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Effects of Sex and MK-801 on Auditory-Processing Deficits Associated With Developmental Microgyric Lesions in Rats

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Neonatally induced microgyric lesions produce defects in rapid auditory processing in adult male rats. Given that females across species are less susceptible to the deleterious effects of neural injury and that treatment with neuroprotective agents at the time of injury can reduce neural damage, the authors tested the effects of sex and neuroprotectant exposure on the behavioral consequences of microgyric lesions in rats. Results showed that sham but not microgyric males were able to perform the task at the fastest rate of stimulus presentation. Microgyric females, in contrast, discriminated at all stimulus conditions and did not differ from female shams. Microgyric males treated with MK-801 had reduced cortical damage and performed the discrimination at the fastest condition. Results suggest that females are less susceptible to the behavioral effects of neocortical microgyria and that MK-801 may ameliorate the behavioral consequences of these lesions in male rats.

Freezing injury to the cortical plate of newborn rats leads to the formation of a focal area of cortical microdysgenesis resembling four-layered microgyria (Dvorák & Feit, 1977; Dvorák, Feit, & Juránková, 1978; Ferrer, Alcántara, Catala, & Zujar, 1993; Humphreys, Rosen, Press, Sherman, & Galaburda, 1991; Marret, Mukendi, Gadisseux, Gressens, & Evrard, 1995; Rosen, Press, Sherman, & Galaburda, 1992; Suzuki & Choi, 1991), a malformation seen in a variety of neurologic disorders, including epilepsy, thantophoric dysplasia, and dyslexia (Barth & van der Harten, 1985; Dekaban, 1965; Ferrer, 1984; Galaburda & Kemper, 1979; Galaburda, Sherman, Rosen, Aboitiz, & Geschwind, 1985; Ho, Chang, Yang, & Chason, 1984; Levine, Fisher, & Caviness, 1974; Norman, 1980).

We have recently demonstrated that male rats with induced-microgyria show auditory-processing deficits on a 2-tone sequence discrimination task, specifically at total stimulus durations of 332 ms or less (Fitch, Tallal, Brown, Galaburda, & Rosen, 1994). These auditory-processing deficits are highly similar to those seen in language-impaired children, who exhibit performance deficits on a similar 2-tone discrimination task at stimulus durations of approximately 350 ms or less (Tallal et al., 1995; Tallal & Piercy, 1973; Tallal, Miller, & Fitch, 1993). It has been suggested that auditory-processing deficits seen in both language-impaired children and microgyric rats may reflect anomalies in the sensory systems that underlie rapid auditory-processing functions critical to phonological perception in humans (Tallal et al., 1993, 1995). Such a hypothesis is consistent with evidence of structural subcortical anomalies in the auditory thalamic nucleus of dyslexic brains (Galaburda, Menard, Rosen, & Livingstone, 1994).

In the current studies we sought to investigate what factors might protect against these detrimental behavioral consequences of early focal neocortical damage. We have shown that neonatal treatment with the neuroprotective agent dizocilpine (MK-801), a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, significantly reduces the amount of cortical damage seen in adult male rats with focal neonatal freezing injury (Rosen, Sigel, Sherman, & Galaburda, 1995). Although questions about the exact mechanism of action of this agent in this system remain, it appears that MK-801 blocks the cascade of neurotoxic steps that follow hypoxic–ischemic injury. Research has also shown that females exhibit a significant advantage over males in cognitive recovery from brain lesions as measured in premature infants (Raz et al., 1995), human adults (McGlone, 1980), and adult rats (Roof, Zhang, Glasier, & Stein, 1993). These findings are consistent with the observation that males are at greater risk for a wide variety of neurodevelopmental disorders (Gualtieri & Hicks, 1985), including language-based disorders with phonological processing components (Finucci, Issacs, Whitehouse, & Childs, 1983; Geschwind & Galaburda, 1985; Gualtieri & Hicks, 1985; Liederman & Flannery, 1993; Neils & Aram, 1986). Evidence of higher male prevalence for language disorder might indicate that males are more susceptible to the effects of early damage to the auditory-processing systems that

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have been shown to be critical to phonological and language development in humans (Tallal et al., 1993, 1995).

To test the effect of these factors on the behavioral consequences of early neocortical focal damage, we conducted two studies. In the first, male rats received focal bilateral neocortical freezing lesions or sham surgery on P1 (Postnatal Day 1), with concurrent saline or MK-801 treatment. In the second, male and female rats received either bilateral neocortical freezing lesions on P1 or sham surgery. All subjects were tested in an auditory-discrimination paradigm modeled on a task that has shown significant differences between language-impaired and control children (Tallal & Piercy, 1973; see also Fitch et al., 1994).

Method

Induction of Focal Necrotic Lesions

In Study 1, 6 pregnant female Wistar rats were obtained (Charles River, Wilmington, MA). On the day after birth (P1), male pups were gathered, randomly assigned to receive either bilateral freezing lesions or sham surgery, and redistributed to mothers in "litters" of 10. Treatment with MK-801 or saline was performed for a whole litter, because of potential interactive effects created by mothers' grooming both treated and untreated pups. However, assignment of subjects to lesion and sham groups was balanced within a litter. Focal necrotic lesions were then induced on the basis of modification of the technique used by Dvorák and colleagues (Dvorák & Feit, 1977; Dvorák et al., 1978) and reported in detail elsewhere (Humphreys et al., 1991; Rosen et al., 1992). In brief, pups were anesthetized by induction of hypothermia, and a small midline incision was made in the skin overlying the skull. For lesion subjects, a cooled (−70 °C) stainless steel probe (2 mm diameter) was placed for 5 s on the skull of lesion subjects, lateral to the midway point between bregma and lambda. The first hemisphere to receive the freezing lesion was randomly assigned. Sham subjects were prepared as described earlier, except that the probe was maintained at room temperature. After placement of the probe, the skin was quickly sutured, subjects were uniquely marked with ink injections to the footpads, warmed under a lamp, and returned to the mother.

In Study 2, 3 female Wistar rats were bred in-house by Glenn D. Rosen to avoid the potential effects of stress during shipping. At birth, litters were culled to 12 pups, evenly distributed by males and females. Subjects were randomly assigned to receive either bilateral freezing lesions or sham surgery (as described earlier), and again treatments were balanced within litters. Litters were weaned on P21 and the subjects were housed in groups (2–3/cage) with like-treat same-sex littermates until P45, when subjects from each study were individually marked with picric acid and shipped to R. Holly Fitch.

MK-801 Injections

Twenty subjects in Study 1 received an intraperitoneal injection of either saline or 2 mg/kg MK-801 (2 mg/ml) 0.5 hr prior to freezing injury and 6 and 14 hr after surgery. Another 17 rats were given MK-801 doses 0.5 hr prior to freezing injury and 6 hr later. Of the 20 subjects given 3 doses of MK-801, 10 did not survive (6 sham and 4 lesioned), a mortality rate consistent with previously published reports (Rosen et al., 1995). All 17 subjects treated with 2 doses of MK-801 survived. The 6 MK-801-treated bilaterally lesioned rats were from the 3 × 2 mg/kg group, as were 4 of the MK-801-treated unlesioned rats. The remaining 2 MK-801-treated unlesioned rats were randomly selected from the 2 × 2 mg/kg group.

Behavioral Testing

On receipt by R. Holly Fitch, subjects were individually housed in tubs. The behavioral testing was performed without awareness of group. At approximately P70, subjects were put on a water-restricted schedule and received ad libitum access to water for only 15 min per day.

The behavioral testing paradigm is described in detail in Fitch et al. (1994). Subjects were introduced to a modified operant-conditioning apparatus for training sessions of 30 to 40 min per day. The test apparatus consisted of a Plexiglas box modified by the attachment of a Plexiglas tube. The face of the tube was affixed to a plate containing a mechanical switch that the rat could operate with its nose, and a drinking tube below the switch. Audio microspeakers were affixed bilaterally over holes drilled in the Plexiglas tube. This apparatus was custom designed to allow shaping of subjects through a series of phases controlled by a Macintosh IIci computer (Apple Computer Inc., Cupertino, CA). Subjects were trained to insert their head into the tube (breaking an emitter-detector beam) and to hold this position for a period of 750 ms before pressing the illuminated nose button to obtain a water reward. Subjects received white-noise feedback to indicate correct positioning. Once able to consistently perform this task (48 trials/session), subjects were introduced to the auditory-discrimination paradigm.

Testing consisted of a go/no-go target identification task. Once in position, the subject was exposed to an auditory stimulus that consisted of a 2-tone sequence. The rat was required to assess whether this stimulus was a target (reinforced) or a nontarget (not reinforced) sequence. The full presentation of the stimulus was contingent on proper head placement of the subject; removal of the head during stimulus presentation resulted in an aborted trial and a 5-s time-out (all lights extinguished). The same tone sequence was then presented on the next trial. If proper head position was maintained for the duration of stimulus presentation, then the nose button was illuminated for a 3-s response interval. A press following the rat's target resulted in the presentation of water, whereas a press following a negative sequence resulted in a time-out of 45 s.

The stimuli were generated by a Macintosh IIci computer and were composed of two ramped sine-wave tones 20 ms in duration, separated by an interstimulus interval (ISI) of 500 ms. The low tone was 1100 Hz and the high tone was 2300 Hz, presented at a suprathreshold intensity of 75 db. Only hi–lo or lo–hi sequences were assigned as targets, and these were counterbalanced across rats and remained constant for each subject across testing sessions. Presentation of target and nontarget stimuli in a test session was random, with the constraint that half of the presentations be target (to maintain motivation), and that no more than 3 target or nontarget sequences occur in succession. Each daily session consisted of 48 trials.

After 6 days of testing at the foregoing stimulus parameters (20-ms tone, 500-ms ISI, 20-ms tone), the ISI for all sequences (including targets and nontargets) was reduced to 350 ms. All other parameters, including the assignment of each subject's target, remained constant. At the end of 6 days, the duration of the tones within the stimulus sequence was reduced from 20 to 16 ms each, and the ISI was reduced from 350 to 300 ms. After 6 days of testing, the tone durations were reduced to 12 ms and the ISI was reduced to 225 ms for another 6 days of testing. Finally, stimulus parameters were returned to the longest duration (20-ms tone, 500-ms ISI, 20-ms tone) for a final 6 days of testing. Thus there

were a total of 30 days of testing, with 6 days at each of 5 conditions defined by incrementally decreased (and finally, increased) stimulus durations.

For each test session the sequence of presentation on each trial and the corresponding response type (hit, false alarm, correct rejection, or miss) and latency to respond were recorded by a Macintosh IIci computer. All phases of training were controlled by programs written in the software program *LabView* specifically for this purpose.

Histology

After the completion of testing, subjects were deeply anesthetized with ketamine and xylazine and were transcardially perfused with 0.9% saline and 10% formalin. The skulls were extracted from the heads, placed in 10% formalin, and shipped to Glenn D. Rosen. There, the brains were removed from the skulls and were placed into fresh 10% formalin for 7 days, before being dehydrated in a series of graded alcohols and embedded in 12% celloidin (cf. Sherman, Galaburda, Behan, & Rosen, 1987). Serial sections were cut coronally at 30 μm, and a series of every 10th section was stained for Nissl substance with cresyllecht violet. Using a drawing tube attached to a photomicroscope (Zeiss Universal, Germany), both neocortical hemispheres were drawn from the frontal to occipital pole. In addition, the damaged area was traced, starting from the first section that showed any architectonic distortion and proceeding until the distortion had unambiguously disappeared. The damaged area was measured from these drawings using *NIH Image v1.54* on a Macintosh Centris 650 computer. Total microgyric volume was determined using Cavalieri's estimation (Rosen & Harry, 1990; see Fitch et al., 1994, for further details). The architectonic location of the lesion was also quantified by overlaying the topographic location on a normalized flattened map of the neocortex derived from Zilles (1985).

Results

We have previously shown that shorter latencies to respond to target as compared with nontarget stimuli provide a sensitive measure of target discrimination (Fitch, Brown, O'Connor, & Tallal, 1993; Fitch et al., 1994). Research with infants using a similar operant auditory-discrimination paradigm has also shown that this latency measure correlates well with percentage correct (Benasich & Tallal, 1994, 1996). Therefore, discrimination was assessed within each group as a function of response type (false alarm/hit) effects on response latency as a function of condition and day. Finally, data were compiled into discrimination indices (derived from the difference between false alarm [FA] and hit latencies for each subject, for each day of testing), which were analyzed using multiple-factor analyses of variance (ANOVAs) with treatment and sex as between-subject variables and condition and day as within-subject variables. In many cases one-tailed tests were used to assess replications or a priori hypothesized effects, and in such cases the use of one-tailed tests is noted.

First, however, we assessed whether data from like-treated groups could be pooled. In Study 1, we found no effect of 2 versus 3 injections of MK-801 on treated shams (mean FA/hit difference for 2× MK-801 = 50.6 ms, 3× MK-801 = 43.7 ms), and no effect of MK-801 versus saline treatment on male sham performance (mean FA/hit difference for MK-801 shams = 46 ms, saline-treated shams = 34.2 ms), hence these groups were pooled. We also found no significant differences between sham male performance for Studies 1 and 2, and hence all sham males from Studies 1 and 2 were pooled into a single group ($n = 18$). We also found no performance differences for untreated bilaterally lesioned males in Studies 1 and 2, and these groups were also pooled ($n = 12$).

Within-Group Analyses

Response latencies to target and nontarget stimuli were analyzed for all groups using multifactor ANOVAs, with treatment and/or study as between-subject variables and condition, day, and response type (false alarms vs. hits) as within-subject variables. Analyses of sham male data showed significant overall discrimination, $F(1, 16) = 27.6$, $p < .001$, and simple effects showed discrimination at each of the 5 conditions: Condition 1, $F(1, 16) = 8.05$, $p < .01$; Condition 2, $F(1, 16) = 9.9$, $p < .005$; Condition 3, $F(1, 16) = 17.4$, $p < .001$; Condition 4, $F(1, 16) = 24.8$, $p < .0001$; Condition 5, $F(1, 16) = 14.1$, $p < .01$; all tests one-tailed. Analyses of data from bilaterally lesioned male rats showed overall significant discrimination, $F(1, 10)$, $p < .02$, and significant discrimination at Condition 1, $F(1, 10) = 6.02$, $p < .02$; one-tailed, Condition 2, $F(1, 10) = 19.3$, $p < .001$; one-tailed, and Condition 3, $F(1, 10) = 3.96$, $p < .05$; one-tailed, but not Condition 4, $F(1, 10) = .24$, $p = .32$. There was near-significant discrimination, however, when lesioned males were returned to the slowest condition, 5, $F(1, 10) = 2.5$, $p = .073$ one-tailed.

Analyses on sham and bilaterally lesioned female rats showed significant discrimination for both groups overall, $F(1, 5) = 6.05$, $p < .05$; $F(1, 5) = 15.23$, $p < .01$. Simple effects showed near-significant or significant discrimination for all 5 conditions for sham females—Condition 1, $F(1, 5) = 3.2$, $p = .07$; Condition 2, $F(1, 5) = 5.4$, $p < .05$; Condition 3, $F(1, 5) = 10.3$, $p < .02$; Condition 4, $F(1, 5) = 5.2$, $p < .05$; Condition 5, $F(1, 5) = 2.9$, $p = .08$—one-tailed—and lesioned females—Condition 1, $F(1, 5) = 6.2$, $p < .05$; Condition 2, $F(1, 5) = 2.1$, $p = .1$; Condition 3, $F(1, 5) = 6.2$, $p < .05$; Condition 4, $F(1, 5) = 19.9$, $p < .01$; Condition 5, $F(1, 5) = 6.39$, $p < .05$, all tests, one-tailed.

The bilaterally lesioned male rats treated with MK-801 showed near-significant overall discrimination ($F(1, 5) = 2.9$, $p = .075$, one-tailed) but failed to show significant discrimination as measured by simple effects at Conditions 1, 2, or 3. Nevertheless, they did show significant discrimination at the fastest condition, Condition 4, $F(1, 5) = 4.4$, $p < .05$, one-tailed, where untreated bilaterally lesioned males had failed to discriminate. MK-801-treated, bilaterally lesioned males also showed significant discrimination when the slowest condition was presented again: Condition 5, $F(1, 5) = 7.6$, $p < .05$, one-tailed. The causes underlying poor performance by MK-801-treated lesioned males in the initial stages of testing is not clear, especially because MK-801 alone exerted no deleterious effects on sham male performance. Nevertheless, it is important to note that these subjects failed to show the rate-specific deficit seen in

untreated lesioned males in the current study and in Fitch et al. (1994).

Between-Groups Analyses

For male rats, treatment (sham vs. lesioned) interacted with condition, $F(4, 112) = 2.0$, $p = .05$, one-tailed, an effect which derived from significantly better performance for sham as compared with untreated lesioned males at the fastest condition, Condition 4, $F(1, 28) = 4.8$, $p < .02$ one-tailed (see Figure 1). This effect replicates a previously reported finding (Fitch et al., 1994). It is interesting that there were no differences between groups when they were returned to the slowest condition, Condition 5, $F(1, 28) = .66$, $p = .21$ one-tailed. These results are important, because they reveal that the decrement in lesioned male performance at Condition 4 was not a reflection of decreasing motivation or "failure to learn," but rather a processing deficit specific to rapid rates of stimulus presentation.

For female rats, there was no effect of treatment (sham vs. lesioned), overall or at any condition. However, when males and females were analyzed together, a main effect of sex, $F(1, 38) = 4.67$, $p < .05$, was observed, with females performing better than males. Analysis of simple effects at each of the five conditions revealed a marginal advantage of lesioned females over lesioned males at Condition 1, $F(1, 38) = 3.3$, $p = .077$, and a significant advantage of lesioned females over lesioned males at the fastest condition, Condition 4, $F(1, 38) = 4.3$, $p < .05$ (see Figure 1). Sham females did not differ significantly from sham males, overall or at any condition.

Analysis of MK-801-treated, bilaterally lesioned males versus untreated lesioned males revealed an interaction between treatment and condition, $F(4, 64) = 2.2$, $p < .05$, one-tailed[1] which reflected in part a near-significant advantage of MK-801-treated lesioned males over untreated lesioned males at Condition 4, $F(1, 16) = 2.3$, $p = .075$ one-tailed (see Figure 2). There were no significant differences between groups at Conditions 1, 2, 3, or 5. These results, when combined with evidence that MK-801-treated lesioned males but not untreated lesioned males showed significant discrimination at Condition 4, suggest that treatment with MK-801 concurrent to neonatal lesion induction ameliorated rate-specific processing deficits seen at the fastest condition.

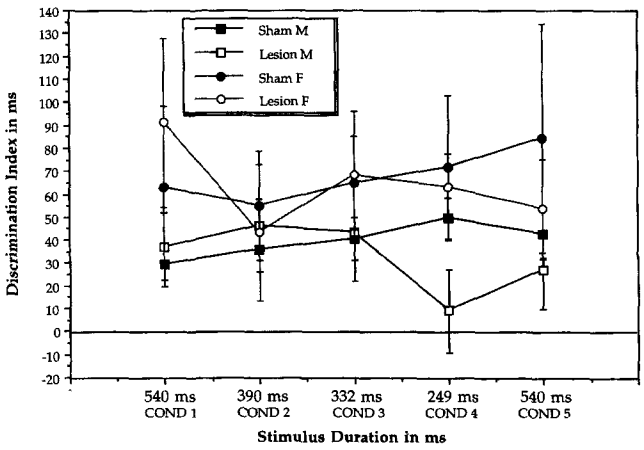

Figure 1. Discrimination indexes (vertical axis), as calculated by the false alarm/hit latency difference (in ms), for sham and lesioned male and female rats at the 5 stimulus duration conditions. Total auditory stimulus duration (horizontal axis) given in milliseconds. (M = male; F = female; COND = condition)

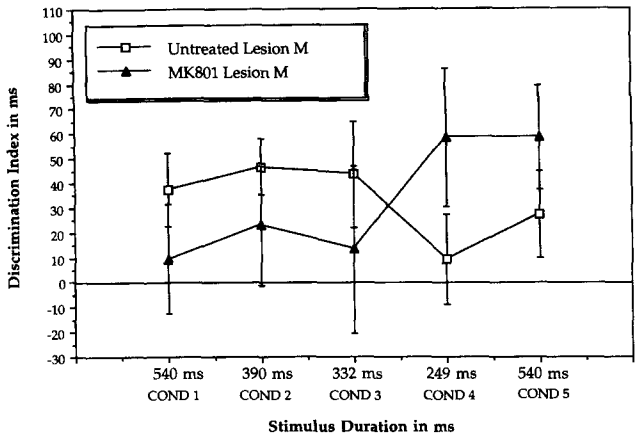

Figure 2. Discrimination indexes (vertical axis), as calculated by the false alarm/hit latency difference (in ms), for untreated and MK-801 treated lesioned male rats at the 5 stimulus duration conditions. Total auditory stimulus duration (horizontal axis) given in milliseconds. (M = male; F = female; COND = condition)

Learning Indexes

As a final note, we analyzed the time and amount of reinforcement required for all subjects to learn the initial operant task (pressing the nose button for water) as a function of experimental group. Analyses revealed that sham and lesioned female rats took significantly longer, $F(1, 38) = 11.23$, $p < .002$, and needed more reinforcement, $F(1, 38) = 6.2$, $p < .02$, to learn the task as compared with sham and lesioned male rats (MK-801-treated lesioned males were not included in this analysis). We interpret this as a reflection of greater exploratory behavior in females as compared with males during the operant sessions, a phenomenon noted and discussed in a prior study with male and female rats (Fitch et al., 1993). It is interesting that lesioned subjects did not require more time to learn the task, and in fact, as measured by amount of reinforcement, took marginally less time, $F(1, 38) = 3.04$, $p = .089$. MK-801-treated lesioned males took the least time and reinforcement of any group to learn the task.

[1] Earlier research has shown that MK-801 significantly reduces the size of microgyric lesions resulting from neonatal freezing damage (Rosen et al., 1995), and which lead to auditory temporal processing deficits in male rats (Fitch et al., 1994). Because a priori evidence predicted that MK-801 would specifically reduce auditory-processing deficits in lesioned males, a one-tailed test was used for this comparison.

Histological Analyses

Analyses were performed on the amount of cortical damage for all lesioned subjects as a function of sex and treatment (MK-801 vs. untreated males). No significant differences were seen between untreated bilaterally lesioned males and females, $F(1, 15) = .09$, *ns* (mean male lesion, total [both hemispheres] with *SEM* = 8.2 ± .94 mm^3; mean female lesion = 7.8 ± 1.2 mm^3). Lesioned areas were centered in the primary somatosensory cortex (Par 1) and the hindlimb (HL) and forelimb (FL) areas, with some encroachment into the lateral borders and the caudomedial portions of the frontal cortex (Fr) and the rostral-most portions of the occipital cortex. Two lesions (1 male and 1 female) extended into the border area between the secondary somatosensory cortex and the primary auditory cortex (see Figure 3).

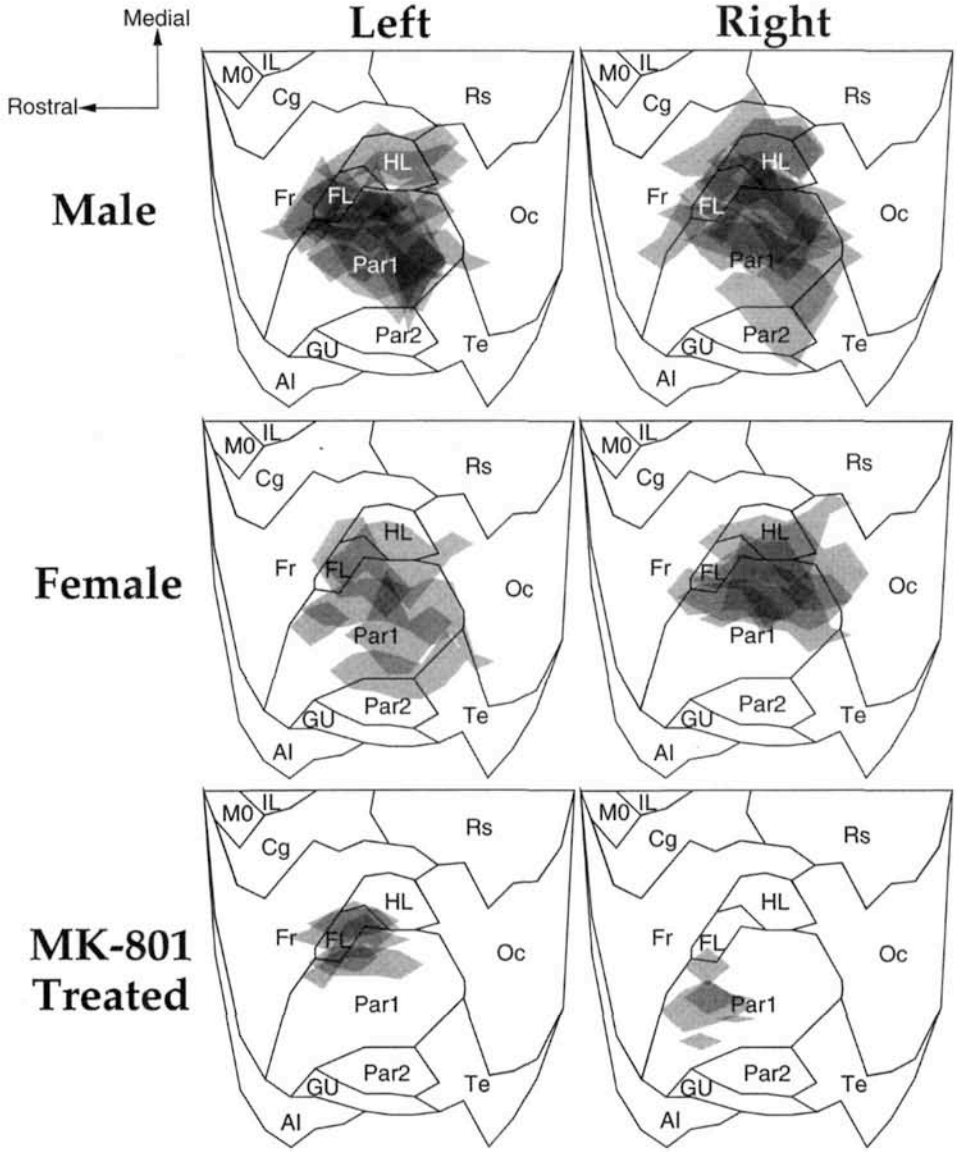

Figure 3. Topographic location of microgyric damage in untreated lesioned males, untreated lesioned females, and MK-801 treated lesioned males. Areas of damage are depicted on a flattened, normalized map of the neocortex derived from Zilles (1985). Each lesion is plotted, and areas of overlap are indicated by progressively darker shades of gray. AI = agranular insular (includes dorsal, posterior, and ventral part); Cg = cingulate cortex (included Cg1-3); FL = forelimb area; Fr = frontal cortex (includes areas Fr11, 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.

Lesioned males treated with MK-801 showed significantly less damage as compared with untreated lesioned males, $F(1, 15) = 12.3, p < .01$ (mean MK-801 lesion = 2.9 ± .58 mm^3), and lesioned areas for this group included predominently Par1, HL, and FL, with some extension into the lateral border with Fr (see Figure 3). (Note that the *df*s reflect the fact that a single brain treated with MK-801 was lost to histology because of unexpected death after behavioral testing but before perfusion.)

Discussion

The critical results presented here are summarized as follows.

1. Male rats that received bilateral, neonatally induced freezing lesions of sensory–motor (SM-I) cortex performed significantly worse than sham male rats on an auditory discrimination task at fast (249 ms) but not slow (332 ms or greater) rates of stimulus presentation (Figure 1). Moreover, although sham males showed significant discrimination at all conditions, lesioned males showed discrimination at the slower conditions but not the fastest (249 ms). These effects replicate previously reported findings (Fitch et al., 1994) and extend them by showing that this effect is not a reflection of decreasing motivation or "failure to learn" in lesioned subjects, because lesioned males showed recovery at the final (slowest) condition.

2. No differences between sham and lesioned female rats were seen at any condition, and lesioned females showed a significant advantage over lesioned males at the fastest condition (Figure 1). Moreover, lesioned females showed significant discrimination at the fastest condition, whereas lesioned males did not. These effects may derive in part from an overall female advantage on the task; although sham female performance was not significantly better than sham male performance, this effect might emerge with a larger *n*. Nevertheless, it is clear that bilaterally lesioned females do not show the rate-specific processing deficit evident for lesioned males.

3. Neonatally lesioned males treated concurrently with the non-competitive NMDA antagonist dizocilpine (MK-801) showed marginally better performance as compared with untreated lesioned males at the fastest rate of stimulus presentation and showed significant discrimination at this condition even though untreated lesioned males did not (Figure 2). Histological analysis confirmed a significant reduction in lesion damage for MK-801-treated subjects.

4. Performance deficits in lesioned male rats at a rapid rate of stimulus presentation do not appear to reflect generalized learning deficits, because lesioned male subjects learned the initial operant component of the paradigm as fast (or faster) than other groups and showed no deficits in performance at slow rates of stimulus presentation at the beginning and end of testing.

In previous studies, we had interpreted the deficit in rapid auditory processing seen for neonatally lesioned males as evidence of a neurophysiologic–behavioral connection between the pathologies seen in human dyslexic brains (Galaburda & Kemper, 1979; Galaburda et al., 1985; Humphreys, Kaufmann, & Galaburda, 1990) and auditory-processing deficits (both phonological and nonlinguistic) evidenced in language-impaired populations (Tallal et al., 1993). Current data from lesioned male rats again show a striking parallel to data obtained from language-impaired children on a comparable two-tone sequence discrimination task, wherein language-impaired children can discriminate longer duration stimuli but, unlike control children, fall to chance levels of performance at stimulus durations of 350 ms or less (Tallal & Piercy, 1973; Tallal et al., 1995). Our findings thus confirm that similar auditory-processing deficits characterize language-impaired children and male rats with induced developmental microgyric lesions.

Our new findings include the observation that treatment with MK-801, an agent that effectively ameliorates the extent of neuroanatomic damage following neonatal freezing injury (Rosen et al., 1995), appears to ameliorate the rate-specific processing deficit in male rats. MK-801-treated lesioned males, however, showed poor performance early in the testing and only successfully discriminated at Conditions 4 and 5. The cause or causes underlying these deficits are unclear, because MK-801 alone apparently had no deleterious effect on sham males. Further, these rats did not significantly differ from unlesioned males at any condition. Clearly this observation will require further study, possibly with other known neuroprotective agents and with a larger number of subjects.

Additionally, we present the intriguing new finding that neonatal freezing lesions do not appear to negatively affect the performance of female rats at rapid rates of stimulus presentation. As noted earlier, the significant advantage of lesioned females over lesioned males at the fastest rate of stimulus presentation may reflect, in part, a general advantage of females over males on the task. Although this argument appears inconsistent with the lack of significant differences between sham males and females, it is supported by the significant overall female advantage when sham and lesioned males and females are examined together. Further studies will be necessary to address this issue. One might also argue that further reduction in stimulus duration could elicit sham–lesion differences in the female group. Pilot data suggest that further reduction in total stimulus duration (to 175 ms) markedly reduces performance in intact subjects and may represent a minimal threshold for performance below which rats cannot discriminate (unpublished data). This remains another point for future study. Nevertheless, whatever the underlying mechanism, the net result of significantly better performance by lesioned females as compared with lesioned males at rapid rates of stimulus presentation is quite intriguing.

Researchers working with populations of developmentally language-disabled children have consistently observed skewed ratios of boys to girls, sometimes ranging as high as 4:1 (Finucci et al., 1983; Gualtieri & Hicks, 1985; Liederman & Flannery, 1993; Neils & Aram, 1986). Although claims of ascertainment bias against boys have rendered these observations controversial (e.g., Shaywitz, Shaywitz, Fletcher, & Escobar, 1990), at least one large study has looked retrospectively at a population obtained prior to

clinical diagnosis (i.e., avoiding ascertainment issues) and found significantly more dyslexic boys as compared with girls (Liederman & Flannery, 1993). Commenting on a possible biological basis for these gender differences, Geschwind and Galaburda speculated in 1985 that perinatal exposure to androgens in mammals may render males more susceptible than their female counterparts to perturbation from the normal course of development, particularly with respect to anomalous cerebral laterality. They further speculated that androgens may act on the male brain by slowing maturation rate and shifting or prolonging the window of susceptibility to neural damage. Research on human dyslexic brains and animal models of developmental neuropathology suggest that focal ischemic neural injury (of unknown origin) occurs in impaired individuals during the latter trimester, and that such injury interferes with critical phases of neuronal migration, possibly resulting in pervasive reorganization of the brain (Galaburda, 1993). An obvious question thus arises regarding the comparability of physiologic damage in impaired males and females. Analysis of human dyslexic brains suggests some differences in the nature of anomalies that characterize female as compared with male dyslexic brains, differences consistent with damage at a later point in neurodevelopment in females (Humphreys et al., 1990). However, the small sample size in the study has restricted interpretations that might be made of these differences.

In our current study, we did not see marked differences in the degree or percentage of damage at the cortical level in lesioned male and female rats, suggesting that the sex effects do not stem from differences in susceptibility to initial cortical damage. Nevertheless, separate analysis of cell size and cell number of the medial geniculate nucleus (MGN) of male and female rats with and without microgyric lesions (Herman et al., 1995, 1996) has shown that there are more small and fewer large neurons in the MGN of bilaterally lesioned males as compared with their unlesioned counterparts. In contrast, there are no differences in cell size between lesioned and unlesioned females. Moreover, correlations between MGN morphology and behavioral performance on the auditory discrimination task at the fastest condition were found for lesioned and sham females and sham males but not lesioned males. Such a finding is extremely interesting in light of evidence of neural anomalies in the MGN of dyslexic humans (Galaburda et al., 1994) and suggests that animal models may in fact provide critical insights into the neurodevelopmental factors that underlie auditory-processing deficits evidenced in language-impaired human populations.

Herman et al. (1996) postulated that sex differences in thalamic morphology associated with cerebral microgyria may reflect underlying sex differences in neural maturation rates, which render female rats less susceptible to perturbation from normal development at the time of P1 cortical damage. In this respect, findings reported herein and by Herman et al. are consistent with speculations made by Geschwind and Galaburda (1985) more than a decade ago, as well as other reports documenting gender differences in maturational rate (e.g., Bachevalier & Hagger, 1991; Maccoby & Jacklin, 1974; Stewart, Kuhnemann, & Rajabi, 1991; Taylor, 1969). Other possible interpretations of the female advantage following neonatal damage on the current task include sex differences in general cerebral organization, a hypothesis supported by an ever-growing wealth of human literature (e.g., Halpern, 1990; Kimura, 1983; Kimura & Harshman, 1984; Kulynych, Vladar, Jones, & Weinberger, 1994; McGlone, 1980; Shaywitz et al., 1995; Witelson, 1991; Wood, Flowers, & Naylor, 1991) and animal literature (e.g., Bachevalier, Brickson, Hagger, & Mishkin, 1990; Bachevalier, Hagger, & Bercu, 1989; Clark & Goldman-Rakic, 1989; Diamond, Dowling, & Johnson, 1981; Fitch et al., 1993; Goldman, Crawford, Stokes, Galkin, & Rosvold, 1974; Roof, Zhang et al., 1993; Stewart et al., 1991; Stewart & Kolb, 1988; see also Breedlove, 1992; Tobet & Fox, 1992). Animal research has also shown sex differences in physiologic response to neural damage (e.g., Hall, Pazara, & Linman, 1991; Kolb & Stewart, 1995; Loy & Milner, 1980). Finally, research with infants supports gender differences in cognitive response to brain injury, as evidenced by significantly better cognitive outcome for premature girls as compared with boys with intracranial bleeds of similar magnitude (Raz et al., 1995). It is not clear, however, whether the latter result reflects neurodevelopmental sex differences, organizational sex differences, or a potential interaction between hormonal milieu, neural organization, and site and timing of damage.

As a final note, progesterone been shown to act as a neuroprotectant against contusion injury in rats as measured by cortical lesion size and behavioral recovery on cognitive tasks (Roof, Duvdevani, Braswell, & Stein, 1994; Roof, Duvdevani, & Stein, 1993). These results suggest that ovarian steroids, which become active in female rats around postnatal days 5–8 (e.g., Funkenstein, Nimrod, & Linder, 1980; Sokka & Huhtaniemi, 1995; Weniger, Zeis, & Chouraqui, 1993), may also play a role in the neural reorganization that accompanies focal neonatal damage. Although in the current experiment we found no differences in gross cortical damage in male and female rats, differences in the distributions of neuronal sizes were seen in the MGN of male but not female lesioned rats (Herman, Fitch, Galaburda, & Rosen, 1995; Herman, Galaburda, Fitch, Carter, & Rosen, 1996). An interesting finding has been reported by Roof et al. (1994), who found that progesterone reduced the amount of neuronal degeneration seen in the medial dorsal thalamic nucleus following damage to the medial frontal cortex. As such, it is possible that ovarian steroids may influence the growth and development of thalamocortical projections and may facilitate preservation or effective reorganization of critical sensory pathways in females subject to perinatal neurodevelopmental injury.

To summarize, the results first suggest that MK-801 may ameliorate both the behavioral and anatomic effects of microgyric lesions in male rats, although this result will clearly require further investigation with more subjects and possibly other neuroprotective agents. Second, neonatally induced microgyric lesions produced auditory-processing deficits in male but not female rats, despite equivalent cortical damage. Related studies suggest that this effect may

relate to anomalies that are seen in the MGN of lesioned male but not female rats (Herman et al., 1995; in review). At a more general level, this result may reflect a decreased susceptibility among females to the behavioral effects of freezing injury to the developing neocortex. Further studies will be required to determine the mechanisms that underlie this female advantage.

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