

# Perceptual auditory gap detection deficits in male BXSB mice with cerebrocortical ectopias

Matthew G. Clark, Gordon F. Sherman,<sup>1</sup> Heather A. Bimonte<sup>2</sup> and R. Holly Fitch<sup>2,CA</sup>

Center for Molecular and Behavioral Neuroscience, Rutgers University; <sup>1</sup>Beth Israel Deaconess Medical Center, Harvard Medical School; <sup>2</sup>Biobehavioral Sciences Graduate Degree Program and Department of Psychology, University of Connecticut, 3107 Horse Barn Hill Rd U-154, Storrs, CT 06269, USA

<sup>CA</sup>Corresponding Author

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Underlying impairments in rapid auditory processing may contribute to disrupted phonological processing, which in turn characterizes developmental language impairment (LI). Identification of a neurobiological feature of LI that is associated with auditory deficits would further support this model. Accordingly, we found that adult male rats with induced cortical malformations were impaired in rapid auditory processing. Since 40–60% of BXSB mice exhibit spontaneous focal

cerebrocortical ectopias (as seen in dyslexics brains), we assessed auditory gap detection in adult male BXSB mice. Ectopic mice were significantly worse than non-ectopics in detecting a 5 ms silent gap, but were not significantly impaired at longer gap durations (10–100 ms). Our results confirm that focal cortical malformations are associated with impairments in rapid auditory processing. *NeuroReport* 11:693–696 © 2000 Lippincott Williams & Wilkins.

**Key words:** Auditory discrimination; Cortex; Dyslexia; Language impairment; Phonological deficits; Reflex modification

## INTRODUCTION

Developmental dyslexia is defined by significant limitations in reading and/or language development that cannot be explained by known factors (e.g. mental retardation, frank brain damage, hearing loss or deprivation). Moreover, dyslexia has consistently been associated with fundamental deficits in phonological processing [1]. Tallal has proposed that impairments in rapid auditory processing may disrupt the developmental perception of speech sounds, contributing in turn to impaired phonological processing as seen in both early childhood LI and later dyslexia [2–5]. However, the underlying etiology of rapid auditory processing impairments and other down-stream perceptual processing deficits remains unclear.

Concomitant postmortem assessment of human brains from individuals with dyslexia has revealed the presence of focal cortical malformations including neocortical ectopias, dysplasias, glial scarring and microgyria [6]. These developmental anomalies are predominantly observed in perisylvian and inferior prefrontal regions. While direct linkage of these focal malformations to behavioral features of dyslexia has proved difficult in humans (given neuroimaging limitations), recent application of a novel animal model has bridged the structural/functional dissociation.

Specifically, Fitch and colleagues [7–9] demonstrated that adult male rats with induced bilateral microgyria in SM-I, occipital or frontal cortices exhibited significant deficits in processing brief auditory stimuli followed in rapid succession by other acoustic information. These same subjects were not impaired in discriminating longer duration stimuli, thus excluding interpretation of more general learning or processing deficits.

BXSB/MpJ-Yaa mice provide a further opportunity to examine the relationship of focal developmental anomalies to auditory processing, since neocortical ectopias occur spontaneously in ~40–60% of these mice [10]. The malformations are primarily located in prefrontal/motor regions and are observed as mushroom-like protrusions of neuronal cell bodies into the molecular layer of cerebral cortex. Previous research has demonstrated that ectopic BXSB mice exhibit differences relative to unaffected littermates on a variety of behavioral learning and memory measures [11]. However, auditory processing functions have not been assessed. Thus, we examined gap detection in male BXSB mice.

Gap detection is a simple and well-accepted method of measuring auditory temporal resolution. In normal human children, Trehub and Henderson [12] found that temporal

resolution in infancy was significantly predictive of subsequent language development in the toddler/preschooler years. Research specifically examining gap detection in children with developmental language disabilities has also demonstrated that some reading impaired children exhibit impaired gap detection relative to non-impaired, age-matched controls [13,14]. Based on these findings, we used an adapted reflex modification paradigm to test the hypothesis that the presence of neocortical ectopias affect gap detection in male BXS<sub>B</sub> mice.

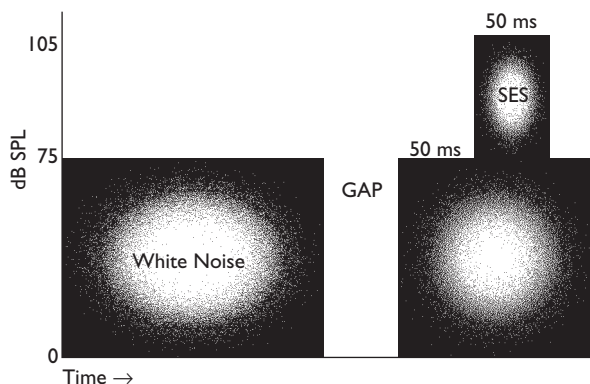
## MATERIALS AND METHODS

**Subjects** The subjects were 33 male BXS<sub>B</sub>/MpJ-Yaa mice born at the Developmental Psychobiology Laboratory at the University of Connecticut. All mice had food and water *ad lib* and were maintained on a 12:12 h light:dark cycle with lights on at 06.00 h.

Behavioral testing took place between 08.00 and 15.00 h. At weaning (4 weeks) the mice were individually housed for the duration of testing.

**Reflex modification paradigm:** The reflex modification paradigm consists of the presentation of a benign stimulus (prestimulus, or pre-pulse stimulus) briefly preceding the presentation of a startle-eliciting stimulus (SES). The SES is a 105 dB white noise burst that causes mice to exhibit an acoustic startle reflex. When the pre-stimulus is detected, the amplitude of the whole-body, acoustic startle reflex is inhibited. This phenomenon is called pre-pulse inhibition. The extent of pre-pulse inhibition is related to the overall detectability of the pre-stimulus. Comparison of reflex amplitudes when a pre-stimulus is present (i.e. a cued trial) *vs* not present (i.e. an uncued trial) provides an objective measure of sensory detection [15].

**Gap detection procedure** The reflex modification paradigm was further adapted to a gap detection procedure. The paradigm consisted of repeated presentation of a SES, with an inter-trial interval (ITI) of 16–24 s [16]. The ITI was variable to prevent anticipation of the SES. A variable duration silent gap (0–100 ms) was presented 50 ms before the SES, with the gap duration on each trial randomly



**Fig. 1.** Single trial schema of the gap detection testing procedure. The duration of the gap varied equally and randomly between the values of 0 (no gap), 2, 5, 10, 20, 30, 40, 50, 75 and 100 ms across 300 trials. SES, startle eliciting stimulus.

selected. As shown in Fig. 1, a given trial occurred every 20 s on average and consisted of 75 dB continuous background white noise, the presentation of a silent gap, 50 ms of additional background white noise, followed by presentation of the SES (a 50 ms, 105 dB broadband white noise burst). This sequence was immediately repeated for the next trial. Trials that did not contain gaps (i.e. uncued trials) were the same as above but the gap had a duration of 0 ms.

A complete testing session contained trials with 0 (no gap), 2, 5, 10, 20, 30, 40, 50, 75 and 100 ms gaps. For the purpose of statistical comparison, the 0 ms or no gap represented the uncued (baseline startle response) condition, while the cued conditions included gap durations of 2–100 ms. Each of the 10 gap conditions were randomly presented 30 times, for a total of 300 trials during a given test session. All subjects were tested once a day over 6 days.

**Apparatus:** Subjects were individually placed in a black-walled, cylindrical cage (12.5 cm diameter, 27.3 cm high) each on a Stoelting electronic activity monitor (model EAM 31404, Stoelting Co., Chicago, IL) during testing. Four monitors were utilized in a quiet testing room (4.5 × 2.3 × 2.7 m). Output voltages from the platforms were sent through a band-pass filter with cutoff frequencies of 1000 Hz and 1 Hz, and passed into a Biopac MP100WS Acquisition system (Biopac Systems, Santa Barbara, CA) to be rectified online on a Power Macintosh 7200/120. This combined apparatus acted to record the amplitude of each subject's whole-body acoustic startle reflex. Incoming voltages (representing transduced movement signals) were acquired at a frequency of 1000 samples/s throughout a session of testing by the Biopac Acquisition system. The epoch of interest was 150 ms in duration, beginning with the onset of the startle-eliciting stimulus. The extracted peak value during this time frame served as each subject's response amplitude for the trial (the dependent variable).

All auditory stimuli were generated on a Macintosh Quadra 700 computer, and output through two Yamaha YST-M100 powered monitor speakers positioned 75 cm above the platforms. The background white noise was presented at 75 dB SPL. The SES was a 50 ms burst of white noise with a 0 ms rise/fall time, presented at 105 dB.

**Anatomical analysis of brains:** Following testing in adulthood, mice were anesthetized and transcardially perfused with 0.9% saline followed by 10% formalin. The heads were removed, placed in 10% formalin, and shipped to Beth Israel Hospital for histological processing and anatomical analysis. See Frenkel *et al.* [17] for details of the histological processing of the brains of the BXS<sub>B</sub> mice.

## RESULTS

**Histology:** Of 33 male BXS<sub>B</sub> mice tested, 12 showed no neuropathology and 21 had one or more neocortical ectopias. Therefore, 63.6% of the male BXS<sub>B</sub> mice tested exhibited ectopias. Subjects were classified as ectopic if they had one or more ectopias, regardless of location or size. The remaining subjects were classified as non-ectopic.

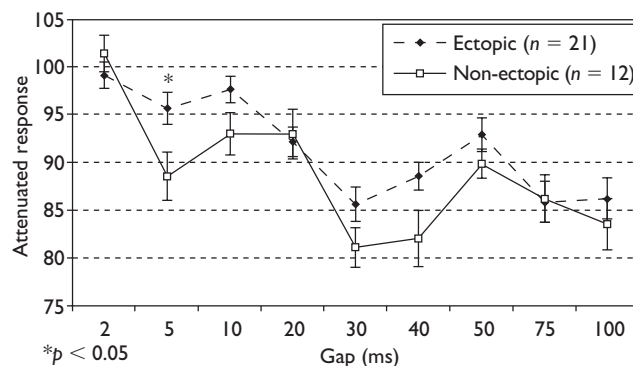
**Gap detection:** The mean startle amplitude for each subject at each condition for each day was computed; grand means were subsequently computed for all subjects within a treatment at each condition for each day. Within each treatment group (i.e. non-ectopic or ectopic) an overall analysis of startle response was performed with two within variables, Gap duration, (10 levels, including the 0ms condition) and day (days 1–6). Absolute reflex response measures for non-ectopic and ectopic BXS mice were analyzed separately to assess whether the mice in the respective groups were exhibiting significant reflex modification at each gap duration (i.e., differential response to cued *vs* uncued trials), which in turn indicates significant detection. In both groups, a significant main effect of Gap Duration was observed (non-ectopic,  $F_{9,81} = 21.40$ ,  $p < 0.0001$ ; ectopic,  $F_{9,81} = 43.39$ ,  $p < 0.0001$ ), indicating that startle response magnitude varied with different stimuli. Based on previous research by Leitner *et al.* [16], there was an *a priori* expectation that if the mice were detecting the gaps there would be a significantly smaller startle to cued (i.e. gap durations of 2, 5, 10, 20, 30, 40, 50, 75 and 100 ms) *vs* uncued (i.e. 0 ms or no gap) stimuli. Therefore, planned comparisons of the average startle response for each subject on uncued versus cued trials were performed. Significant differences between uncued and cued response were evident at gap durations down to 5 ms, but not at 2 ms, in both non-ectopic and ectopic groups.

Responses were then converted to percentages, specifically representing the cued response as a percentage of baseline (uncued) response for each subject, for each condition. If no advantage was conferred by a given gap duration (i.e. no detection), the cued response should approximate the uncued one (i.e. 100%). An analysis was performed on these converted measures (called attenuated response measures) using pathology (non-ectopic, ectopic) as the between variable, and day (days 1–6) and gap duration as within variables (but with only nine levels of gap duration, since all values at the 0 gap condition converted to 100% and this condition was not included). Again, there was a significant effect of gap duration ( $F_{8,216} = 38.40$ ,  $p < 0.001$ ). However, there was a significant interaction of pathology with gap duration ( $F_{8,216} = 2.46$ ,  $p < 0.02$ ). To further investigate this interaction, a simple effects analysis of pathology at each gap duration was performed. This analysis revealed ectopic mice were significantly impaired relative to non-ectopic mice at only the 5 ms gap condition ( $F_{1,27} = 5.52$ ,  $p < 0.05$ ). Ectopic and non-ectopic gap detection performance did not differ significantly at the remaining longer gap durations of 10–100 ms (Fig. 2).

## DISCUSSION

Ectopic and non-ectopic male BXS mice demonstrated significant gap detection in broadband white noise down to gap durations as low as 5 ms. These results approximate thresholds observed in other species including humans [16] and rats [18]. At the shortest detectable gap (5 ms), however, ectopic mice showed, on average, significantly less detection than non-ectopics.

Previously we showed that male rats with induced cerebrocortical microgyria are impaired in processing two-tone stimuli at rapid, but not slower, presentation rates [7–



**Fig. 2.** Grand means with SEM of ectopic and non-ectopic subjects attenuated response at each gap duration. Ectopic BXS mice are significantly impaired relative to non-ectopic BXS mice at only the 5 ms gap duration. Attenuated Response is computed by dividing the startle response to cued trials (i.e., gap durations of 2, 5, 10, 20, 30, 40, 50, 75 and 100 ms) by the startle response to uncued trials (i.e. no gap condition)  $\times 100$ .

9]. The current findings of impaired gap detection in ectopic male mice, paired with our previous findings from microgyric male rats, substantiates the view that focal cortical malformations (e.g. ectopias and microgyria) are related to impairments in rapid auditory processing. Finally, the current results are consistent with data obtained from children with developmental dyslexia [13,14], wherein longer gap duration thresholds have been seen for affected individuals than for controls.

Specific mechanisms through which auditory processing deficits might arise from distal developmental cortical anomalies, which are displaced from primary or secondary auditory cortices, remain to be uncovered. Previously, we demonstrated that rats with induced microgyria (a similar developmental focal cortical anomaly) exhibit impaired rapid auditory processing and concurrently exhibit differences in thalamic cell size distribution [7], much like thalamic cell size reductions observed in the brains of individuals with dyslexia [19]. This link between focal cortical malformations and changes in the distribution of thalamic cell sizes has been hypothesized to be due to connective changes resulting from neonatal focal damage to the developing neocortex [7]. Similarly, the presence of neocortical ectopias in BXS mice has been shown to be related to alterations in neuronal size in the thalamus [20]. Focal cortical malformations (e.g. neocortical ectopia and microgyria) may, therefore, disrupt the formation of not only the afferent and efferent connections associated with the damaged region [21], but may also result in the maintenance of otherwise transient connections [22].

While it has been demonstrated that there are changes in connectivity related to the presence of neocortical ectopias [23], there is little direct evidence to substantiate a claim of changes in connectivity specifically involving primary auditory brain regions (e.g. auditory cortex, medial geniculate body, inferior colliculus, etc.). Moreover, neurophysiological research with human dyslexics [24] and ectopic BXS mice [17] has demonstrated aberrant auditory evoked responses to brief acoustic stimuli. Human dyslexics, for example, demonstrated differences in evoked magnetoencephalographic (MEG) responses to two-tone

stimuli when the stimuli were presented at rapid, but not slower, rates [24]. Similarly, ectopic BXSB mice demonstrated aberrant auditory evoked potentials (AERP) to the second of two 10 kHz tones only at short interstimulus intervals in a 10.5 kHz/5.6 kHz/10.5 kHz tone presentation procedure [17]. Such aberrant neurophysiological responses suggest differences in neural connectivity and function, which may be related in turn to the presence of focal cortical malformations such as neocortical ectopias. It is important to address the lack of differences in AERPs observed by Frenkel and colleagues using a 10.5 kHz/gap/10.5 kHz presentation procedures. Disregarding procedural differences (i.e. two-stimulus AERP detection is different from gap detection in continuous white noise), note that AERPs were assessed for gap stimuli  $\geq 12$  ms, while our behavioral differences occurred at a 5 ms silent gap. Research is currently under way to assess auditory processing in ectopic mice utilizing additional stimulus parameters as employed by Frenkel and colleagues. Ongoing research will continue to address issues of (1) localization of impaired function (i.e. whether degraded processing occurs at the level of cortex, thalamus, or below); and (2) specific neural mechanisms that could explain degraded auditory event related potentials to brief acoustic stimuli [17] as well as perceptual deficits in detecting brief acoustic stimuli in subjects with these focal cortical malformations.

## CONCLUSION

Developmental dyslexia has been characterized by behavioral evidence of auditory and phonological processing deficits [2–5,25] and anatomical evidence of focal cortical malformations [6]. One venue to assess the etiology of the linking behavioral and anatomical features of dyslexia is through an animal model [7–9]. Since BXSB mice exhibit spontaneously occurring neocortical ectopias [10] that are similar to those observed in the postmortem analyses of human dyslexic brains, we designed an experiment to assess the auditory gap detection abilities of ectopic and non-ectopic male BXSB mice. Gap detection is a simple means of assessing auditory temporal acuity. Results

indicated ectopic mice were impaired relative to non-ectopic mice in detection of a brief, 5 ms gap in white noise, but were not impaired at the longer gap duration of 10–100 ms. The results support the hypothesis that focal malformation impair auditory gap detection in male BXSB mice in a manner similar to results observed in children with dyslexia [13,14].

## REFERENCES

1. Wagner RK and Torgesen JK. *Psych Bull* **101**, 192–212 (1987).
2. Tallal P and Piercy M. *Nature* **241**, 468–469 (1973).
3. Tallal P and Piercy M. *Neuropsychologia* **12**, 83–93 (1974).
4. Tallal P. *Brain Lang* **9**, 182–198 (1980).
5. Tallal P, Miller S and Fitch RH. *Ann N Y Acad Sci* **682**, 27–47 (1993).
6. Galaburda AM, Sherman GF, Rosen GD *et al.* *Ann Neurol* **18**, 222–233 (1985).
7. Herman AE, Galaburda AM, Fitch RH *et al.* *Cerebr Cortex* **7**, 453–464 (1997).
8. Fitch RH, Tallal P, Brown CP *et al.* *Cerebr Cortex* **4**, 260–270 (1994).
9. Clark MG, Rosen GD, Tallal P *et al.* *J Cog Neurosci* **12** (2000).
10. Sherman GF, Morrison L, Rosen GD *et al.* *Brain Res* **532**, 25–33 (1990).
11. Denenberg VH, Sherman GF, Schrott LM *et al.* *Brain Res* **562**, 98–104 (1991).
12. Trehub SE and Henderson JL. *J Speech Hear Res* **39**, 1315–1320 (1996).
13. McCroskey RL and Kidder HC. *J Learn Disabil* **13**, 69–76 (1980).
14. Ludlow CL, Cudahy EA, Bassich C *et al.* Auditory processing skills of hyperactive, language-impaired, and reading-disabled boys. In: Lasky EZ and Katz J, eds, *Central Auditory Processing Disorders*. Baltimore: University Park Press, 1983: 163–184.
15. Wecker JR, Ison JR and Foss JA. *Neurobehav Toxicol Teratol* **7**, 733–738 (1985).
16. Leitner DS, Hammond GR, Springer CP *et al.* *Percept Psychophys* **54**, 395–405 (1993).
17. Frenkel M, Sherman GF, Bashan KA *Neuroreport* **11** (2000).
18. Ison JR and Pinckney LA. *Percept Psychophys* **34**, 84–88 (1983).
19. Galaburda AM, Menard MT and Rosen GD. *Proc Natl Acad Sci USA* **91**, 801 08013 (1994).
20. Sherman GF, Hinton WR and Galaburda AM. *Soc Neurosci Abstr* **24**, 561 (1998).
21. Goldman PS and Galkin TW. *Brain Res* **152**, 451–485 (1978).
22. Innocenti GM and Berbel P. *J Neur Transplant* **2**, 29–54 (1991).
23. Sherman GF, Stone JS, Press DM *et al.* *Brain Res* **529**, 202–207 (1990).
24. Nagarajan S, Mahncke H, Salz T *et al.* *Proc Natl Acad Sci USA* **96**, 6483–6488 (1999).
25. Reed MA. *J Exp Child Psychol* **48**, 270–292 (1989).