bilateral) and females (five control, four unilateral, and five bilateral) were trained in a skilled reaching task (Whishaw and Pellis, 1990; Whishaw et al., 1991, 1993). Rats were placed on a restricted diet the day before training began, and maintained at 90% normal body weight. For control and bilateral lesion groups, each rat was permitted to establish a preferred forelimb during the first few days of training. In the case of unilateral operates, the left forelimb, contralateral to the lesion hemisphere, was trained at the task. Unilateral lesion animals initially preferred to use the ipsilateral to lesion forelimb (right forelimb), and required shaping during the first one to four sessions. Shaping entailed placing a bracelet (Elastoplast bandage, BSN Medical Inc., St. Laurent, QC, Canada) onto the forelimb ipsilateral to the lesion to prevent the forelimb from advancing through the apparatus aperture, but not to obstruct weight support or other movements such as grooming.

Training took place in clear Plexiglas boxes (25×35×30 cm) allowing unobstructed experimenter observation. Outside the front wall of the training box was a Plexiglas shelf 3 cm above the floor, with two surface dimples off-center of the aperture. A food pellet (45 mg, Bioserv Inc., Frenchtown, NJ, USA) was consistently placed onto the apparatus shelf dimple contralateral to the reaching forelimb. Each rat was placed inside the box and trained to reach through an aperture (1 cm width) at the front of the apparatus for a pellet of food on the shelf, and then to return to the back of the testing box after each attempt. Each training session consisted of a brief warm-up followed by the presentation of 20 pellets, during which the number of reach attempts (total number of attempts through the aperture) and success (number of pellets retrieved to the mouth) were recorded. The training was daily with one session per day totaling 14 sessions. Each rat was digitally videotaped on session 14 using a Cannon ZR 30 MC digital video camcorder with shutter speed at 1000th of a second with additional lighting provided by a cool florescent (Lowel Caselight 4, Brooklyn, NY, USA) studio light source mounted on a stand behind the camera, as well as a fiberoptic light directly illuminating the paw during a reach.

Movement components

To further investigate movements of the forelimb during reaching, a qualitative kinematics analysis was conducted (Whishaw et al., 1993). Briefly, a successful reach was subdivided into 10 movement elements: (1) limb lift: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body; (2) digits close: as the limb is lifted, the digits are semi-flexed and the paw is supinated so that the palm faces the midline of the body; (3) aim: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth; (4) advance: the head is lifted and the limb is advanced directly forward above and beyond the food pellet; (5) digits open: as the limb is advanced the digits are extended and opened; (6) pronate: using a movement of the upper arm, the elbow is adducted while pronating the paw over the pellet; (7) grasp: as the pads of the palm or digits touch the pellet, the pellet is grasped by closure of the digits; (8) supination I: as the