

Severity of focal microgyria and associated rapid auditory processing deficits

Ann M. Peiffer, Melissa M. McClure, Steven W. Threlkeld, Glenn D. Rosen¹ and R. Holly Fitch^{CA}

Department of Psychology; Behavioral Neuroscience Division, University of Connecticut, 3107 Horse Barn Hill Rd. U-4154, Storrs, CT 06269-4154;

¹Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

^{CA}Corresponding Author: roslyn.h.fitch@uconn.edu

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Data from rodent models of induced microgyria suggest that bilateral damage leads to more severe rapid auditory processing deficits than unilateral damage. It is unclear whether this reflects the degree, or bilateral/unilateral nature, of damage. The current study evaluates the effects of microgyric severity by assessing rats with single- vs double-pair bilateral focal microgyric lesions, using auditory discrimination and MGN measures. Behavioral data show a

significant auditory processing deficit on rapid processing tasks for microgyric as compared to control subjects, and also reveal more severe deficits for double- than for single-pair bilateral microgyrics. Greater disruptions are also seen in the MGN of double-pair compared to single-pair bilateral microgyric subjects. *NeuroReport* 15:1923–1926 © 2004 Lippincott Williams & Wilkins.

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INTRODUCTION

Approximately 7–8% of preschool children are diagnosed with language disability of unknown origin [1]. Research has consistently shown rapid auditory processing deficits in this population [2,3], as well as in adult dyslexics (see [4] for review). It has been suggested that auditory processing deficits may trigger a cascading disruption of speech perception and production, and ultimately, language and reading development [2]. Such assertions are consistent with evidence of anomalous neural activation patterns during processing of both speech and non-speech stimuli in human dyslexics [5]. From a diagnostic perspective, consistent neurological features of language disability remain largely unknown. However, post-mortem analyses of the brains of human dyslexics have revealed the presence of neocortical malformations [6]. Similar malformations can be induced in rodents [7,8], and the presence of these anomalies has been associated with rapid auditory processing deficits in both male rats and mice [9–13].

Evidence has shown that the location of a malformation (e.g., frontal, parietal, or occipital cortex) does not influence the subsequent auditory processing deficit in microgyric male rats [14], suggesting that early focal cortical damage leads to subtle and pervasive disruption of neural networks critical to sensory processing. Evidence also suggests that increased neocortical damage may be associated with more significant rapid auditory processing impairments, since rats with bilateral microgyria were found to be more impaired than unilateral microgyric rats on an operant conditioning auditory discrimination task [13]. Studies of

premature infants also support a link between severity of brain injury sustained by intracranial hemorrhage and degree of subsequent cognitive/language impairments [15,16].

In order to test the hypothesis that an increased degree of early damage may be associated with more severe behavioral deficits (absent a confound of unilateral *vs* bilateral damage), the current study investigated rapid auditory processing ability in male rats with induced double-pair bilateral microgyria as compared to rats with single-pair bilateral microgyria, or sham surgery. Given evidence that morphology of the medial geniculate nucleus (MGN) is altered in microgyric male rats [14] and dyslexic humans [17], analyses of cell size distributions in the MGN of microgyric subjects were also performed.

MATERIALS AND METHODS

Subjects: Subjects included 46 male Wistar rats born from purchased dams (Charles River) at the University of Connecticut. At weaning (P21), subjects were uniquely marked and pair-housed using a 12:12h light/dark cycle, with food and water available *ad lib*. At P50 (adulthood) subjects were individually housed for behavioral testing. 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.

Induction of focal microgyria: On P1 litters were culled to 10 pups (8 male, 2 female), with male pups randomly designated to receive single- or double-pair bilateral freezing lesion or sham surgery (treatment balanced within litters). Based on a modification of the technique employed by Dvorák and associates [18], focal microgyric lesions were induced as either a single pair (one to each hemisphere) or a double-pair (two to each hemisphere) as described previously [19]. 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.

Behavioral testing: startle reduction: The modified startle reduction paradigm (described in detail elsewhere [10,11]) comprises the presentation of a benign auditory pre-stimulus (cue) prior to a loud startle-eliciting stimulus (SES). If the cue is detected, the amplitude of the acoustic startle response is reduced relative to uncued trials, and the degree of reduction is proportional to the detectability of the cue. During testing, each subject is placed on a load-cell platform (MED Associates, Georgia, VT, USA). The platform's output voltage is 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 are extracted following the onset of the SES, and auditory stimuli are generated on a Power Macintosh 6100 using custom programmed software. Stimulus files (one file per session) are played using SoundHack 0.881NF and presented through powered Yamaha YHT-M100 speakers, with sound intensity levels checked by sound level meter (see [10,11] for more details). Attenuated responses are calculated from absolute response measures using the formula $[\text{cued response}/\text{uncued response}] \times 100$, where absolute response scores reflect load-cell displacement indices for each subject's startle reflex on each trial. Planned multivariate ANCOVAs are then performed on both absolute and attenuated measures for each task.

In the current study, all subjects received five sessions (1/day) of a single tone procedure. Individual scores on this task were used as a covariate, to control for baseline startle response differences on subsequent procedures. Next, a standard oddball procedure was employed which included four stimulus conditions (1 condition/session/day). Stimulus conditions were defined by the interstimulus interval (ISI) between repeating two-tone sequences (using tones of 7 ms duration, and 2.3 or 1.1 kHz), and included ISIs of 225, 75, 40, or 10 ms. The repeating tone sequences were further separated by a fixed between-sequence ISI, defined as the within-sequence ISI plus 200 ms. These parameters remained constant within each test session. The repeated presentation of the standard two-tone sequence (high/low) served as background, and on cued trials, the cue (standard stimulus in reverse order, low/high) was presented just prior to the SES. The interval between each SES presentation was variable, but averaged 20 s (range 16–24 s). After 4 days of standard oddball testing (one day per four conditions), a modified oddball procedure was employed. This procedure used within-sequence ISIs comparable to those used in the standard version of the task, but between-sequence ISIs that

were reduced in duration (i.e. speeded). This modified oddball procedure employed the following ISI conditions: (1) within ISI=40 ms, between ISI=140 ms; (2) 20 ms/70 ms; and (3) 10 ms/60 ms (Fig. 2a). Each ISI condition was tested over 5 days (rather than 1, as above) due to the more difficult nature of this version of the procedure. Finally, a three-tone sequence oddball procedure was employed. Here the 2-tone sequence was extended with the addition of a third 7 ms high tone for both background (high/low/high) and cue (low/high/high) sequences. Testing again was performed at each ISI duration for 5 days. ISI conditions for the 3-tone oddball were: (1) within ISI=60 ms, between ISI=260 ms; (2) 30 ms/100 ms; and (3) 10 ms/60 ms.

Brain analysis: Following behavioral testing, subjects were weighed, anesthetized with ketamine/xylazine (100/15 mg/kg), and transcardially perfused with saline followed by formalin. Heads were removed, placed in formalin, and shipped to GDR for anatomical analysis. The brains were removed, weighed, lesions confirmed and location assessed visually.

Stereological analysis of the MGN: All stereological measurements were performed on a computer-controlled Zeiss Axiophot microscope interfaced to Stereo Investigator (MicroBrightField Inc., Colchester, VT, USA) software. Cell-packing densities and cross-sectional cell areas were measured in the MGN of all subjects using the optical fractionator and nucleator probes. Volume of the MGN was estimated using the Cavalieri probe. Coefficients of error for all measures were <0.05 .

RESULTS

Brain analysis: Post mortem analysis confirmed 4-layered bilateral microgyria in all subjects exposed to the P1 freezing lesion treatment, and no malformations were seen in any control subjects. For single bilateral microgyrics, lesions were located in sensorimotor cortex (SM-I) and occasionally impinged on the occipital cortex. For double bilateral microgyrics, lesions were found mostly in SM-I, with some extension into frontal, temporal, or occipital cortices. The

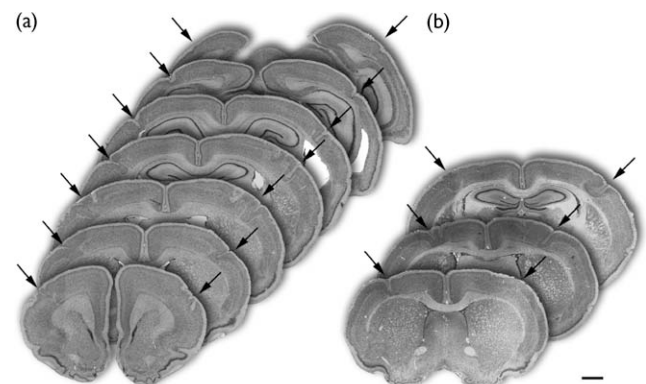


Fig. 1. Double- and single-pair bilateral microgyria, coronal sections, adult. (a) Two 5 s freezing lesions to each hemisphere (sections separated by ~ 1.2 mm). (b) A single 5 s freezing lesion to each hemisphere (sections separated by ~ 0.9 mm). Arrows denote malformation in each hemisphere; bar=1 mm.

majority of double lesions appeared as one continuous severe lesion (Fig. 1).

Brain weight: Consistent with previously reported findings [20], a single-pair of bilateral microgyria significantly reduced brain weight when compared with control littermates ($F(1,29)=6.77$; $p<0.02$). A double-pair of bilateral microgyria also significantly reduced brain weight compared with control littermates ($F(1,27)=27.24$; $p<0.001$), and these subjects showed a further reduction of brain weight compared with single-pair microgyrics ($F(1,27)=9.71$; $p<0.01$).

Standard two-tone sequence oddball results: Oddball results showed that all three treatment groups significantly detected the oddball tone pair at all ISI durations. Treatment showed no significant main effect ($F(2,43)<1$), and failed to interact significantly with ISI condition ($F(6,129)=1.75$; $p=n.s.$). There were no attenuated response performance differences between treatments at any ISI for any group, though a trend towards worse performance in the microgyric groups at the shorter duration conditions was seen (consistent with prior findings [10–12]).

Modified two-tone sequence oddball results: Overall, higher attenuated response scores were seen on the modified oddball, which employed shorter between-ISI durations relative to the standard oddball (indicating that this task had much greater task demand). Controls significantly detected all durations, while single- and double-pair microgyrics only detected the oddball at the 10/60 ms ISI condition ($F(1,15)=16.95$; $p<0.001$; and $F(1,13)=4.92$; $p<0.05$, respectively). Attenuated response scores indicated that controls performed better than the single-pair microgyrics at the 20/70 ms ISI condition ($p<0.04$) and the double-pair microgyrics at the 20/70 and 10/60 ms ISI conditions ($p<0.01$). Single- and double-pair microgyrics also differed at the 10/60 ms ISI condition ($p<0.04$), with single-pair microgyrics performing better than double-pair (Fig. 2a).

Three-tone oddball results: Again, higher attenuated response scores were seen for the 3-tone oddball than for the standard oddball version (indicating higher task demand). All groups, however, significantly detected all ISI durations. Attenuated response scores for the 60 and 30 ms ISI indicated similar performance between all groups. At the 10 ms ISI, however, controls performed better than double-pair microgyrics ($p<0.003$), and single-pair microgyrics were also better than double-pair microgyrics ($p<0.02$; Fig. 2b).

MGN morphology: Analysis of cell size distribution within the MGN for experimental and control subjects was generally consistent with prior evidence that microgyric male rats show a shift to more small and fewer large cells in the MGN [14]. There was a shift in cell size distribution towards more small and fewer large cells for microgyric subjects overall, compared with controls ($\chi^2=16$, $df=11$, $p=0.07$, one-tail). Within the microgyric group, subjects with double-pair bilateral microgyria had a significantly greater shift in cell size distribution than did single-pair microgyric subjects ($\chi^2=25.95$, $df=11$, $p<0.01$), indicating that more

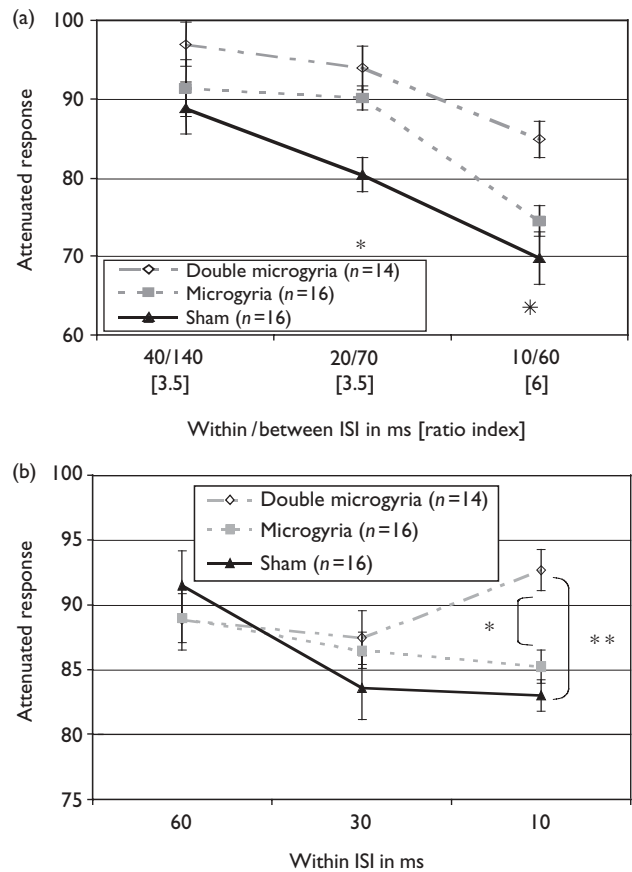


Fig. 2. (a) Modified oddball results (collapsed across 5 days for each ISI), attenuated response scores. At the 20/70 ms ISI condition, controls performed better than single- and double-pair microgyrics ($*p<0.04$, $p<0.01$). At the 10/60 ms ISI condition, double-pair microgyrics performed worse than control and single-pair microgyrics ($*p<0.01$, $p<0.04$). Controls detected all ISI conditions, while single- and double-pair microgyrics only detected the 10/60 ms ISI condition. (b) Three-tone oddball results (collapsed across 5 days for each ISI), attenuated response scores. At the 60 and 30 ms ISI conditions all groups performed similarly, while at the 10 ms ISI condition, controls performed better than double-pair microgyria ($**p<0.003$) but not single-pair microgyria ($p=0.45$), and single-pair microgyria were better than double-pair microgyria ($*p<0.02$).

severe cortical damage was related to more profound anomalies at the thalamic level. This interpretation is supported by findings that double- but not single-pair microgyrics had significantly fewer cells in the MGN ($p<0.02$) and a smaller MGN volume ($p<0.03$) than controls.

DISCUSSION

Impairments associated with disturbances in neocortical migrational (such as microgyria) are observed on tasks characterized by sufficient auditory temporal demand. We now show that doubling the damage by adding a second pair of bilateral microgyria appears to worsen the deficit seen in single-pair bilateral microgyric littermates. This effect is evident in more severe deficits on the shortest conditions of the modified 2-tone and 3-tone oddball discrimination procedures. Moreover, anomalies at the

thalamic level were significantly greater in double-pair microgyric subjects.

The mechanistic link between cortical malformations and subsequent rapid auditory processing deficits remains unknown. One possibility could involve induced changes in developmental neural connectivity. In support of this view, both afferent and efferent thalamic connections are disrupted by focal cortical anomalies [21], and morphological thalamic changes are seen in dyslexic humans and in male microgyric rats [14,17]. These changes may be more pervasive in subjects with increased disruption of cortical developmental processes.

CONCLUSION

We have reaffirmed a tripartite association between focal disruptions of neocortical migration, anomalous morphology of the MGN, and disruptions in rapid auditory processing. Also, we have shown that more severe cortical disruption is related to significantly greater behavioral deficits, and greater disruptions of MGN morphology, suggesting in turn that a higher incidence of focal anomalies in the cortex of developing humans may predict more severe language difficulties later in life. This hypothesis clearly requires further assessment, for example through ongoing neuroimaging studies of human dyslexic populations.

REFERENCES

- Leonard LB. *Children With Specific Language Impairment*. Cambridge, MA: MIT Press; 1998.
- Tallal P and Piercy M. Defects of non-verbal auditory perception in children with developmental aphasia. *Nature* 1973; **241**:468–469.
- Tallal P and Stark R. Speech acoustic cue discrimination abilities of normally developing and language impaired children. *J Acoust Soc Am* 1981; **69**:568–574.
- Farmer ME and Klein RM. The evidence for a temporal processing deficit linked to dyslexia: a review. *Psychonom Bull Rev* 1995; **2**:460–493.
- Temple E, Poldrack RA, Protopapas A, Nagarajan S, Salz T, Tallal P *et al*. Disruptions of the neural response to rapid acoustic stimuli in dyslexia: evidence from fMRI. *Proc Natl Acad Sci USA* 2000; **97**:13907–13912.
- Galaburda AM, Sherman GF, Rosen GD, Aboitiz F and Geschwind N. Developmental dyslexia: four consecutive cases with cortical anomalies. *Ann Neurol* 1985; **18**:222–233.
- Humphreys P, Rosen GD, Press DM, Sherman GF and Galaburda AM. Freezing lesions of the newborn rat brain: a model for cerebrocortical microgyria. *J Neuropathol Exp Neurol* 1991; **50**:145–160.
- Rosen GD, Press DM, Sherman GF and Galaburda AM. The development of induced cerebrocortical microgyria in the rat. *J Neuropathol Exp Neurol* 1992; **51**:601–611.
- Fitch RH, Brown C, Tallal P and Rosen G. Effects of sex and MK-801 on auditory processing deficits associated with developmental microgyric lesions in rats. *Behav Neurosci* 1997; **111**:404–412.
- Clark M, Rosen G, Tallal P and Fitch RH. Impaired processing of complex auditory stimuli in rats with induced cerebrocortical microgyria. *J Cogn Neurosci* 2000; **12**:828–839.
- Peiffer AM, Rosen GD and Fitch RH. Rapid auditory processing and MGN morphology in rats reared in varied acoustic environments. *Dev Brain Res* 2002; **138**:187–193.
- Peiffer AM, Rosen GD and Fitch R.H. Sex differences in auditory processing deficits in microgyric rats. *Dev Brain Res* 2004; **148**:53–57.
- Fitch RH, Tallal P, Brown C, Galaburda A and Rosen G. Induced microgyria and auditory temporal processing in rats: a model for language impairment? *Cerebr Cortex* 1994; **4**:260–270.
- Herman AE, Galaburda AM, Fitch RH, Carter AR and Rosen GD. Cerebral microgyria, thalamic cell size and auditory temporal processing in male and female rats. *Cerebr Cortex* 1997; **7**:453–464.
- Frisk V and Whyte H. The long-term consequences of periventricular damage on language and verbal memory. *Dev Neuropsychol* 1994; **10**:313–333.
- Levy ML, Masri LS and McComb JG. Outcome for preterm infants with germinal matrix hemorrhage and progressive hydrocephalus. *Neurosurgery* 1997; **41**:1111–1118.
- Galaburda AM, Menard MT, Rosen GD and Livingstone MS. Evidence for aberrant auditory anatomy in developmental dyslexia. *Proc Natl Acad Sci USA* 1994; **91**:8010–8013.
- Dvorák K, Feit J and Juránková Z. Experimentally induced focal microgyria and status verrucosus deformis in rats — Pathogenesis and interrelation histological and autoradiographical study. *Acta Neuropathol (Berlin)* 1978; **44**:121–129.
- Rosen GD and Galaburda AM. Single cause, polymorphic neuronal migration disorders: an animal model. *Dev Med Child Neurol* 2000; **42**:652–662.
- Peiffer AM, Fitch RH, Thomas J and Rosen GD. Brain weight differences associated with induced focal microgyria. *BMC Neurosci* 2003; **4**:12.
- Rosen GD, Burstein D and Galaburda A. Changes in efferent and afferent connectivity in rats with induced cerebrocortical microgyria. *J Comp Neurol* 2000; **418**:423–440.

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