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Research report

Sex differences in rapid auditory processing deficits in microgyric rats

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Abstract

Early neocortical injury has been associated with rate-specific auditory processing deficits using rodent models. In the few cases where females were studied, they appeared less vulnerable than males to the behavioral consequences of early neocortical injury. In the current study, male rats with neocortical microgyria were found to exhibit significant impairments in detecting tone sequences at short but not long inter-stimulus intervals (ISI) as compared to sham-operated male littermates. Microgyric females, however, performed similarly to sham-operated female littermates on this task at all durations. Current findings support an association between focal cortical malformations and impaired rapid auditory processing in males, and less vulnerability in females to the behavioral consequences of these malformations on a task eliminating confounds of motivation, experience, and estrus.

Theme: Disorders of the nervous system
Topic: Developmental disorders

Keywords: Acoustic startle response; Auditory discrimination; Dyslexia; Language impairment; Neuromigrational anomaly; Estrous cycle

1. Introduction

Males are diagnosed with neurodevelopmental disorders (including but not limited to epilepsy, hyperactivity, autism, cerebral palsy, mental retardation and dyslexia) more often than females [13]. Within the dyslexic population, numerous researchers report a predominance of males, and some have postulated the existence of a biological "risk factor" putting males at greater risk than females (see [2,13,17,27]); although others suggest that a non-biological reason, such as an assessment bias, causes more males to be identified as dyslexic (see [24]). In a recent population-based birth cohort study, however, boys were two to three times more likely than girls to be identified as reading disabled regardless of the identification method employed (non-regression or regression-based discrepancy or low achievement; [17]). Post-mortem analysis of the brains of dyslexic humans indicates the high occurrence of focal neocortical anomalies (e.g. microgyria, heterotopias) located predominately in frontal and peri-sylvian regions of the left hemisphere [9,10].

Behaviorally, dyslexics and children with language impairments have deficits in processing rapidly occurring stimuli in many sensory modalities. Similarly, infant auditory processing thresholds (as assessed by the detection of the shortest gap between two complex tone pairs) predicts later language outcome [3,4]. Further, infants with a positive family history for language impairments exhibit higher auditory processing thresholds than infants with negative family histories, and subsequently, have poorer language scores when assessed at 16, 24, and 36 months of age [3,4]. In general, children with specific language impairment (SLI) show deficits at processing rapidly occurring sensory events, regardless of whether the stimuli are verbal or non-verbal (e.g. [19,26]). Once in elementary school, up to 80% of children diagnosed with SLI will meet the criteria for dyslexia [25].

By assessing rapid auditory processing abilities, rodent models of the neocortical malformations seen in dyslexic brains provide an ability to empirically address both the association between cortical anomalies and processing

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impairments, as well as putative sex differences associated with language disabilities. For example, Fitch et al. reported that male, but not female, microgyric rats were impaired at discriminating their target at short inter-stimulus intervals (ISI) in a go/no-go operant two-tone auditory discrimination task [8]. Interestingly, morphometric analysis revealed that the microgyric lesions produced in both female and male rats involved comparable amounts of cortical damage [14]. More recently, Clark et al. [5] used an acoustic startle response paradigm (which eliminates confounds of motivation and experience from testing) to confirm that male microgyric rats exhibit impaired discrimination of an oddball tone pair for short (<40 ms) but not long ISI. The current study extends the Clark et al. [5] study to include both male and female subjects. In addition, female subjects' estrous cycles were monitored to test whether the hormonal state of females might affect the ability to attenuate in the startle paradigm.

2. Methods

2.1. Subjects

Subjects included a total of 67 male and 56 female Wistar rats from three separate studies born to time mated dams (Charles River Laboratories, Wilmington, MA) at the University of Connecticut. All studies received similar treatment with respect to the induction of the focal microgyric lesion on postnatal day 1 (P1; see below). Subjects in Study 1 and 2 included microgyric males and females (Study 1 and 2: $n=10$ males, 10 females) and sham-operated littermates (Study 1: $n=10$ males, 10 females; Study 2: $n=5$ males, 5 females). In Study 3 subjects were comprised of males and females that received pre- and/or post-natal injections of sesame oil (as control for a treatment not reported here), along with P1 microgyric or sham treatment ($n=16$ microgyric males, 16 sham males, 11 microgyric females, 10 sham females). Prenatal injections (s.c. 0.5 ml sesame oil) were given to the dam on embryonic days 16–21, and postnatal injections (s.c. 0.05 ml sesame oil) were given to pups on P1–5.

All pups were weaned on P21, and housed using a 12-h light:12-h dark cycle with food and water available ad libitum. Testing began in adulthood after P60. University of Connecticut's Institutional Animal Care and Use Committee (IACUC) approved all procedures. Procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, including adequate measures to minimize pain or discomfort to the animals.

2.2. Induction of focal microgyria

On P1 litters were culled to 10 pups, which were randomly designated to receive bilateral freezing lesion or sham surgery, balancing treatment groups within litters. Based on a modification of the technique employed by Dvorák and associates [6,7], similarly sized and shaped focal microgyric lesions were induced. In brief, pups in the lesion condition received hypothermic anesthesia followed by a small midline incision over the skull. A cooled stainless steel probe (-70 °C; 2 mm diameter) was placed on the skull for 5 s, approximately 2 mm lateral of the sagittal suture and 2 mm caudal of bregma. Following the initial lesion in a randomly determined hemisphere, an identical lesion was placed in the opposite hemisphere with a second probe. Sham surgeries were identical with the exception that the probe was maintained at room temperature. Following treatment, the skin was rapidly sutured and subjects marked with ink footpad injections, warmed under a lamp, and returned to the dam.

2.3. Behavioral testing—startle reduction

The modified startle reduction paradigm (described in detail elsewhere; [5,20]), presents a benign pre-stimulus prior to a startle-eliciting stimulus (SES). If the pre-stimulus is detected, the amplitude of the acoustic startle response is reduced, compared to uncued trials, in proportion to the overall detectability of the pre-stimulus. The trial interval for all procedures averaged 20 s (range of 16–24 s). All subjects received 5 days of a single tone procedure. Single tone data for Study 1 was analyzed for estrous effects (see below). Additionally, for all subjects, Day 1 attenuated results were used as a covariate measure to control for baseline startle response differences on the oddball procedure. Attenuated response was calculated by (cued response/uncued response × 100). The Oddball procedure employed four stimulus conditions using 225, 75, 40, or 10 ms as the ISI between the two-tones (2.3 and 1.1 kHz). These parameters remained constant within the 1-day test session (i.e. one ISI employed each day of testing). The repeated presentation of a standard stimulus (high/low two-tone sequence) served as background. On cued trials, the cue was the standard stimulus in reverse order (low/high) presented just prior to the SES.

2.3.1. Apparatus

During testing, each subject was placed on a load-cell platform (MED Associates, Georgia, VT). The platform's output voltage was passed through a linear amplifier (Med Associates Model#250-60) and into a Biopac MP100WS Acquisition system connected to a Power Macintosh 7200 to record the amplitude of the subject's acoustic startle response. Maximum peak values were extracted during the 150 ms directly following the onset of the SES, representing the subject's response amplitude for that trial (dependent variable). Auditory stimuli were generated on a Power Macintosh 6100 using custom programmed software with programmable frequency output. Stimulus files (one file per session) were played using SoundHack 0.881NF and pre-

sented through powered Yamaha YHT-M100 speakers. Sound intensity levels were checked at subject level before testing using a hand held sound level meter (Radio Shack).

2.4. Estrous cycle assessment

A subset of female animals (from Study 1) was assessed following daily startle testing to establish day of estrus. Day of cycle was confirmed by examining the cellular composition of individual vaginal smears (see [1]). Startle reduction data was then analyzed for putative estrous effects using day of cycle as a within variable.

2.5. Brain analysis

Following behavioral testing, subjects were weighed, anesthetized and transcardially perfused with fixative (10% buffered formalin phosphate). Heads were removed, placed in formalin, and shipped to GDR for anatomical analysis. The brains were removed, lesions confirmed and location visually assessed.

3. Results

3.1. Brain analysis

Post-mortem analysis confirmed four-layered bilateral microgyria in all subjects exposed to the P1 freezing lesion treatment (located in sensorimotor cortex (SM-I) including regions Par1, Par2, HL, and FL). No differences were observed between microgyria in males and females (similar to previous findings [8]; see Fig. 1), and no malformations were seen in any sham subject.

3.2. Estrous cycle results

Data from Study 1 females are shown as an average of the 5 days of repeated single tone, which comprised all four phases of estrus for all subjects (see Table 1). No main effect of Estrus ($F_{3,61} < 1$), or interaction with Condition ($F_{3,61} < 1$) or Day ($F_{12,61} < 1$), was found. A significant Day effect was found ($F_{4,61} = 21.9$; $P < 0.001$), reflecting the improvement in startle reduction in response to the cue across test sessions (i.e. days). No differences between sham and microgyric rats were seen ($F_{1,61} < 1$). Oddball results also showed no effect of Estrus or interaction with Condition (data not shown). Therefore, further analyses were performed without consideration of estrus.

Table 1
No significant startle result differences on the single tone procedure over estrus

Day of estrus	Attenuated response (± S.E.)
Proestrus	46.7 (± 3.54)
Estrus	48.9 (± 3.23)
Metestrus	48.3 (± 3.29)
Diestrus	46.0 (± 4.42)

3.3. Oddball results

Cued and uncued scores were compared separately for all groups across all studies. Cued scores were significantly lower ($P < 0.01$) than uncued scores for all study groups at all ISI conditions, indicating significant detection of the oddball cue at all ISI tested. For study and group comparisons, attenuated responses were tabulated, and Day 1 attenuated response scores from the single tone procedure were used as a covariate to control for baseline startle differences.

Analyses were performed on males and females separately to assess whether data from Studies 1 to 3 could be pooled. For males, a MANOVA of the oddball attenuated responses, using the variables of Study (three levels), Condition (two levels, sham/lesion), and ISI (four levels), showed a main effect of Study ($F_{2,60} = 25.4$, $P < 0.001$) but no interaction of Study × Condition ($F_{2,60} < 1$) nor Study × Condition × ISI ($F_{6,183} = 1.02$, n.s.). Therefore, we concluded that male data from the three studies could be pooled. A similar MANOVA

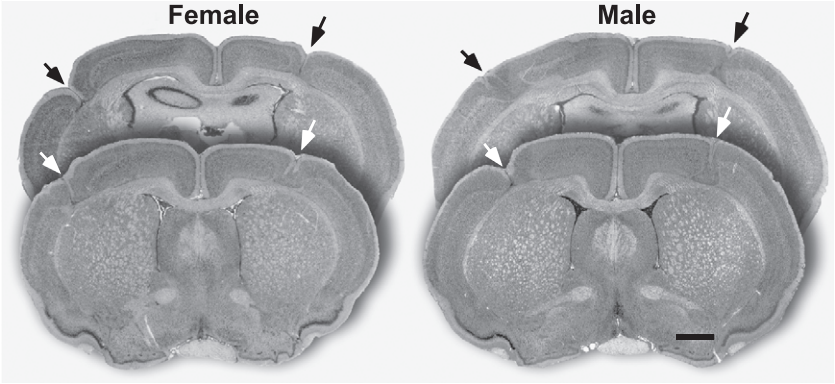

Fig. 1. Brightfield photomicrographs of matched rostral and caudal Nissl-stained sections from a female and a male rat with induced bilateral microgyria (arrows). There are no sex differences in size or location of the malformation (bar = 1 mm; see [8] for further details).

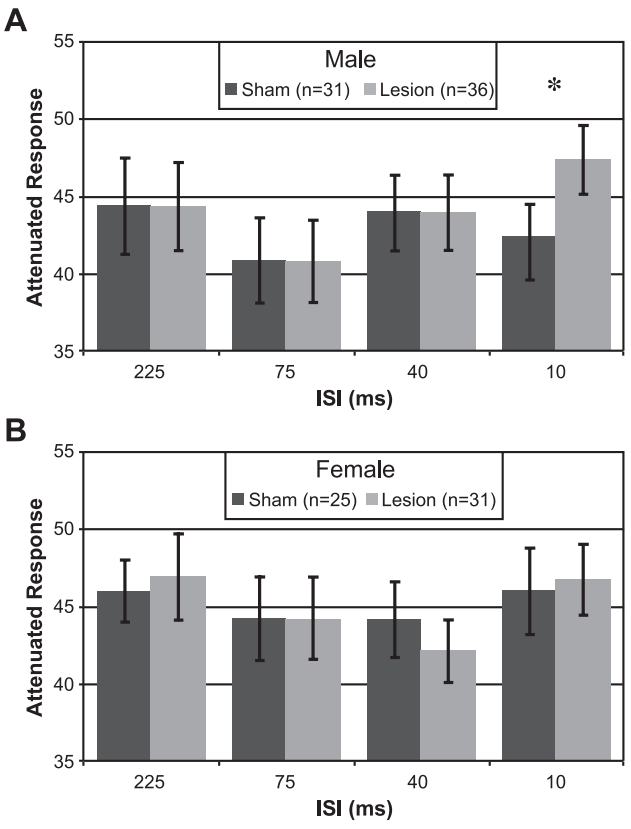

Fig. 2. Oddball results are presented as adjusted means by co-varying each subject's attenuated response on the first day of single tone. Attenuated response is the cued response divided by the uncued response and multiplied by 100. Sham and lesion males (A) differed only at the shortest ISI tested (*$P<0.03$, one-tailed), with lesion males worse than shams. Sham and lesion females, (B) did not significantly differ at any ISI tested. Error bars depict S.E.

analysis of female oddball attenuated responses revealed a significant main effect of Study ($F_{2,47}=6.58$, $P<0.01$), but no interaction of Study × Condition ($F_{2,47}<1$) or Study × Condition × ISI ($F_{6,144}<1$), and the subjects from the three studies were pooled.

Performance for shams and lesions was assessed at each ISI for males and females separately (see Fig. 2). Previous research indicates allows the directional prediction that microgyric subjects will show poorer performance than sham littermates [5,8,20]; therefore, one-tailed tests for significance were used for analysis of performance. Male LSD test indicated that lesion males performed significantly worse than sham males at the 10 ms ISI ($F_{1,58}=4.77$, $P<0.03$). However, no differences were found at the longer ISI durations. In the female LSD test, no significant differences were found between sham and lesion females at any ISI duration (for 10 ms $F_{1,47}<1$, $P=0.52$).

4. Discussion

We report that male but not female microgyric rats demonstrate impairments in detecting quickly changing acoustic stimuli as compared to their respective sham littermates, even though the microgyric injury is similar in both sexes. Interestingly, microgyric and sham males did not differ in discriminating more slowly changing stimuli for this same task. These findings are consistent with data from other species exhibiting neuromigrational anomalies (i.e. ectopic BXSB/MpJ mice, see [20]), and support prior research on microgyric rats using a different testing paradigm (see [8]). Taken together, these results support an association between rapid auditory processing deficits and neuromigrational anomalies, specifically in males.

An important aside in the current study is that no apparent estrous cycle effects were seen on acoustic startle results for females, although estrous effects have been shown to affect pre-pulse inhibition or startle reduction in rats (see [18]). It is possible that strain/supplier differences associated with startle behavior may also affect how the estrous cycle influences pre-pulse inhibition. The current study used both a different strain of rat and a different supplier than Koch [18]. The lack of a hormone effect in this case does not reflect on sex differences in auditory processing deficits associated with injury, but merely reflects a lack of activational effects by estrogens on the acoustic startle circuit and baseline auditory acuity in female rats.

The specific mechanisms by which cortical anomalies lead to auditory processing deficits remain to be established. One potential mechanism, supported by evidence of morphological thalamic changes in humans with dyslexia [10], could involve changes in neural connectivity as a result of cortical malformations forming during neuromigration [11,12,16,23]. Similar effects are seen in male, but again not female, microgyric rats and ectopic mice [8,16]. These findings suggest that afferent and efferent thalamic connection and callosal projections may be disrupted by focal cortical anomalies, and/or otherwise transient connections present at the time of injury may be maintained [11,12,23]. One potential biological mechanism that could mediate this differential response between the sexes includes differential gonadal hormones circulating before, during, and after perinatal injury (see [22]).

The post-mortem analysis of dyslexic brains suggests that the proportion of focal neural anomalies that occur in the brains of male and female dyslexics may differ [2,9,10,15]: males tend to develop more neuromigrational anomalies (e.g. microgyria, heterotopias) while females develop more glial scarring [2,10]. Some researchers have speculated that the events predicating neuromigrational anomalies in human fetuses occur during the 4th or 5th month of gestation, and that the tendency for females to mature faster than males may underlie the discrepancy between the sexes for the type of anomaly seen in the post-mortem analysis [2]. In addition, males may be more susceptible to the adverse effects of injury than females, and therefore, develop more severe forms of neuromigrational anomalies from similar injury. Along these lines, evidence for the greater susceptibility of males as compared to

females for neurodevelopmental impairment continues to mount. With respect to cognitive outcome following intracranial hemorrhages, the most common form of insult in the premature infant, boys perform significantly worse than girls on standardized intelligence tests, even when perinatal risk factors and degree of insult are controlled [21]. The disadvantageous outcome in males may stem from enhanced vulnerability of the central nervous system to early insult, and this vulnerability may also compromise later functional recovery explaining the greater incidence of neurological disorders diagnosed in males.

In conclusion, male microgyric rats are impaired compared to male shams at detecting rapidly, but not slowly, changing auditory stimuli. Female microgyric rats, on the other hand, do not exhibit an auditory processing deficit as compared to female shams for short duration stimuli. These results support previous evidence of sex differences in the behavioral response to the types of early neocortical injury responsible for neuromigrational anomalies that are similar to those seen in the brains of dyslexics. These results support the hypothesis that sex differences in the incidence of neurodevelopmental disorders may be related to increased susceptibility of the male brain to injury during critical periods of brain development.

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