Sex differences in rapid auditory processing deficits in ectopic BXSB/MpJ mice

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Received 8 August 2002; accepted 8 October 2002

DOI: 10.1097/01.wnr.0000044223.79663.3b

Prior research with rodent models, performed predominantly in males, has demonstrated a significant association between focal neocortical malformations (e.g. ectopias and microgyria) and rate-specific auditory processing deficits. In the current study and consistent with prior findings, we report that ectopic male BXSB/MpJ mice exhibit impairments in detecting a two-tone oddball stimulus at short but not long inter-stimulus interval durations when compared to non-ectopic male littermates. However, ectopic female littermates showed no rapid auditory processing deficit when compared to non-ectopic females on this same task. Current results add growing support to: (1) an association between focal cortical malformations and impaired auditory processing in males; and (2) the existence of sex differences in the behavioral consequences of focal cortical malformations. *NeuroReport* 13:2277–2280 © 2002 Lippincott Williams & Wilkins.

Key words: Acoustic startle response; Auditory discrimination; Cortical malformations; Dyslexia; Language impairments; Neuromigrational anomalies

INTRODUCTION

Males are reported to be at a disadvantage compared to females in the incidence of neurodevelopmental disorders. That is, males appear to develop disorders such as epilepsy, autism, hyperactivity, mental retardation, cerebral palsy, and dyslexia significantly more often than females [1]. With respect to dyslexia, other researchers have also reported a higher incidence in males [2], although this finding remains controversial [3].

Research in adult dyslexics has also revealed auditory processing deficits in this population (see [4] for review). A rapid auditory processing deficit is defined as an inability to correctly process and comprehend quickly occurring or changing acoustic stimuli [5]. The auditory processing deficits seen in dyslexics are comparable to those found in children identified as having specific language impairment (SLI or LI; [5]). Not surprisingly, research has shown that up to 80% of LI children go on to be diagnosed with reading disabilities in elementary school (i.e. dyslexia) [6]. Tallal and colleagues [7] have suggested that auditory processing deficits may be one causal factor in disrupting language acquisition and may impose cascading effects on the development of other language related skills.

Neurobiological research has yet to reveal clear and consistent diagnostic features of LI and dyslexia. The brains of a few dyslexic individuals (some with suggested language disorder) have been analyzed post-mortem, and shown to exhibit focal neuromigrational cortical anomalies, including focal microgyria, molecular layer ectopias (ectopias), and neocortical dysplasias [8]. Evidence further suggests a sex difference in the properties of these cortical anomalies, with males having the tendency to develop migrational anomalies (e.g. microgyria or ectopias), and females to develop focal glial scarring and ectopias [9,10]. At the present time, statistical analysis of this observation is not possible due to the small numbers studied.

Research on similar cortical malformations in rodent models has in turn revealed an association with auditory processing deficits. For example, numerous studies have demonstrated deficits in two-tone sequence discrimination in adult male rats with induced focal microgyria, specifically for short but not long stimulus durations [11–13]. Moreover, research has shown that female littermates do not exhibit these behavioral deficits following induced focal microgyria, even though the amount of cortical damage per se was equivalent to males [12,14]. In BXSB/MpJ and NZB/BINJ mice, which exhibit spontaneous developmental ectopias (morphologically similar to those found in dyslexics), additional evidence of auditory processing deficits in males has been reported, although ectopic females have not previously been assessed [15,16]. BXSB/MpJ mice develop ectopias in frontal cortex, while NZB/BINJ mice develop ectopias in the SM-I cortical region. Although these strains exhibit malformations in different cortical locations, a rapid auditory processing impairment has been demonstrated in ectopic males of both strains as measured in an

acoustic startle reflex paradigm, and with auditory event related potentials (AERP; [15–17]).

In the current study, both male and female ectopic and non-ectopic BXSB/MpJ mice were assessed with a behavioral auditory processing task (two-tone oddball) to further characterize the consequences of focal cortical malformations as a function of sex.

MATERIALS AND METHODS

Subjects: Subjects were 35 male and 20 female BXSB/MpJ mice born at the University of Connecticut. Mice received food and water ad lib and were maintained on a 12:12 h light:dark cycle (lights on at 06.00 h). At weaning, mice were individually housed for the duration of testing, beginning around postnatal day 35 (P35). All testing was performed blind to histological condition, which was identified at histological assessment. Due to equipment limitations, subjects were tested in two sets at different times. Data analyses revealed that Set failed to interact with Histology on any measure, and thus Set was dropped as a variable from final analyses.

Reflex modification paradigm: The reflex modification paradigm consists of the presentation of a benign auditory pre-stimulus (cue) just prior to a startle-eliciting stimulus (SES). The SES is a 50 ms 105 dB white noise burst that elicits an acoustic startle reflex (ASR). When the cue is detected, the amplitude of the whole-body ASR elicited by the SES is reduced or attenuated (also called pre-pulse inhibition). The extent of attenuation is related to the overall detectability of the cue.

During testing, each subject was placed on a PHM-250 load cell platform (Med Associates, Georgia, VT). The platform's output voltage was passed through a PHM-250-60 linear load cell amplifier and into a Biopac MP100WS Acquisition system (Biopac Systems, Santa Barbara, CA) connected to a Power Macintosh 7200 to record the amplitude of the subject's ASR. Maximum peak values were extracted during the 150 ms directly following the onset of the SES and represent the subject's response amplitude for that trial (dependent variable). Auditory stimuli were generated on a Power Macintosh 6100 and played via powered Yamaha YHT-M100 speakers.

Repeated presentation of a standard stimulus, consisting of a 75 dB high/low two-tone sequence (2300 Hz and 1100 Hz, respectively), served as background. Tones (7 ms) were separated by a within-stimulus inter-stimulus interval (ISI) of 500, 225, 75, 50, or 10 ms duration, which remained constant within a test session. All two-tone sequences were separated by a between-sequence ISI, which was always 200 ms greater than the within-stimulus ISI in order to maintain the perceptual contiguity of the tone pair. Test sessions included 104 trials and were presented across five consecutive days at each within stimulus ISI. On uncued trials, the standard stimulus preceded the 105 dB SES by 50 ms. For cued trials, an oddball stimulus (comprising the same tones used in the standard sequence but in reverse order, i.e. low/high) was presented 50 ms before the SES (see sample trials in Fig. 1). As reported previously [13], data were pooled to assess performance at long (500 and 225 ms) *vs* short (75, 40, and 10 ms) ISI.

Attenuated response values are calculated by dividing the cued response by the uncued and multiplying by 100.

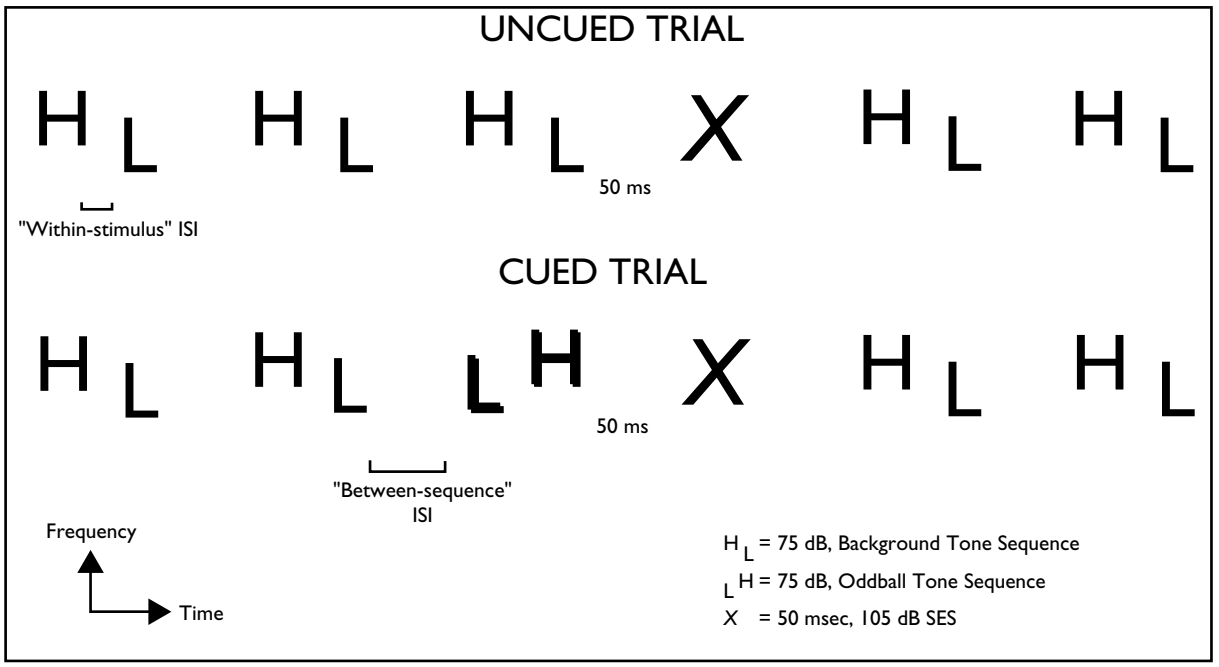

Fig. 1. An illustration of the startle Two-Tone Oddball procedure used in behavioral testing (modified from [13]). H indicates the high tone at 2300 Hz and L indicates the low tone at 1100 Hz. Within-stimulus ISI was presented at 500, 225, 75, 40 or 10 ms for 1 week (between-stimulus ISI = within-stimulus ISI + 200 ms). If a subject detects the oddball stimulus in a cued trial, the response elicited by the SES will be reduced as compared to that of uncued trials. Therefore, attenuation of the startle response provides an index of cue detection.

Therefore a higher attenuated response reflects less attenuation by the pre-pulse cue (i.e. poorer discrimination), while lower attenuated response reflects greater auditory cue discrimination. All ANOVAs on male data were performed as one-tailed tests because previous results [13,16] allowed directional predictions in the present data set (one-tailed p-values, denoted $\hat{p}$).

Anatomical analysis of brains: At the conclusion of testing, mice were anesthetized and transcardially perfused with fixative (phosphate-buffered 10% formalin, Fischer Scientific). Heads were removed, placed in fresh fixative, and shipped to GDR at Beth Israel Hospital for histological processing and anatomical analysis. The brains were removed, placed in fresh fixative (one week) and dehydrated (ethanol and ethanol/ether). Brains were then embedded in celloidin and serially sectioned at 30 μm in the coronal plane. Every fifth section was mounted in series on glass slides and stained (cresyl violet for Nissl substance). Sections were examined for the presence and location of cortical ectopias or other neuroanatomical abnormalities. Hemispheric and architectonic location of the ectopias, and any other abnormalities, were recorded.

RESULTS

Histology: Of 35 male BXSB/MpJ mice tested, 18 showed no neuropathology and 17 (48.6%) had one or more neocortical ectopias (see Fig. 2 for sample). 29 total ectopias were identified in males (six subjects had multiple ectopias: five doubles and one triple), located in the frontal cortex (nine right, five left), primary somatosensory cortex (nine right, four left), right secondary somatosensory cortex (one), and left occipital cortex (one). Of 20 female BXSB/MpJ mice tested, eight showed no neuropathology and 12 (60%) had one or more neocortical ectopias. Thirteen total ectopias were identified in females (one subject had two ectopias), located in the frontal cortex (two right, nine left), right primary somatosensory cortex (one), and right occipital cortex (one).

Two-tone oddball detection: All subject groups showed significant startle attenuation on cued trials at all ISI conditions ($p < 0.01$). Comparing attenuated response values for long and short ISI, a marginal main effect of sex ($F(1,50) = 3.65$, $p = 0.06$) was found, with females overall performing better than males. Further analyses were performed separately for the sexes. Females showed no main effect of ectopia ($F(1,18) < 1$, ns), nor interaction between ectopia and ISI duration ($F(1,18) < 1$, ns). Males showed no main effect of ectopia ($F(1,32) = 1.09$; $\hat{p} = 0.15$, ns), but the interaction between ectopia and ISI duration was nearly significant ($F(1,32) = 2.62$; $\hat{p} = 0.06$). A Scheffé test of this interaction (Fig. 3) shows that at long duration ISIs, male ectopic and non-ectopic mice did not differ ($p = 0.5$), but at short durations ectopic males were significantly worse than non-ectopics ($p < 0.01$).

As an aside, the possibility was noted that apparent ectopia effects in males but not females could reflect the higher incidence of multi-ectopias in males. To investigate this possibility, data was re-analyzed using single ectopic animals only (thus six multi-ectopic males and one multi-ectopic female were dropped). Results showed that significantly worse performance on short duration stimuli was still seen for single ectopic males compared with non-ectopic males ($p < 0.01$); and performance scores for ectopic females were unchanged. Further comparing multi-ectopic to single-ectopic males, performance scores for long and short duration stimuli were similar (indeed, single ectopic males had a mean performance score slightly worse than multi-ectopic male littermates at short durations (83.5 *vs* 84.7, respectively), but this group difference was not significant; $F < 1$). These results are consistent with prior findings indicating that number of ectopias does not alter

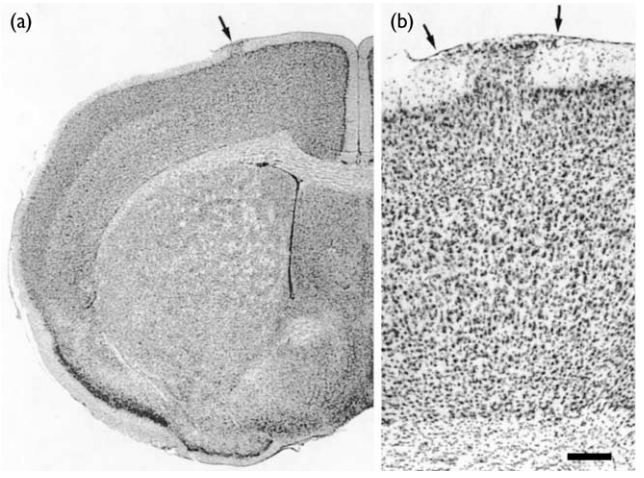

Fig. 2. Ectopic collection of neurons in a BXSB/BINJ mouse (arrows). BXSB/MpJ ectopias are typically found in prefrontal or motor cortical layer I areas. (**b**) The same ectopia as (**a**) but at higher magnification to show the ectopia's typical mushroom-like appearance. Bar = 400 μm (a), 100 μm for b.

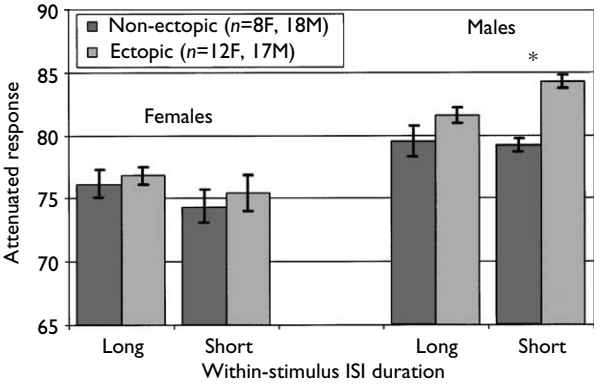

Fig. 3. Ectopic *vs* non-ectopic attenuated response to the variable ISI durations presented in the two-tone oddball procedure. Comparing attenuated response values for long (500 and 225 ms) and short (75, 40, and 10 ms) conditions, ectopic and non-ectopic females had no main effect of ectopia ($F(1,18) = 0.05$; $p = 0.82$, ns). Males show no main effect of ectopia ($F(1,32) = 1.09$; $p = 0.15$, ns) but the interaction between ectopia and ISI duration was near significant ($F(1,32) = 2.62$; $p = 0.06$). A Scheffé test of the interaction revealed that at long duration ISI male ectopic and non-ectopic mice did not differ ($p = 0.5$), but at short duration ISI ectopics were significantly worse than non-ectopics (*$p < 0.01$).

behavioral deficits in mice [15,17] and supports the view that this variable does not account for sex differences in the behavioral consequence of ectopias. This interpretation is also consistent with evidence that similar malformations (microgyric lesions) cause auditory processing deficits in male but not female rats, despite equivalent damaged cortical area [12]. However, future studies will continue to consider this issue.

DISCUSSION

Deficits in rapid auditory processing, which seem to be functionally related to impaired speech perception and overall language development (including reading), are consistently seen in behavioral studies on individuals with developmental language disabilities. Concurrent post-mortem studies of the brains of dyslexics have revealed focal neocortical malformations. A line of evidence is also emerging that suggests that males are more susceptible than females to the adverse effects of these neocortical malformations. The current study employs an animal model to further examine putative relationships between basic auditory processing, cortical malformations, and sex. Using a reflex modification procedure, we found that ectopic male but not female BXSB/MpJ mice exhibit impaired detection of a two-tone oddball at short ISI durations, while at longer durations male and female ectopic detection of the two-tone oddball did not differ from their respective non-ectopic littermates.

Taken with previously reported findings of sex differences in the behavioral effects of induced focal microgyria in a rat model [12,14], we see that the pattern of an impairment in males but not females, and for short but not long duration stimuli, is remarkably consistent, even though the malformations and species differ. The specific mechanisms by which cortical anomalies lead to the auditory processing deficits observed here still remain to be elucidated. One potential mechanism, supported by evidence of morphological thalamic changes in dyslexic humans, as well as male but not female microgyric rats and male ectopic mice, could involve changes in neural connectivity as a result of cortical malformations [14,19,20]. That is, afferent and efferent thalamic connections may be disrupted by focal cortical anomalies [21], and/or otherwise transient connections present at the time of injury may be maintained [22].

A sex difference in response to cortical malformations may further reflect differences in gonadal steroid hormones. Once bound to a receptor, steroids alter gene expression, potentially leading to alterations in cell growth, proliferation, or death that can affect a brain area's size, cell number, or packing density (see [23] for review). Early migrational patterns, myelination, and dendritic growth can also be influenced [24]. Interestingly, Rosen and colleagues [25] found that the morphological changes seen in the auditory thalamus (MGN) of microgyric males were also seen in testosterone treated microgyric females. Oil-treated microgyric females did not show a morphological thalamic change as compared to sham females [14,25], suggesting that the early presence of testosterone directly modifies reorganizational response to early injury and associated behavioral consequences [25].

CONCLUSIONS

The current study provides additional behavioral evidence that developmental focal anomalies in the cerebral cortex are associated with basic rapid auditory processing deficits in male but not female subjects. Specifically, male ectopic BXSB/MpJ mice have poorer discrimination than non-ectopic male littermates of an oddball tone pair when the ISI is short (75 ms) but not long ($>$ 75 ms). Female ectopic BXSB/MpJ show no discrimination impairments at any duration as compared to non-ectopic female littermates. Results support the notion that the response to early neuromigrational anomalies in the presence of testosterone may be more deleterious than in the absence of testosterone. This phenomenon may contribute to reports of disparity between males and females in the incidence and expression of LI and dyslexia.

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Acknowledgements: This research was supported by NIH Grant HD20806.