Sex Differences in the Effects of Early Neocortical Injury on Neuronal Size Distribution of the Medial Geniculate Nucleus in the Rat Are Mediated by Perinatal Gonadal Steroids

Glenn D. Rosen, Amy E. Herman and Albert M. Galaburda

Dyslexia Research Laboratory and Charles A. Dana Research Institute, Beth Israel Deaconess Medical Center, Division of Behavioral Neurology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston MA 02215 and Harvard Medical School, Boston, MA 02115, USA

Freezing injury to the cortical plate of rats induces cerebrocortical microgyria and, in males but not females, a shift toward greater numbers of small neurons in the medial geniculate nucleus (MGN). The purpose of the current study was to examine a hormonal basis for this sex difference. Cross-sectional neuronal areas of the MGN were measured in male rats, untreated female rats and female rats treated perinatally with testosterone propionate, all of which had received either neonatal cortical freezing or sham injury. Both male and androgenized female rats with microgyria had significantly smaller MGN neurons when compared to their sham-operated counterparts, whereas untreated females with microgyria did not. These differences were also reflected in MGN neuronal size distribution: both male and androgenized female rats with microgyria had more small and fewer large neurons in their MGN in comparison to shams, while there was no difference in MGN neuronal size distribution between lesioned and sham females. These findings suggest that perinatal gonadal steroids mediate the sex difference in thalamic response to induction of microgyria in the rat cortex.

Introduction

Focal four-layered microgyria can be induced in otherwise normal brains by injury to the cortical plate during late neocortical neuronal migration, such as by the placement of freezing probes on the skull of newborns (Dvorák and Feit, 1977; Dvorák *et al.*, 1978; Humphreys *et al.*, 1991; Suzuki and Choi, 1991; Rosen *et al.*, 1992; Jacobs *et al.*, 1996; Luhmann and Raabe, 1996), the injection of ibotenic acid into the developing cortex (Innocenti and Berbel, 1991a; Marret *et al.*, 1995) or prenatal X-irradiation (Ferrer *et al.*, 1993). These malformations can have profound behavioral effects that mimic those seen in animals with spontaneous malformations (Sherman *et al.*, 1990; Schrott *et al.*, 1992; Rosen *et al.*, 1995b; Boehm *et al.*, 1996).

In a recent series of experiments, we noted an intriguing sex difference in the behavioral and anatomical consequences of early freezing injury to the neocortex. Male rats with microgyria were found to have difficulties in performing a fast, but not a slow, auditory discrimination task (Fitch *et al.*, 1994, 1997). In contrast, female littermates with an identical microgyria discriminated successfully at both the fast and slow discrimination conditions (Fitch *et al.*, 1997; Herman *et al.*, 1997). Further, when the medial geniculate nuclei (MGN) of these animals were examined, male rats with microgyria had more small and fewer large neurons as compared to unlesioned controls. For females, MGN neuronal size distribution did not differ between microgyric animals and shams. We suggested that this sex difference in MGN neuronal size distribution, despite comparable microgyria, might account for the disparity between lesioned males and females in the auditory temporal discrimination task.

The observation that changes in the MGN are present in lesioned males but not females, and that males and females show different behavioral effects after comparable injury, merits further investigation. In the current experiment, we tested the hypothesis that the differential response of the MGN to early cortical injury is hormonally mediated. Specifically, neuronal size distribution in the MGN in male and female rats, with or without microgyria, was compared to those in female rats treated perinatally with testosterone propionate (TP). Findings indicate that females treated with TP had MGN neuronal size distribution more similar to males than to untreated females.

Materials and Methods

Protocol

Time-mated pregnant Wistar rats were obtained from Charles River Laboratories (Wilmington, MA), or females were bred in house from stock acquired from the same source. Embryonic day 1 (E1) corresponded to the day sperm was detected on vaginal smear. Pregnant female rats were divided into three groups. Group 1 rats were injected s.c. daily with 2 mg TP suspended within the vehicle (0.1 ml sesame oil) on E16 until E21. Group 2 rats were injected s.c. daily with the vehicle on E16 until E21. Group 3 rats received no injections. On the day after birth (P1), animals were randomly assigned to receive bilateral freezing injuries to the parietal cortex or to a sham condition. Daily treatment was continued on the pups from groups 1 and 2 commencing on P1 and ending on P5. Pups from group 1 were injected with 0.1 mg TP suspended within the vehicle (0.05 ml sesame oil). Pups from group 2 were injected with the vehicle only. At P80, pups were killed, gender classification confirmed by post-mortem examination for testes, and their brains were processed for histological and morphological examination. The male rats from group 1 (TP-treated) were excluded from further analysis. Using stereological techniques, the volumes of the microgyria and the MGN were determined, and neuronal size and number were determined in the MGN.

Induction of Microgyria

Microgyria were induced based on a modification of a technique by Dvorák and colleagues (Dvorák and Feit, 1977; Dvorák *et al.*, 1978), and reported in detail elsewhere (Humphreys *et al.*, 1991; Rosen *et al.*, 1992). Pups were anesthetized with hypothermia, and a small incision was made in the anteroposterior plane of the skin over midline, exposing the skull. A cooled (–70°C) 2 mm diameter stainless steel probe was placed on the skull of lesion subjects over one or the other hemisphere for 5 s. The probe, targeted at the presumptive parietal cortex, was placed 1 mm caudal to bregma and 2 mm lateral of the sagittal suture. After placement of the probe, an identical lesion was placed in the opposite hemisphere. The side of the first probe placement was randomly determined. Animals receiving sham surgery were treated identically to those receiving freezing injury except that the probe was maintained at room temperature. Following the second probe placement, the skin was quickly sutured, and subjects were marked with a unique pattern of ink injection to the footpads, warmed under a lamp, and returned to the mother. On P21, litters were weaned and the subjects were housed with littermates (two or three per cage).

Histology

On approximately P80 subjects were anesthetized (87% Ketamine/13% Xylazine; 100 mg/kg i.p.) and were transcardially perfused with 0.9% saline and 10% formalin. The brains were removed, placed in 10%

formalin for at least 7 days before being dehydrated in a series of graded alcohols and embedded in 12% celloidin. Serial sections were cut coronally at 30 μm. Series of every fifth section were stained with cresyl violet, mounted on glass slides and coverslipped with Permount.

Morphometry

For all morphological measures of the MGN, sections were coded so that the experimenter would be blind to sex, hormone treatment and surgical group.

Microgyric Volume

Volumes of microgyria were determined from systematic serial sections using point counting and Cavalieri's rule. Images from the sections were projected on a video screen and point counting was performed using NIH Image v. 1.55 interfaced to a Macintosh Centris 650 computer (Apple Computer, Cupertino, CA). Total microgyric volume was determined using Cavalieri's estimation (Gundersen and Jensen, 1987). If the equispaced criteria for Cavalieri's estimator were not met (due to missing or damaged sections), a measurement method involving piecewise parabolic integration was employed (Rosen and Harry, 1990). The architectonic location of the lesion was quantified by overlaying the topographic location on a normalized flattened map of the neocortex derived from Zilles (1985).

MGN Volume

Volume of the MGN (inclusive of each subnucleus) was determined using the method described above for microgyric volume.

MGN Neuronal Measures

Cross-sectional neuronal areas and cell-packing densities were measured in the MGN of all rats using the three-dimensional counting methods and software of Williams and Rakic (1988). Three fields were measured on each of three slides, sampling from the rostral to the caudal extent and in the dorsal, ventral and medial portions of the nucleus (Winer, 1991) of both hemispheres for a total of 18 fields per brain as described previously (Herman *et al.*, 1997).

Images taken at 500× on a Zeiss Universal microscope (Carl Zeiss, Inc., Thornwood, NY) were projected onto a Sony GVM 1310 monitor (Sony Corporation, Park Ridge, NJ) that was connected to the Macintosh Centris 650 workstation. A counting square (95 × 85 μm) was overlaid on these images. Movement in the *Z*-axis was read by a Heidenhain MP-25 photoelectric micrometer (Heidenhain Corporation, Schaumburg, IL) interfaced to a National Instrument NB-GPIB card (National Instruments Corporation, Austin, TX) in the Macintosh. The base of the section was set to a *Z*-axis reading of zero. A red opaque overlay precluded cell counting below the confines of the optical box. As the plane of focus moved to 5 μm above the base, the screen became transparent. On a digitizing tablet, those neurons whose single nucleoli were in focus were traced. Neurons touching the top and right side of the counting box were omitted. The screen turned green when the fine focus rose above 25 μm, precluding measurement of neurons above the optical box. Thus, a perfect 20 μm thick slab of tissue was measured. Both the neuronal areas and cell packing density (neurons/mm^3) were output to the computer. Total neuronal MGN neuronal number for each hemisphere was determined by multiplying the cell-packing density for each hemisphere by its volume.

Analysis

There were two independent measures: 'Sex' (Male, Female, Female + TP) and Surgical Group (Lesion and Sham). Anatomically dependent measures included MGN neuronal size, MGN neuronal number, MGN volume and microgyric volume. Statistical analyses of all data were performed using ANOVA and chi-square tests.

Results

Preliminary Analysis

Examination of the 50 brains assigned to the lesioned condition showed that ten brains (five females, four androgenized females and one male) had lesions not fully restricted to the parietal region and had to be removed from the analysis. Analysis of the distribution of excluded and included subjects indicated no bias in the dependent variables among the three groups (χ^2 = 2.96, df = 2, NS). Hormone treatment induced masculinization as illustrated by the fact that female rats exposed to androgens had wider ano-genital distances. Subsequent post-mortem examination for the presence of testes confirmed gender identification.

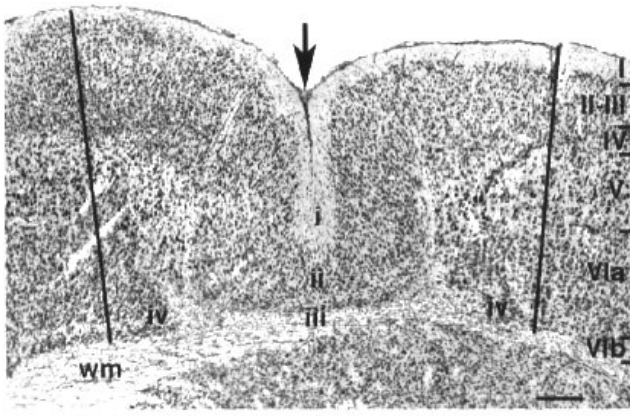

Figure 1. Low-power photomicrograph of a typical region of induced microgyria in the parietal cortex. In comparison to the adjacent undamaged cortex (right) with six layers, microgyric cortex has four layers. Layer i is contiguous with the molecular layer of the undamaged cortex and fuses to form a microsulcus (arrow). Layer ii is contiguous with layers II and III of undamaged cortex, but is unlaminated. Layer iii (lamina dissecans) is a glial scar that is the remnant of the original injury. Layer iv is contiguous with layer VIb of intact cortex. Solid lines show the medial and lateral borders of the microgyric area for the purposes of determining microgyric volume. The dorsal border is the pial surface and the ventral border is defined by the border between the white matter (wm) and layer iv of the microgyria. Bar = 200 μm.

Histology

All subjects receiving the freezing lesion to the cortical plate in the neonatal period exhibited focal cortical malformations resembling four-layered microgyria, and their appearance was identical to that reported in previous work (see Fig. 1). As expected, animals exposed to sham surgery showed no cerebral malformation. The location and mean volume of the microgyria are depicted in Figure 2. An overall ANOVA demonstrated no difference in volume of lesioned cortex between males, females and TP females [$F(2,37)$ = 1.30, NS]. Further comparisons showed no difference in volume of lesioned cortex between males and females [$F(1,25)$ = 0.47, NS], males and TP-treated females [$F(1,26)$ = 2.56, NS], and females and TP-treated females [$F(1,23)$ = 0.76, NS]. The degree of greatest lesion overlap occurred in Par1 (primary somatosensory cortex) and at the border of Par1 with HL (hindlimb region) and FL (forelimb region, Zilles, 1985).

Morphometry

In order to control for the possibility of effect of perinatal manipulation, it was first determined whether there were any differences in morphometric measures between vehicle-injected and control females. As expected, there was no difference in MGN neuronal area, MGN volume or MGN neuronal number between the two groups and their data were pooled for further analysis (F < 1, NS for all comparisons).

In order to analyze the effects of gender and lesion on MGN neuronal size, MGN volume and MGN neuronal number, a series of three two-way ANOVAs were performed. A two-way ANOVA

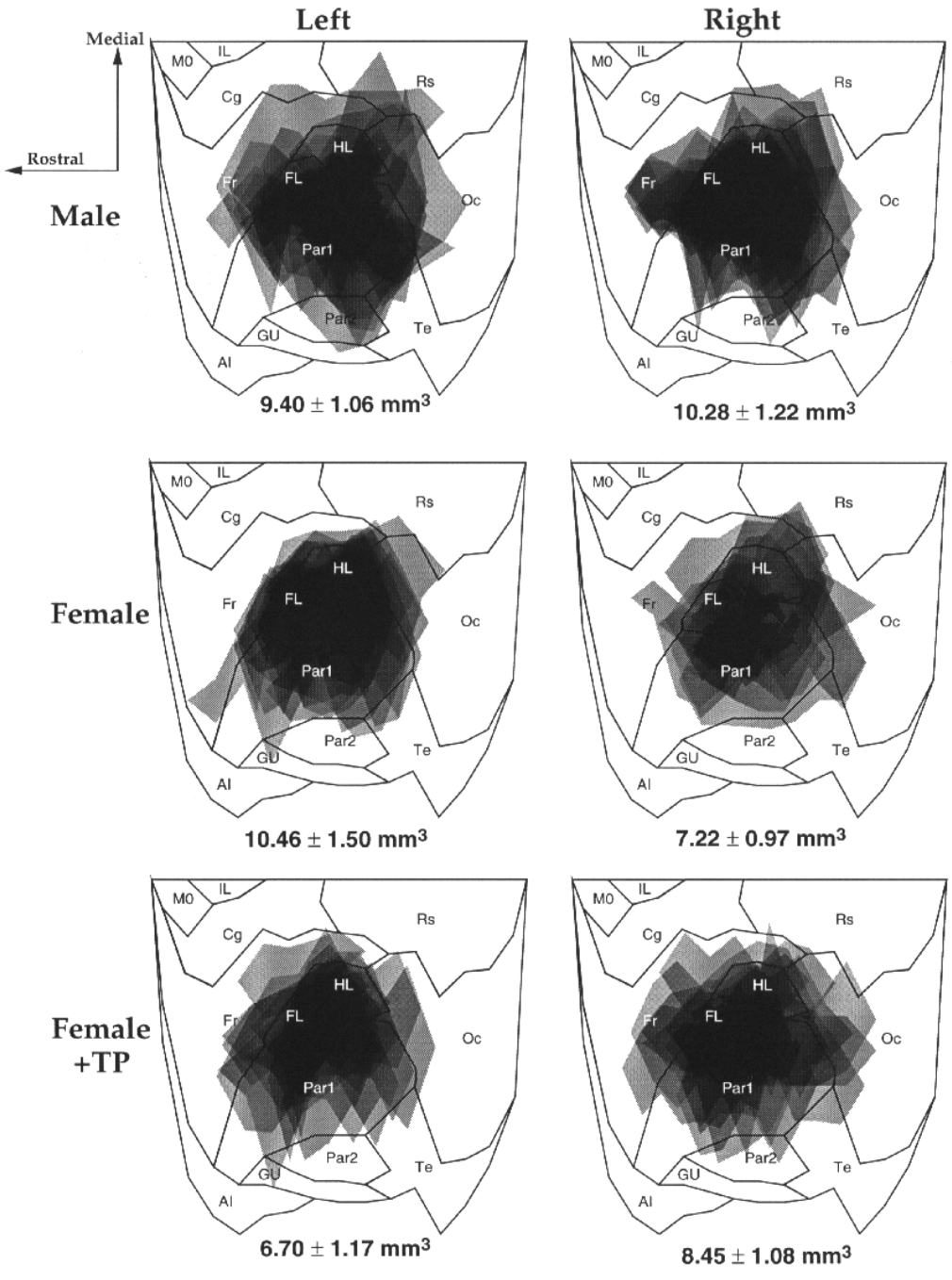

Figure 2. Topographic location of freezing lesions showing similarity in lesion placement among the experimental groups. Lesions from the left and right hemispheres of rats are plotted on a flattened, normalized map of the neocortex derived from Zilles (1985). Each lesion is plotted and areas where lesions overlap are indicated by progressively darker shades of gray. The means ± SEM (in mm^3) for the volume of the microgyri are found below each map. Abbreviations: Al, agranular insular (includes dorsal, posterior and ventral part); Cg, cingulate cortex (included Cg1–3); FL, forelimb area; Fr, frontal cortex (includes areas Fr1, Fr2 and Fr3); Gu, gustatory cortex; HL, hindlimb area; IL, infralimbic area of the medial frontal cortex; MO, medial orbital area; Oc, occipital cortex (includes all subdivisions of Oc1 and Oc2); Par1 and Par2, primary and secondary somatosensory cortices respectively; Rs, retrosplenial cortex (includes granular and agranular subdivisions); Te, primary auditory cortex, and temporal areas 2 and 3.

with 'Sex' (Male versus TP-treated Female) and Surgical Group (Lesion versus Sham) as the independent measures showed no significant main effects and no interactions for MGN volume and neuronal number. There was a significant main effect of Surgical Group for MGN neuronal size [$F(1,58) = 4.24$, $P < 0.05$], indicating that sham animals in both groups had larger neurons than lesioned animals (see Fig. 3). A two-way ANOVA with Sex (Male versus Female) and Surgical Group (Lesion versus Sham) as the independent measures showed no significant main effects or interactions for either MGN neuronal area or number. There was a significant Sex effect for MGN volume [$F(1,60) = 4.54$, $P < 0.05$] with males having larger MGN volumes than females. There was also a significant Sex × Surgical Group interaction for this variable [$F(1,60) = 4.13$, $P < 0.05$], and a *post-hoc* analysis

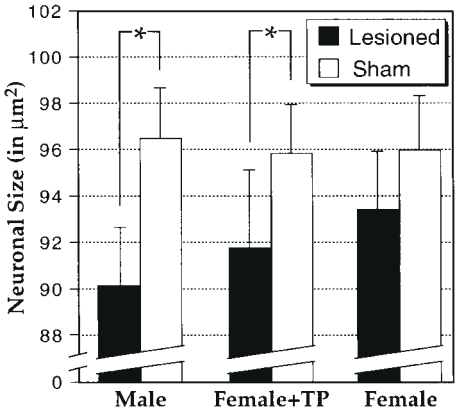

Figure 3. Histogram of MGN neuronal size in lesioned (black bars) and sham (white bars) Males (left), Females + TP (center) and Females (right). There is a significant difference in neuronal size between lesioned and sham animals in Males and Females + TP, but not in Females. Asterisk indicates $P < 0.05$.

indicated that MGN volume was larger in male shams as compared to female shams, but that there was no such difference between males and females with lesions (Table 1). Finally, a two-way ANOVA with 'Sex' (Female versus TP-treated Female) and Surgical Group (Lesion versus Sham) as the independent measures showed no significant main effects or interactions for any variable.

The ANOVA suggested that there were mean MGN neuronal size differences between sham and lesioned animals in the male and androgenized female groups, but not in untreated females. In order to more fully describe the potential differences in the groups, and to relate these findings to those found previously (Herman *et al.*, 1997), we assessed the distribution of neuronal sizes in the MGN. Thus, the frequency distribution of neurons in consecutive bins increasing by 15 mm^2 and ranging from 25 to >190 mm^2 was determined. As in the previous study (Herman *et al.*, 1997), chi-square values were calculated for the distribution of neurons in these bins in each of the three groups. To compensate for the sensitivity of this test, we set $\alpha = 0.001$ for rejection of the null hypothesis. The distribution of MGN neuronal sizes differed between lesioned and sham males, with the lesioned animals having more small and fewer large neurons ($\chi^2 = 116.62$, df = 11, $P < 0.001$). On the other hand, there was no difference in the distribution of MGN neuronal sizes between lesioned and sham females ($\chi^2 = 27.77$, df = 11, NS). These results replicate the previous study (Herman *et al.*, 1997). Androgenized females displayed a pattern similar to males: lesioned TP-treated females had more small and fewer large neurons than sham TP-treated females ($\chi^2 = 62.48$, df = 11, $P < 0.001$). These results are summarized in Figure 4.

To protect against the possibility that a few lesioned male subjects with large numbers of small neurons could shift the distribution of the entire population to the left, the percentage distribution of neurons for each individual subject was computed, using the same 12 bins as described above. A mean percentage frequency distribution was derived for each of the groups. Using the total number of measured neurons for each group, an 'unweighted' distribution of neuronal sizes was computed by multiplying the percentage for each bin by the total neuronal number. These unweighted distributions were then compared by chi-square analysis. As before, lesioned males had more small and fewer large MGN neurons than shams ($\chi^2 = 126.46$, df = 11, $P < 0.001$). Likewise, androgenized females

Table 1
Means ± SEM for morphometric measures of MGN

Experimental group	Microgyric volume (mm^3)	MGN volume (mm^3)	MGN neuronal number (×1000)	*n*
Male sham		1.91 ± 0.11[a]	146.48 ± 9.36	19
Male lesion	19.68 ± 1.99	1.65 ± 0.07	129.45 ± 6.21	15
Female sham		1.47 ± 0.07[a]	119.51 ± 9.15	18
Female lesion	17.68 ± 2.12	1.63 ± 0.17	144.70 ± 19.77	12
Female sham + TP		1.52 ± 0.13	120.97 ± 9.32	15
Female lesion + TP	15.14 ± 1.99	1.64 ± 0.11	126.89 ± 10.28	13

[a]Differ from each other ($P < 0.05$).

with lesions had more small and fewer large MGN neurons than shams ($\chi^2 = 61.48$, df = 11, $P < 0.001$). In contrast with the previous analysis, a significant difference in neuronal size distribution between lesioned females and shams was found ($\chi^2 = 53.68$, df = 11, $P < 0.001$). Inspection of the *post-hoc* cell contributions revealed that the last three bins accounted for the difference in distribution, yet those bins contained <3% of the total number of neurons. Therefore, these last three bins were excluded for all comparisons, and the chi-squares computed. As before, neurons were shifted towards smaller sizes in lesioned males versus shams ($\chi^2 = 120.45$, df = 8, $P < 0.001$) and in lesioned androgenized females versus shams ($\chi^2 = 57.15$, df = 8, $P < 0.001$). There was no difference in the distribution of MGN neuronal sizes between lesioned and sham females ($\chi^2 = 19.94$, df = 8, NS).

In order to test the distribution of the MGN neurons in the three subnuclei, the same chi-square analysis described above was performed within each subnucleus of the MGN. There was no difference in MGN neuron size distribution for any region in control females (dorsal $\chi^2 = 27.07$, ventral $\chi^2 = 9.81$, medial $\chi^2 = 20.59$; df = 11, NS). Lesioned males had more small and fewer large neurons in the dorsal, ventral and medial subnuclei of the MGN (dorsal $\chi^2 = 54.21$, ventral $\chi^2 = 47.68$, medial $\chi^2 = 32.86$; df = 11, $P < 0.001$ for all subnuclei). Lesioned TP-treated females had more small and fewer large neurons in the medial subnucleus of the MGN only (dorsal $\chi^2 = 24.76$, df = 11, NS; ventral $\chi^2 = 23.83$, df = 11, NS; medial $\chi^2 = 48.30$, df = 11, $P < 0.001$).

Discussion

In males and in androgenized females, animals with surgically induced cerebrocortical microgyria had smaller neurons in their MGN than their sham-operated counterparts. There was no such difference in untreated females. Further analysis of the distribution of neuronal sizes demonstrated that lesioned males had more small and fewer large MGN neurons. In contrast, there was no difference in neuronal size distribution between lesioned and sham females. These results replicate the findings of Herman *et al.* (1997). Furthermore, androgenized female rats with microgyria have more small and fewer large neurons than androgenized female shams, thereby suggesting that the thalamic sex difference in response to early injury to the cortical plate is mediated by perinatal gonadal steroids

Gonadal Steroids and Brain Injury

These results, taken together with those reported previously (Herman *et al.*, 1997), suggest that the reaction of the female brain to early injury is different from that of the male. If perinatal exposure to androgens acts to modify the brain's response to early injury, then the question can be asked as to the mechanism

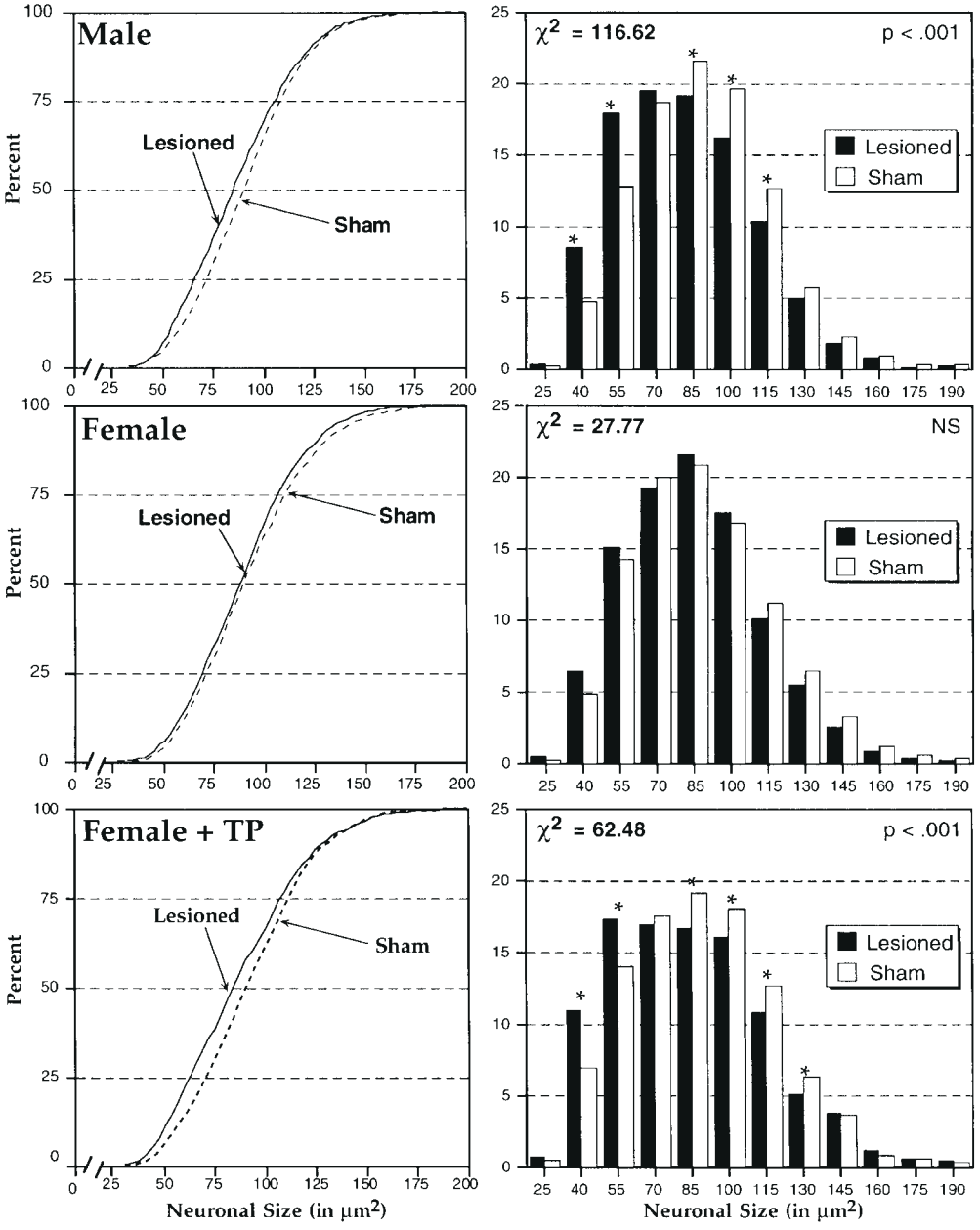

Figure 4. Cumulative percent (left) and histograms (right) illustrating the distribution of neuronal size in the MGN of males (top), females (middle) and androgenized females (bottom). In the cumulative percent graphs, note the nearly total overlap of distribution of the lesioned and sham females. In comparison, the distribution of the male lesioned MGN cells is significantly deviated toward the left (i.e. smaller) when compared with male shams. Similarly, the distribution of the female + TP lesioned MGN cells is significantly deviated toward the left (i.e. smaller) when compared with female TP shams. In the histograms, asterisks indicate a bin's significant *post-hoc* contribution to the χ^2.

for this change. It is well known that exogenous testosterone can affect the central nervous system either directly as testosterone, or through its metabolites estradiol and dihydrotestosterone (cf. MacLusky and Naftolin, 1981; Pomerantz *et al.*, 1985). The design of the current experiment does not allow us to distinguish which of these steroids, acting either alone or in some combination, are responsible for the effects reported here. Evidence from the literature, however, suggests a number of possibilities.

Gonadal steroids and their metabolites have been shown to play an important role in the reorganization of lesioned neural tissue. Injections of testosterone, estradiol or progesterone attenuate the reactive astrocytic response to penetrating wounds to the parietal cortex and underlying hippocampal structures (Garcia-Estrada *et al.*, 1993). Post-lesion treatment with TP decreased neuronal loss following axotomy of the hypoglossal and facial nerves (Yu, 1989). Others have shown that progesterone acts as a neuroprotectant (Roof *et al.*, 1992, 1993), and a variety of endogenous and exogenous androgens were shown to attenuate the effects of glutamate toxicity on spinal neurons (Ogata *et al.*, 1993). Male gerbils display greater postischemic neuronal necrosis than females, perhaps because of an antioxidant effect of endogenous estrogen (Hall *et al.*, 1991).

It should be pointed out, however, that the studies cited above investigated the efficacy of gonadal steroids as potential neuroprotective agents in the mature nervous system. The current study, in contrast, introduced exogenous testosterone and induced microgyria during the perinatal period. We found that there was no difference in lesion size between males and

females, but rather in secondary changes in the thalamus. Thus, the findings suggest that the sex differences in MGN neuronal size distribution could not be the result of sex differences in lesion size. Rather, the lack of a local neuroprotective effect (e.g. Rosen *et al.*, 1995a; Fitch *et al.*, 1997) by circulating androgens in this developmental brain injury model lends support to the hypothesis that the male/female differences reported here, as well as possibly the sex differences in the behavioral consequences of this type of induced cortical malformation, are the result of the effects of androgens on the reorganization of neuronal connectivity and trophic activity following early injury rather than on the modulation of the size or severity of the brain injury itself.

Connectivity, Early Brain Injury and Testosterone

Early damage to the developing brain can result in profound changes in connectivity. In hamsters, neonatal lesions of the superior colliculus result in altered retinal projections (Schneider, 1979, 1981; Finlay *et al.*, 1979). Maintenance of otherwise transient projections follows the induction of microgyria in cats (Innocenti and Berbel, 1991a,b), and unilateral removal of facial whiskers in newborn rats results in the maintenance of a normally transient cross-modal projection between the MGN and somatosensory cortex (Nicolelis *et al.*, 1991). Neonatal ischemia results in an excess of callosal projections from visual areas 17 and 18 in cats (Miller *et al.*, 1993), and an increase in callosal connectivity has been noted in a microgyric rat (Rosen *et al.*, 1989).

We had previously hypothesized that the changes in MGN neuronal size distribution in male rats with microgyria resulted from changes in connectivity following induction of microgyria (Herman *et al.*, 1997). If perinatal gonadal steroids mediate these post-injury changes in connectivity, then there are a number of potential mechanisms by which they could do so. It could be, as discussed above, that there is a protective effect of endogenous androgens in the females. Alternatively, exposure to testosterone might enhance the process of normal plasticity, thereby encouraging extraordinary restructuring of connectivity. Still further, some combination of these two factors could be at play. In the human, Raz *et al.*, (1995) reported that the cognitive sequelae of perinatal intracranial hemorrhage was greater in males than in females, thus supporting the notion of a greater vulnerability of the male brain to the effects of early injury. In rats, Kolb and Stewart (1995) found that neonatal TP treatment of females reduced the brain's ability to recover from the trauma of early prefrontal cortex lesions. In non-human primates, early damage to orbital prefrontal cortex had differential effects on the two sexes (Goldman *et al.*, 1974) that appeared to be mediated by gonadal steroids (Clark and Goldman-Rakic, 1989). Specifically, lesioned males performed worse than unlesioned males on an object discrimination task, whereas there was no difference between lesioned and unlesioned females. Treating females postnatally with testosterone masculinized their behavior (Clark and Goldman-Rakic, 1989).

There are several possibilities that may explain how gonadal steroids may modulate neuronal plasticity during normal and pathological conditions. One mechanism might be the effect of gonadal steroids on the formation and/or the elimination of synaptic contacts by modulation of the number of pre- and post-synaptic neurons (Garcia-Segura *et al.*, 1994). The effects of gonadal steroids on glial cells may also be important in neuronal remodeling, as described above (Garcia-Estrada *et al.*, 1993). Alternatively, sex steroids may mediate changes in the formation, growth and shape of dendrites, dendritic spines and axons. Thus, studies of organotypic cultures of the developing rodent hypothalamus, preoptic area and cerebral cortex reveal that estrogen elicits a striking enhancement of neurite growth, involving both axons and dendrites (Toran-Allerand, 1991). Gonadal steroids may, therefore, promote extensive remodeling of neuronal tissue after a brain insult, and not only in neuroendocrine regions of the brain (e.g. Nichizuka and Arai, 1982; Hammer and Jacobson, 1984; Ferreira and Caceres, 1991).

The combined activity of a variety of endogenous neurotrophic agents also regulates neuronal development, survival and repair after an injury (Barde, 1989, 1990). The forebrain regions of both sexes are potential targets for neurotrophins and estrogen, as well as sites of neurotrophin and estrogen synthesis (Toran-Allerand, 1996). Estrogen receptors co-localize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain (Toran-Allerand *et al.*, 1992). In addition, there is evidence for co-expression of mRNAs for both estrogen and neurotrophin receptors (Miranda *et al.*, 1993b), as well as evidence that estrogen target neurons in the developing rodent forebrain co-localize the mRNAs for the neurotrophins and their receptors (Miranda *et al.*, 1993a). Thus, working either in combination or cooperatively, estrogen and neurotrophins play crucial roles in neuronal development, survival and plasticity (Toran-Allerand, 1996).

Considering the current results in the light of these findings then, it could be that high levels of circulating androgens in the developing male brain may render it 'hyper-reactive', so that it responds to injury with extensive neuronal remodeling that is ultimately detrimental. One would predict, therefore, that any changes in connectivity associated with induction of microgyria would be greater in males than in females, and this hypothesis is currently being investigated. On the other hand, an argument could be made that circulating androgens in the male brain accelerate the process of connectional formation in such a way that it no longer responds to injury and is, in contrast to the hypothesis above, 'hypo-reactive'. If these neonatal rats were indeed hypo-plastic, then one would posit that they would have formed their adult patterns of connectivity prior to the lesion, and that the effects of neonatal lesions in these rats on MGN neuronal size would be identical to those following lesions in adulthood. While we consider this possibility to be unlikely, it cannot be excluded until the effects of adult lesions to the parietal cortex on cell size of the MGN are investigated.

Alternatively, it could be argued that estradiol in physiological doses may act as a neuroprotectant. As discussed above, we have no evidence that endogenous estrogens affect the freezing injury itself – the size of the microgyria is no different between males and females. Thus, any neuroprotective effect would have to occur during the purported connectional reorganization that follows the original injury. Future research aimed at eliminating endogenous estrogens without the introduction of exogenous testosterone will enable us to answer this question definitively.

An additional alternative is the possibility that the effects of androgens on the distribution of neuronal sizes in the MGN are activational rather than organizational. In this scenario, the cyclic ovarian hormone release post-pubertally in females would act to 'rescue' the neurons of the MGN from a change in distribution in reaction to the freezing lesion. Although there is evidence that exogenous treatment with TP in gonadectomized ferrets enlarges the soma of hypothalamic neurons (Cherry *et al.*, 1992), there is no evidence that estrogen or progesterone in physiological dosages affect neuronal size activationally. While

we think that the effects of circulating androgens acting activationally is unlikely, it cannot be excluded until the current experiment is replicated with the addition of pre-pubertal gonadectomies for all groups.

Summary and Conclusions

Previous investigation had indicated that freezing injury to the neocortex of neonatal male rats induces cerebrocortical microgyria and was associated with defects in fast auditory processing and a shift toward smaller neuronal sizes in the MGN (Herman *et al.*, 1997). No difference was observed in the MGN neuronal size distribution in microgyric females. The current study replicates these results and demonstrates that gonadal steroid hormones play a role in modulating the effects of freezing injury to the cortical plate on MGN neuronal size distribution. Specifically, early administration of TP to female rats eliminates the aforementioned sex difference in MGN neuronal size distribution. These findings suggest that the presence of circulating androgens in the developing male brain permits sex differences in the reorganization of the thalamus in response to cortical injury as compared to the female. While the current experiment cannot directly address the issue of mechanism underlying this gonadal steroid-mediated difference, we hypothesize that the presence of circulating androgens in the developing male brain may generate a 'hyper-plastic' condition such that there is more extensive neuronal remodeling after injury than in the female brain. Additional investigations will be required to delineate further how gonadal steroids modulate the brain response to early injury.

Notes

The authors gratefully acknowledge Drs Steven Yellon and R. Holly Fitch for their comments on an earlier draft of the manuscript. Heinz Windzio and Lisa Garcia provided expert technical assistance. This work was supported, in part, by PHS grant HD20806 and from a grant from the New England Branch of the Orton Dyslexia Society.

Address correspondence to Glenn D. Rosen, Department of Neurology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA. Email: glenn_rosen@bidmc.harvard.edu.

References

Barde YA (1989) Trophic factors and neuronal survival. Progr Growth Factor Res 2:1525–1534.

Barde YA (1990) The nerve growth factor family. Progr Growth Factor Res 2:237–48.

Boehm GW, Sherman GF, Hoplight BJ, Hyde LA, Waters NS, Bradway DM, Galaburda AM, Denenberg VH (1996) Learning and memory in the autoimmune BXSB mouse: effects of neocortical ectopias and environmental enrichment. Brain Res 726:11–22.

Cherry JA, Tobet SA, DeVoogd TJ, Baum MJ (1992) Effects of sex and androgen treatment on dendritic dimensions of neurons in the sexually dimorphic preoptic/anterior hypothalamic area of male and female ferrets. J Comp Neurol 323:577–85.

Clark AS, Goldman-Rakic PS (1989) Gonadal hormones influence the emergence of cortical function in nonhuman primates. Behav Neurosci 103:1287–1295.

Dvorák K, Feit J (1977) Migration of neuroblasts through partial necrosis of the cerebral cortex in newborn rats – contribution to the problems of morphological development and developmental period of cerebral microgyria. Acta Neuropathol 38:203–212.

Dvorák K, Feit J, Juránková Z (1978) Experimentally induced focal microgyria and status verrucosus deformis in rats – pathogenesis and interrelation histological and autoradiographical study. Acta Neuropathol 44:121–129.

Ferreira A, Caceres A (1991) Estrogen-enhanced neurite growth: evidence for a selective induction of Tau and stable microtubules. J Neurosci 11:392–400.

Ferrer I, Alcantara S, Marti E (1993) A 4-layered lissencephalic cortex induced by prenatal x-irradiation in the rat. Neuropathol Appl Neurobiol 19:74–81.

Finlay BL, Wilson KG, Schneider GE (1979) Anomalous ipsilateral retinotectal projections in Syrian hamsters with early lesions: topography and functional capacity. J Comp Neurol 183:721–740.

Fitch RH, Tallal P, Brown C, Galaburda AM, Rosen GD (1994) Induced microgyria and auditory temporal processing in rats: a model for language impairment? Cereb Cortex 4:260–270.

Fitch RH, Brown CP, Tallal P, Rosen GD (1997) Effects of sex and MK-801 on auditory-processing deficits associated with developmental microgyric lesions in rats. Behav Neurosci 111:404–412.

Garcia-Estrada J, Del Rio JA, Luquin S, Soriano E, Garcia-Segura LM (1993) Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. Brain Res 628:271–8.

Garcia-Segura LM, Chowen JA, Parducz A, Naftolin F (1994) Gonadal hormones as promoters of structural synaptic plasticity: cellular mechanisms. Progr Neurobiol 44:279–307.

Goldman PS, Crawford HT, Stokes LP, Galkin TW, Rosvold HE (1974) Sex-dependent behavioral effects of cerebral cortical lesions in the developing rhesus monkey. Science 186:540–542.

Gundersen HJG, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. J Microsc 147:229–263.

Hall ED, Pazara KE, Linseman KL (1991) Sex differences in postischemic neuronal necrosis in gerbils. J Cereb Blood Flow Metab 11:292–298.

Hammer RP, Jacobson CD (1984) Sex differences in dendritic development of the sexually dimorphic nucleus of the preoptic area in the rat. Int J Dev Neurosci 2:77–96.

Herman AE, Galaburda AM, Fitch HR, Carter AR, Rosen GD (1997) Cerebral microgyria, thalamic cell size and auditory temporal processing in male and female rats. Cereb Cortex 7:453–464.

Humphreys P, Rosen GD, Press DM, Sherman GF, Galaburda AM (1991) Freezing lesions of the newborn rat brain: a model for cerebrocortical microgyria. J Neuropathol Exp Neurol 50:145–160.

Innocenti GM, Berbel P (1991a) Analysis of an experimental cortical network: (i) architectonics of visual areas 17 and 18 after neonatal injections of ibotenic acid; similarities with human microgyria. J Neur Transplant 2:1–28.

Innocenti GM, Berbel P (1991b) Analysis of an experimental cortical network: (ii) connections of visual areas 17 and 18 after neonatal injections of ibotenic acid. J Neur Transplant 2:29–54.

Jacobs KM, Gutnick MJ, Prince DA (1996) Hyperexcitability in a model of cortical maldevelopment. Cereb Cortex 6:514–523.

Kolb B, Stewart J (1995) Changes in the neonatal gonadal hormonal environment prevent behavioral sparing and alter cortical morphogenesis after early frontal cortex lesions in male and female rats. Behav Neurosci 109:285–294.

Luhmann HJ, Raabe K (1996) Characterization of neuronal migration disorders in neocortical structures. 1. Expression of epileptiform activity in an animal model. Epilepsy Res 26:67–74.

MacLusky NJ, Naftolin F (1981) Sexual differentiation of the central nervous system. Science 211:1294–1303.

Marret S, Mukendi R, Gadisseux J, Gressens P, Evrard P (1995) Effect of ibotenate on brain development: an excitotoxic mouse model of microgyria and postthypoxic-like lesions. J Neuropathol Exp Neurol 54:358–370.

Miller B, Nagy D, Finlay BL, Chance B, Kobayashi A, Nioka S (1993) Consequences of reduced cerebral blood flow in brain development. I. Gross morphology, histology, and callosal connectivity. Exp Neurol 124:326–42.

Miranda RC, Sohrabji F, Toran-Allerand CD (1993a) Estrogen target neurons co-localize the mRNAs for the neurotrophins and their receptors during development: a basis for the interactions of estrogen and the neurotrophins. Mol Cell Neurosci 4:510–525.

Miranda RC, Sohrabji F, Toran-Allerand CD (1993b) Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. Proc Natl Acad Sci USA 90:6439–6443.

Nichizuka M, Arai Y (1982) Synapse formation in response to estrogen in the medial amygdala developing in the eye. Proc Natl Acad Sci USA 79:7024–7026.

Nicolelis MAL, Chapin JK, Lin RCS (1991) Neonatal whisker removal in rats stabilizes a transient projection from the auditory thalamus to the primary somatosensory cortex. Brain Res 567:133–139.

Ogata T, Nakamura Y, Tsuji K, Shibata T, Kataoka K (1993) Steroid

hormones protect spinal cord neurons from glutamate toxicity. Neuroscience 55:445–449.

Pomerantz SM, Fox TO, Sholl SA, Vito CC, Goy RW (1985) Androgen and estrogen receptors in fetal rhesus monkey brain and anterior pituitary. Endocrinology 116:83–89.

Raz S, Lauterbach MD, Hopkins TL, Glogowski BK, Porter CL (1995) A female advantage in cognitive recovery from early cerebral insult. Dev Psychol 31:958–966.

Roof RL, Duvdevani R, Stein DG (1992) Progesterone treatment attentuates brain edema following contusion injury in male and female rats. Restor Neurol Neurosci 4:425–427.

Roof RL, Duvdevani R, Stein DG (1993) Gender influences outcome of brain injury: progesterone plays a protective role. Brain Res 607:333–336.

Rosen GD, Harry JD (1990) Brain volume estimation from serial section measurements: a comparison of methodologies. J Neurosci Methods 35:115–124.

Rosen GD, Galaburda AM, Sherman GF (1989) Cerebrocortical microdysgenesis with anomalous callosal connections: a case study in the rat. Int J Neurosci 47:237–247.

Rosen GD, Press DM, Sherman GF, Galaburda AM (1992) The development of induced cerebrocortical microgyria in the rat. J Neuropathol Exp Neurol 51:601–611.

Rosen GD, Sigel EA, Sherman GF, Galaburda AM (1995a) The neuroprotective effects of MK-801 on the induction of microgyria by freezing injury to the newborn rat neocortex. Neuroscience 69:107–114.

Rosen GD, Waters NS, Galaburda AM, Denenberg VH (1995b) Behavioral consequences of neonatal injury of the neocortex. Brain Res 681:177–189.

Schneider GE (1979) Is it really better to have your brain lesion early? A revision of the 'Kennard principle'. Neuropsychologia 17:557–583.

Schneider GE (1981) Early lesions and abnormal neuronal connections. Trends Neurosci 4:187–192.

Schrott LM, Denenberg VH, Sherman GF, Waters NS, Rosen GD, Galaburda AM (1992) Environmental enrichment, neocortical ectopias, and behavior in the autoimmune NZB mouse. Dev Brain Res 67:85–93.

Sherman GF, Morrison L, Rosen GD, Behan PO, Galaburda AM (1990) Brain abnormalities in immune defective mice. Brain Res 532:25–33.

Suzuki M, Choi BH (1991) Repair and reconstruction of the cortical plate following closed cryogenic injury to the neonatal rat cerebrum. Acta Neuropathol 82:93–101.

Toran-Allerand CD (1991) Organotypic culture of the developing cerebral cortex and hypothalamus: relevance to sexual differentiation. Psychoneuroendocrinology 16:7–24.

Toran-Allerand CD (1996) Mechanisms of estrogen action during neural development: mediation by interactions with the neurotrophins and their receptors? J Steroid Biochem Mol Biol 56:169–78.

Toran-Allerand CD, Miranda RC, Bentham WD, Sohrabji F, Brown TJ, Hochberg RB, MacLusky NJ (1992) Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. Proc Natl Acad Sci USA 89:4668–4672.

Williams RW, Rakic P (1988) Three-dimensional counting: an accurate and direct method to estimate numbers of cells in sectioned material. J Comp Neurol 278:344–352.

Winer JA (1991) Anatomy of the medial geniculate body. In: Neurobiology of hearing: the central auditory system (Altschuler RA, Hoffman DW, Bobbin RP, Clopton BM eds), pp. 293–333. New York: Raven Press.

Yu WH (1989) Administration of testosterone attenuates neuronal loss following axotomy in the brain-stem motor nuclei of female rats. J Neurosci 9:3908–14.

Zilles K (1985) The cortex of the rat: a stereotaxic atlas. Berlin: Springer-Verlag.