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HISTOMETRIC CHANGES AND CELL DEATH IN THE THALAMUS AFTER NEONATAL NEOCORTICAL INJURY IN THE RAT

G. D. ROSEN,[1a,b]* B. MESPLES,[a,b] M. HENDRIKS[a,b] AND A. M. GALABURDA[1a,b]

[a]*Dyslexia Research Laboratory and Charles A. Dana Research Institute, Department of Neurology, Division of Behavioral Neurology, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA*

[b]*Harvard Medical School, Boston, MA 02115, USA*

Abstract—Freezing injury to the developing cortical plate results in a neocortical malformation resembling four-layered microgyria. Previous work has demonstrated that following freezing injury to the somatosensory cortex, males (but not females) have more small and fewer large cells in the medial geniculate nucleus. In the first experiment, we examined the effects of induced microgyria to the somatosensory cortex on neuronal numbers, neuronal size, and nuclear volume of three sensory nuclei: ventrobasal complex, dorsal lateral geniculate nucleus, and medial geniculate nucleus. We found that there was a decrease in neuronal number and nuclear volume in ventrobasal complex of microgyric rats when compared with shams, whereas there were no differences in these variables in the dorsal lateral geniculate nucleus or medial geniculate nucleus. We also found that there were more small and fewer large neurons in both ventrobasal complex and medial geniculate nucleus. In experiment 2, we attempted to determine the role of cell death in the thalamus on these histometric measures. We found that cell death peaked within 24 h of the freezing injury and was concentrated mostly in ventrobasal complex. In addition, there was evidence of greater cell death in males at this age. Taken together, these results support the notion that males are more severely affected by early injury to the cerebral cortex than females.

Key words: cell death, dorsal lateral geniculate nucleus, medial geniculate nucleus, microgyria, stereology, ventrobasal thalamus.

Neuronal migration disorders of the cerebral cortex, such as heterotopias, microgyria, porencephaly, and lissencephaly, have been associated with a wide variety of disorders including intractable epilepsy (Meencke and Janz, 1984; Palmini et al., 1991a,b; Crino, 2004), dyslexia (Galaburda and Kemper, 1979; Galaburda et al., 1985), and developmental delay (Barkovich et al., 1988; Barkovich and Raybaud, 2004). Animal models have been developed for many of these neuronal migration disorders. Some of these are the result of spontaneous genetic mutations, such as the *reeler* or *scrambler* mouse (Caviness et al., 1972; Goffinet, 1984; Sweet et al., 1996; D'Arcangelo et al., 1997; Rice et al., 1998) or the Tish rat (Lee et al., 1997; Chen et al., 2000), whereas others are induced in otherwise normal rodents such as by prenatal injection with methylazoxymethanol (Ferrer et al., 1982; Chevassus-au-Louis et al., 1999) or neurotrophin-4 (Brunstrom et al., 1997).

Malformations have also been induced by mechanical disturbances of the cortical plate. Thus, focal collections of neurons in the molecular layer can be induced by puncture wounds at or around birth (Rosen et al., 1992b), and freezing injury to the developing cortical plate induces malformations that resemble human four-layered microgyria (Dvorák and Feit, 1977; Dvorák et al., 1978; Humphreys et al., 1991; Rosen et al., 1992a; Ferrer et al., 1993; Marret et al., 1995). These induced malformations have been used to model a variety of disorders, including epilepsy (Luhmann et al., 1998; Chevassus-au-Louis et al., 1999; Jacobs et al., 1999a; Jacobs and Prince, 2005) and developmental dyslexia (Humphreys et al., 1991; Fitch et al., 1994, 1997a; Herman et al., 1997; Rosen et al., 1999; Peiffer et al., 2004).

The effects of these relatively small focal malformations extend beyond the obvious distortion of the cerebral cortex. Recordings from slices of cortex containing microgyria induced by freezing injury to the cortical plate, for example, reveal epileptogenic discharges as far as 2–4 mm away from the malformation (Jacobs et al., 1996, 2000; Luhmann and Raabe, 1996; Jacobs and Prince, 2005). Using the same model, we and others have demonstrated a variety of connectional, anatomic, and behavioral alterations associated with the presence of this type of malformation. For example, both cortico-cortical and thalamo-cortical connections are disturbed not only in the malformation itself, but in areas both proximal and distal to it (Giannetti et al., 1999, 2000; Rosen et al., 2000). Anatomically, induction of microgyria dramatically decreases brain weight and neocortical volume globally (Peiffer et al., 2003). Microgyria in the somatosensory cortex not only disrupts the formation of barrel fields in that hemisphere (Jacobs et al., 1999b), but also distorts the barrel field of the opposite hemisphere (Rosen et al., 2001). Behaviorally, we have reported that adult and young male subjects with induced microgyria have defects in rapid auditory

[1] These authors contributed equally to this work.

*Correspondence to: G. D. Rosen, Department of Neurology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA. Tel: +1-617-667-3252; fax: +1-617-667-5217.
E-mail address: grosen@bidmc.harvard.edu (G. D. Rosen).

Abbreviations: dLGN, dorsal lateral geniculate nucleus; FJB, Fluoro-Jade B; MGN, medial geniculate nucleus; P, postnatal day; PBS, phosphate-buffered saline; PFA, paraformaldehyde; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling; VB, ventrobasal complex; VPL, ventroposterolateral nucleus of the thalamus.

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processing—a defect also seen in many individuals with language impairment and developmental dyslexia (Tallal and Piercy, 1974; Fitch et al., 1997b)—when compared with controls with no malformations. In comparison, adult females with identical malformations have no difficulty in processing this type of stimuli (Fitch et al., 1994; Clark et al., 2000a; Peiffer et al., 2004).

Because studies of the brains of individuals with developmental dyslexia had exhibited changes in cell size in the medial geniculate nucleus (MGN) and dorsal lateral geniculate nucleus (dLGN) of the thalamus (Livingstone et al., 1991; Galaburda et al., 1994), we measured cell size in these two nuclei in male and female rats with and without induced microgyria (Herman et al., 1997). We found more small and fewer large neurons in the MGN of male microgyrics when compared with their sham littermates. There was no difference between microgyrics and shams in the females. In addition, there were no differences in cell size distribution among any of the groups in the dLGN. We further reported that the difference in the MGN resulted from perinatal effects of gonadal hormones—female microgyrics exposed to testosterone in the perinatal period had cell size distributions in the MGN similar to those of males (Rosen et al., 1999).

These findings raise a number of questions. We have established that damage to neonatal somatosensory cortex changes cell size distribution in a thalamic nucleus to which it is not connected, at least during adulthood. What is not yet known is how these changes compare with those in thalamic nuclei directly connected to the area of injury. Moreover, what effects might be seen in other thalamic nuclei that do not connect with the somatosensory cortex? By what process are these changes in thalamic cell size distribution occurring? Is cell death affecting one neuronal population more than another, or could differences in cell proliferation, whereby one cell type is affected out of proportion to others, underlie these differences? In the present study, we first focused on stereologically assessing histometric features in the thalamus following early somatosensory cortical freezing injury. We examined effects in a directly connected thalamic nucleus (the ventrobasal complex (VB)), a nucleus that receives transient developmental connections from the somatosensory cortex (MGN, Nicolelis et al., 1991), and a nucleus that never connects directly with the somatosensory cortex under normal conditions (the dLGN). We then examined the patterns of cell death throughout the thalamus following freezing injury to the developing cortical plate in an effort to determine the contribution of this variable to the histometric changes.

EXPERIMENTAL PROCEDURES

Two experiments were conducted in this study. Experiment 1 investigated changes in neuron number, neuron size, and regional volume in three thalamic nuclei following postnatal freezing injury to the somatosensory neocortex. Experiment 2 investigated cell death in the thalamus following postnatal freezing injury to the somatosensory cortex. All procedures involving animal care and experimentation were carried out in accordance with guidelines provided by the National Institutes of Health and approved by the institutional animal care committee at Beth Israel Deaconess Medical Center. Care was taken to minimize the number of animals used, as well as their pain and suffering.

Experiment 1 protocol

On postnatal day (P) 1, rats were randomly assigned to receive freezing injury to the cerebral cortex or to a sham surgery. In adulthood, the subjects were killed, their brains removed, embedded in celloidin, sliced in the coronal plane, stained with Cresyl Violet, and every 5th section mounted on glass slides. Using stereologic probes, we estimated the number of neurons, the sizes of neurons, and the volume of VB, MGN, and dLGN nuclei.

Subjects

A subset of 24 subjects (six male lesioned, six male shams, six female lesioned, and six shams) was randomly chosen from subjects of a previous experiment and prepared as described previously (Rosen et al., 1999). In brief, timed pregnant rats were obtained from Charles River Laboratory (Wilmington, MA, USA) in the last week of gestation. The pregnant females were singly housed under a 12-h light/dark cycle and were provided with food and water *ad libitum*. On P1, male and female pups were randomly assigned either to receive bilateral freezing injury to the somatosensory cortex or to a sham condition. Subjects were anesthetized by placement on ice for 2 min. A small incision on the scalp was made midsagittally. For those subjects receiving freezing lesions, a cooled (−70 °C) probe was placed over the presumptive somatosensory cortex (directly on bregma, approximately 2 mm medial to the sagittal suture) for 5 s. The procedure was repeated on the opposite hemisphere with a second cooled probe. Animals receiving sham surgery were treated identically, with the exception that the probe was at room temperature. The incision was sutured, ink was injected into the footpads for identification, and the pups were warmed before being returned to their mother.

At P70-100, the subjects were deeply anesthetized (xylazine/ketamine 100 mg/ml) and killed by transcardial perfusion with 0.9% saline followed by 10% formalin. The brains were removed from the skull and allowed to post-fix for at least one week prior to embedding. Afterward, the brains were dehydrated in graded ethanols and embedded in 12% celloidin. They were then cut in the coronal plane at 30 μm, stained with Cresyl Violet, and every 5th section was mounted on glass slides with Permount.

Table 1. Parameters for stereologic probes for experiment 1

Thalamic nuclei	Optical fractionator and nucleator			Point counting, sampling frequency (μm)	Section periodicity
	Counting frame (μm)	Disector height	Sampling frequency (μm)		
VB	30×30	20	250×250	250×250	Every 10th
MGN	25×25	20	250×250	250×250	Every 5th
dLGN	25×25	20	225×225	200×200	Every 5th