Impaired detection of variable duration embedded tones in ectopic NZB/BINJ mice

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Received 13 June 2001; accepted 12 July 2001

Utilizing rodent models, prior research has demonstrated a significant association between focal neocortical malformations (i.e. induced microgyria, molecular layer ectopias), which are histologically similar to those observed in human dyslexic brains, and rate-specific auditory processing deficits as seen in language impaired populations. In the current study, we found that ectopic NZB/BINJ mice exhibit significant impairments in detecting a variable duration 5.6 kHz tone embedded in a 10.5 kHz continuous background, using both acoustic reflex modification and auditory event-related potentials (AERP). The current results add further support to the association between focal cortical malformations and impaired auditory processing, and the notion that these auditory effects may occur regardless of the cortical location of the anomaly. *NeuroReport* 12:2875–2879 © 2001 Lippincott Williams & Wilkins.

Key words: Auditory discrimination; Auditory event-related potentials; Cortex; Dyslexia; Language impairments; Phonological deficits; Reflex modification

INTRODUCTION

Between 5 and 10% of children fail to develop normal language skills in the appropriate developmental time-frame. In the absence of other known causes (e.g. deafness, seizures), these children are identified as having specific language impairment (SLI or LI). Research has shown that children with LI also exhibit rapid auditory processing deficits–defined as an inability to correctly process and comprehend quickly changing or occurring acoustic stimuli [1]. Tallal and colleagues [2] have suggested that this auditory processing deficit may be one causal factor in disrupting language acquisition and may impose cascading effects on the development of other language-related skills. Accordingly, research has shown that up to 80% of LI children go on to be labeled in elementary school with reading disabilities (i.e. dyslexia) [3]. Moreover, auditory processing deficits have also been identified in adult dyslexics (see [4] for review).

At a neurobiological level, research has not yet revealed clear and consistent diagnostic features of LI and dyslexia. However, post-mortem analysis of the brains of dyslexic individuals (some with suggested language disorder) has identified the occurrence of neuromigrational cortical anomalies, including focal microgyria, molecular layer ectopias (ectopias), and neocortical dysplasias [5].

Research on such malformations has in turn revealed an association with auditory processing deficits in rodents. For example, numerous studies have demonstrated deficits in two-tone sequence discrimination in adult male rats with induced focal microgyria for short but not long stimulus durations [6–8]. In addition, BXSB/MpJ mice with spontaneously developing ectopias in frontal cortex (morphologically similar to those found in dyslexics) exhibit impairments in threshold silent gap detection (5 ms) when compared to non-ectopic littermates, but not at longer duration gaps (10–100 ms) [9]. Further, research examining auditory event-related potentials (AERPs) to variable duration embedded tones shows that ectopic BXSB/MpJ have a reduced response to the onset of a second 10.5 kHz tone when preceded by a short embedded 5.6 kHz tone, while ectopic and non-ectopic littermates exhibit similar AERPs at longer embedded tone durations [10].

In order to extend these results, we recently examined the NZB/BINJ inbred mouse strain, which also develops spontaneous ectopias, but mainly in the SM-I cortical region. Ectopic and non-ectopic NZB/BINJ were assessed in a behavioral auditory processing task, as well as in an AERP task similar to that used by Frenkel and colleagues [10].

MATERIALS AND METHODS

Subjects: The subjects were 43 male NZB/BINJ mice born at the University of Connecticut. A randomly selected subset of 10 was used for AERP analysis. All 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, which began around postnatal day 35 (P35). All testing was performed blind to histological condition, which was not confirmed until 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 just prior to a startle-eliciting stimulus (SES). The SES is a 50 ms 105 dB broad band white noise burst that elicits an acoustic startle reflex. When the pre-stimulus is detected, the amplitude of the whole-body acoustic startle reflex elicited by the SES is reduced or attenuated (also called pre-pulse inhibition). The extent of pre-pulse inhibition is related to the overall detectability of the pre-stimulus. By comparing reflex amplitudes when a pre-stimulus is present (a cued trial) *vs* not present (an uncued trial), an objective measure of sensory detection can be obtained [11].

Apparatus: During testing, each of the subjects was placed on a movement transducer platform (Stoelting Co. model EAM 31404). The output voltages from the platforms were passed into a Biopac MP100WS Acquisition System connected to a Power Macintosh 7200 to record the amplitude of the subject's acoustic startle reflex. Maximum peak values were extracted during the 150 ms directly following the onset of the SES. This value represents the subject's response amplitude for that trial (dependent variable). Auditory stimuli were generated on a Power Macintosh 6100 and were played via powered Yamaha YHT-M100 speakers.

Variable duration embedded tone procedure: A 105 dB SES with an average inter-trial interval of 20 s was presented [12], with a variable duration 5.6 kHz tone embedded in the 10.5 kHz background tone 50 ms before each SES. All tones were presented at 75 dB. The embedded tone duration on each of the 300 trials in a session was randomly selected from 0, 2, 5, 10, 20, 30, 40, 50, 75 and 100 ms. A sample trial is represented in Fig. 1. A 0 ms trial represents an uncued condition, while the cued conditions include all other tone durations. All subjects received one session across each of 5 days of testing.

Auditory event-related potentials: Subjects were surgically implanted with gold-plated surface electrodes. For surgery, mice were anesthetized with ketamine/xylazine (40 mg/kg: 8 mg/kg, i.p.) with a local anesthetic injected over the scalp. Electrodes were then secured to the skull with Crazy Glue and dental cement (see [10] for further details).

Recording sessions started 24 h after surgery. Mice were sedated, placed in a mouse restrainer in an insulated, sound attenuated chamber, and allowed to acclimatise for 30 min. Auditory stimuli were delivered at 84 dB by speakers directly in front of the mice, and generated by a stimulus synthesizer developed in the lab with a programmable Parralax Basic chip. The auditory stimulus consisted of an initial 360 ms, 10.5 kHz tone followed by a randomly selected interval (12, 36, 72, 144, or 288 ms) of a 5.6 kHz tone. Following this interval, a second 120 ms second 10.5 kHz stimulus was delivered. An inter-trial interval of 600–700 ms was maintained. EEG signals were amplified with a differential AC amplifier (A-M systems). Signals were digitized at 2 kHz (Instrutech), and averaged online with Superscope II (GW Instruments). Each AERP was calculated from 100 averaged trials at a given stimulus condition.

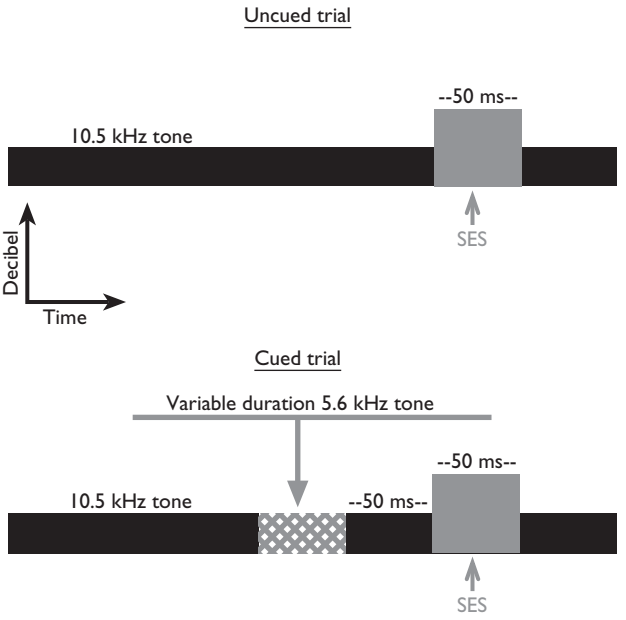

Fig. 1. An illustration of the startle paradigm used in behavioral testing. All tones are presented at 75 dB, while the SES (startle-eliciting stimulus) is presented at 105 dB. If a subject detects the embedded 5.6 kHz tone in a cued trial, the acoustic startle response elicited by the SES will be reduced compared with that elicited in uncued (0 ms) trials. Therefore, attenuation of the startle response provides an index of cue detection.

Anatomical analysis of brains: Following testing, mice were anesthetized and transcardially perfused with fixative (phosphate-buffered 10% formalin, Fischer Scientific). The heads were removed, placed in fresh fixative, and shipped to GDR at Beth Israel Deaconess Medical Center for histological processing and anatomical analysis. The brains were removed, placed in fresh fixative for 1 week, and then dehydrated in ethanol and ethanol/ether. The brains were embedded in celloidin, and were serially sectioned at 30 μm in the coronal plane. A series of every fifth section was mounted on glass slides and stained with cresyl violet for Nissl substance. The sections were examined for the presence and location of cortical ectopias or other neuroanatomical abnormalities. Hemispheric and architectonic location of the ectopias, along with any other abnormalities, were recorded.

RESULTS

Histology: Of 43 male NZB/BINJ mice tested, 35 showed no neuropathology and eight (18.6%) had one or more neocortical ectopias (see Fig. 2 for sample). Ten total

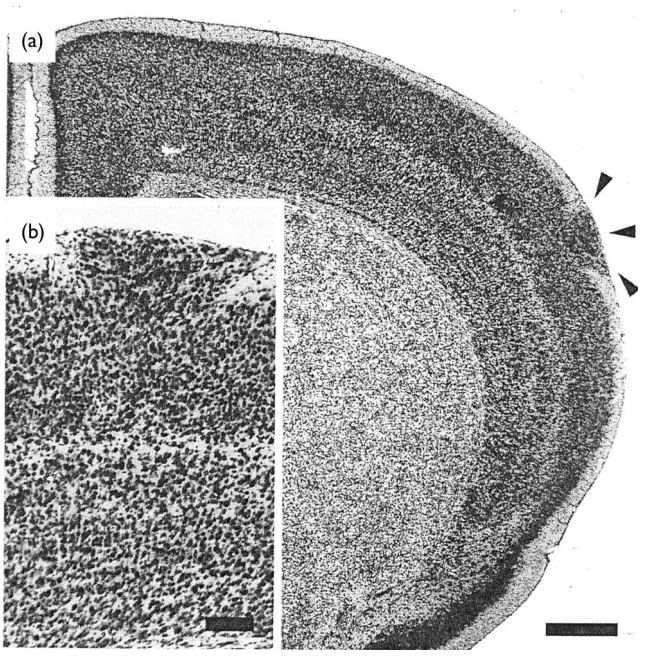

Fig. 2. Ectopic collection of neurons in the NZB mouse. (**a**) High power photomicrograph of NZB mouse with molecular layer ectopia (arrowheads). (**b**) Higher magnification of molecular layer ectopia seen in panel A illustrating typical mushroom-like appearance. Bar = 400 μm (a); 100 μm (b).

ectopias were identified (two subjects had multiple ectopias), located in the primary somatosensory cortex (3 right, 4 left), left secondary somatosensory cortex (1), left frontal cortex (1), and left gustatory cortex (1). Of the 10 males undergoing AERP analysis, seven showed no neuropathology and three (30%) had one neocortical ectopia (primary somatosensory cortex (1 right, 2 left).

Variable duration embedded tone detection: Uncued (0 ms) baseline response amplitudes for ectopic and non-ectopic groups showed comparable means and ranges and did not differ significantly ($F(1,41) = 0.30$, $p =$ n.s.), indicating no difference in motility between the two groups. Planned comparison of actual response amplitudes on cued trials *vs* uncued trials were then performed for all cue durations. Non-ectopic NZB/BINJ males detected all embedded tones as measured by significant startle attenuation at all conditions within each group. Ectopic NZB/BINJ males failed to detect short embedded tone durations of 2 and 5 ms, but detected significantly at longer durations (Fig. 3). Actual responses were then converted to percentages, which represent the cued response as a percentage of baseline (uncued) response (called attenuated response). Overall ectopic and non-ectopic performance was compared on this measure. A main effect of ectopia ($F(1,42) = 5.08$, $p < 0.03$) was found for attenuated response, with no significant interaction with the length of the embedded 5.6 kHz tone ($F(8,328) = 0.53$, $p =$ n.s.).

AERP: Amplitude of the deflection in response to the onset of the first and second 10.5 kHz tone was compared using a ratio of the second response over the first response (A2/A1 × 100). A main effect of ectopia was found, indicating that non-ectopics responded more to the second onset than ectopics ($F(1,8) = 5.49$, $p < 0.05$). Response to the second onset indicates that the embedded tone duration was long enough for the subject to discriminate the second onset as separate from the first. Simple effects analysis of the ectopia effect showed that: (1) at the long condition

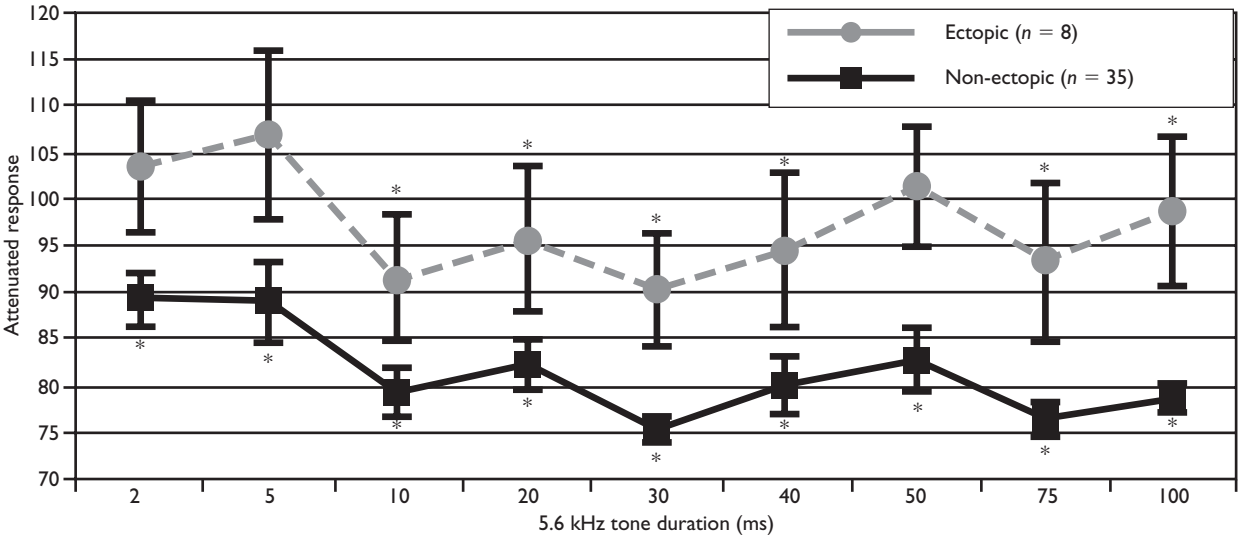

Fig. 3. Ectopic *vs* non-ectopic attenuated response to variable duration embedded tones. An asterisk indicates the embedded tone durations where ectopic and non-ectopic subjects were able to significantly attenuate their startle on cued trials ($^* p < 0.01$). Ectopic NZB/BINJ were unable to detect the short durations (2 and 5 ms) but could detect longer tones. Comparing attenuated response values, a main effect of ectopia ($F(1,42) = 5.08$; $p < 0.03$) was found, with no significant interaction with the length of the embedded tone. This revealed that the non-ectopic attenuation was significantly better than ectopic attenuation at all conditions.

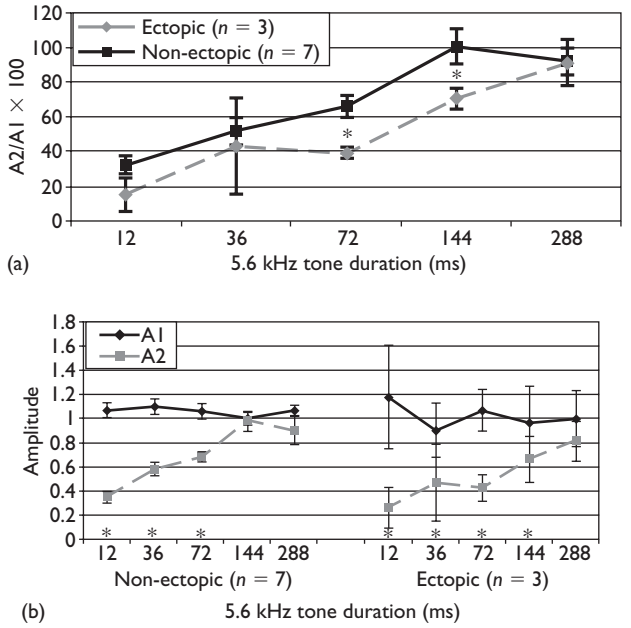

Fig. 4. AERP results. (**a**) AERP response by embedded tone duration. A2 = AERP amplitude to second onset; A1 = AERP amplitude to first onset. A lower (A2/A1 × 100) value indicates a smaller A2 compared with A1. An overall main effect of ectopia ($p < 0.048$) was found, indicating that non-ectopics responded more to the second onset of the 10.5 kHz tone than ectopics. Simple effects analyses of ectopia at each tone duration show that NZB/BINJ male ectopic and non-ectopic mice respond equally to the onset of the second 10.5 kHz tone at long durations (288 ms), whereas at the shorter durations of 144 and 72 ms ectopics respond significantly less to the second onset than non-ectopics ($^{*}p < 0.04$). At the shortest of durations the response does not differ significantly. (**b**) AERP for A1 *vs* A2. A comparison of the response to A1 versus A2 revealed that while non-ectopics responded equivalently at 144 and 288 ms, ectopics responded similarly only at the 288 ms duration. At the 144 ms duration, ectopics showed a significant decrease in response to A2 ($^{*}p < 0.03$). This decrease suggests that the embedded tone duration was not long enough for the subjects to respond to A2 as a unique onset from A1. At all other durations non-ectopics and ectopics showed a significant decrease in amplitude for A2 compared with A1.

(288 ms), ectopics and non-ectopics did not differ in their response to the second 10.5 kHz onset; and (2) at the shorter conditions (72 and 144 ms), non-ectopics responded more to the second onset than ectopics (72 ms, $p < 0.04$; 144 ms, $p < 0.03$; Fig. 4a).

We directly compared the response deflection for A1 with the deflection for A2 (Fig. 4b). At the 288 ms condition both ectopic and non-ectopic groups showed no significant difference between the A1 and A2 response. This indicates that both groups responded equally to both the first and second 10.5 kHz tone onsets. In other words, the 5.6 kHz tone was long enough to perceptually separate the end of the first 10.5 kHz tone from the start of the second, and create a temporal separation of the 10.5 kHz tone. At 144 ms non-ectopics had no significant difference in A1 *vs* A2 response, yet ectopics' response was significantly higher for A1 than A2 ($p < 0.04$). These findings show that ectopics and non-ectopics differ in the length of the 5.6 kHz tone needed to achieve temporal separation of the 10.5 kHz tone (i.e. ectopic animals need a longer separation than non-ectopic littermates). For the 74, 36 and 12 ms durations, both non-ectopic and ectopic subjects had a greater response to A1 than to A2.

DISCUSSION

Behavioral studies on individuals with developmental language disabilities have shown reliable deficits in rapid auditory processing, which seem to be functionally related to impaired speech perception and overall language development, including reading. Concurrent postmortem studies of the brains of dyslexics have revealed focal neocortical malformations. The current studies use an animal model to further examine a putative relationship between basic auditory processing and cortical malformations. Specifically, we found that ectopic male NZB/BINJ mice exhibit impaired detection of embedded tones at short durations (using a reflex modification procedure). However, they could detect significantly at longer durations, albeit not as well as non-ectopic littermates. In addition, ectopic male NZB/BINJ mice respond as well to the second onset of a 10.5 kHz tone following the insertion of a 5.6 kHz tone of 288 ms duration, but are less responsive than non-ectopic littermates at shorter durations of 72 and 144 ms. Ectopic NZB/BINJ do not exhibit an overall hearing impairment, as evidenced by equivalent ectopic and non-ectopic response to the 288 ms tone duration in the AERP, as well as significant attenuation performance equivalent to non-ectopic littermates on another auditory perception task presented in the startle reflex procedure (unpublished data). These collective findings suggest that if the behavioral startle procedure had been extended to include embedded tones longer than 100 ms (i.e. between 144 and 288 ms), we may have found a duration where ectopic and non-ectopic attenuated response would be equivalent in this behavioral paradigm. Future research will assess this possibility.

Combining these findings with previously reported findings from the BXSB/MpJ mouse strain [9,10] we see that the pattern of an impairment evident at short but not long durations is consistent, even though the cortical location of the ectopias differ between the BXSB/MpJ and NZB/BINJ strains. Such dissociation between auditory processing deficit and the location of the anomaly is also evident within a rat model, where induced microgyria in frontal, parietal, and occipital cortices all resulted in equivalent rapid auditory impairments compared with sham littermates [13].

Specific mechanisms by which these cortical anomalies lead to the processing deficits observed here still remain to be elucidated. One possible mechanism includes changes in neural connectivity [14], a hypothesis supported by evidence of morphological thalamic changes in humans with dyslexia [15], as well as microgyric rats [13], ectopic NZB/BINJ and NXSM-D/EiJ mice [14] and ectopic BXSB/MpJ mice [16]. Connectional changes have been specifically associated with the presence of ectopias [14,17]. Further, focal cortical anomalies may disrupt afferent and efferent thalamic connections [18], in addition to maintaining otherwise transient connections present at the time of injury [19]. Anomalous neural connectivity is also supported by evidence from neurophysiological assessments (i.e. AERP and evoked MEG responses). Specifically, AERP evidence from the BXSB/MpJ strain [10] and now the NZB/BINJ

strain indicate altered neural connectivity in mice with neocortical ectopias, which is associated with an impaired ability to respond to a second onset of 10.5 kHz tone after a brief embedded 5.6 kHz tone. Similarly, evoked MEG responses in humans with dyslexia differ from non-dyslexic counterparts when two-tone stimuli are presented at rapid but not slower rates [20].

CONCLUSION

This study provides additional behavioral and electrophysiological evidence that developmental focal anomalies in the cerebral cortex are associated with basic rapid auditory processing deficits, and importantly, shows behavioral and electrophysiological evidence of auditory processing deficits for the same animals. Specifically, male ectopic NZB/BINJ mice have poorer discrimination of embedded tones than non-ectopic littermates, and poorer evoked responses to the second onset of a 10.5 kHz tone when preceded by short but not long embedded 5.6 kHz tones. Since NZB/BINJ ectopias occur in a different cortical area than those of the BXSB/MpJ strain, the current study further supports the notion that these auditory effects may occur regardless of the location of the cortical anomaly. As such, these findings do not appear to reflect the direct effects of cortical damage, but rather, suggest a developmental propagation mechanism that may ultimately affect other brain structures or systems.

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Acknowledgements: We thank Drs Matthew Clark and Gordon Sherman for technical assistance and helpful discussion. This research was supported by NIH Grant HD20806.